

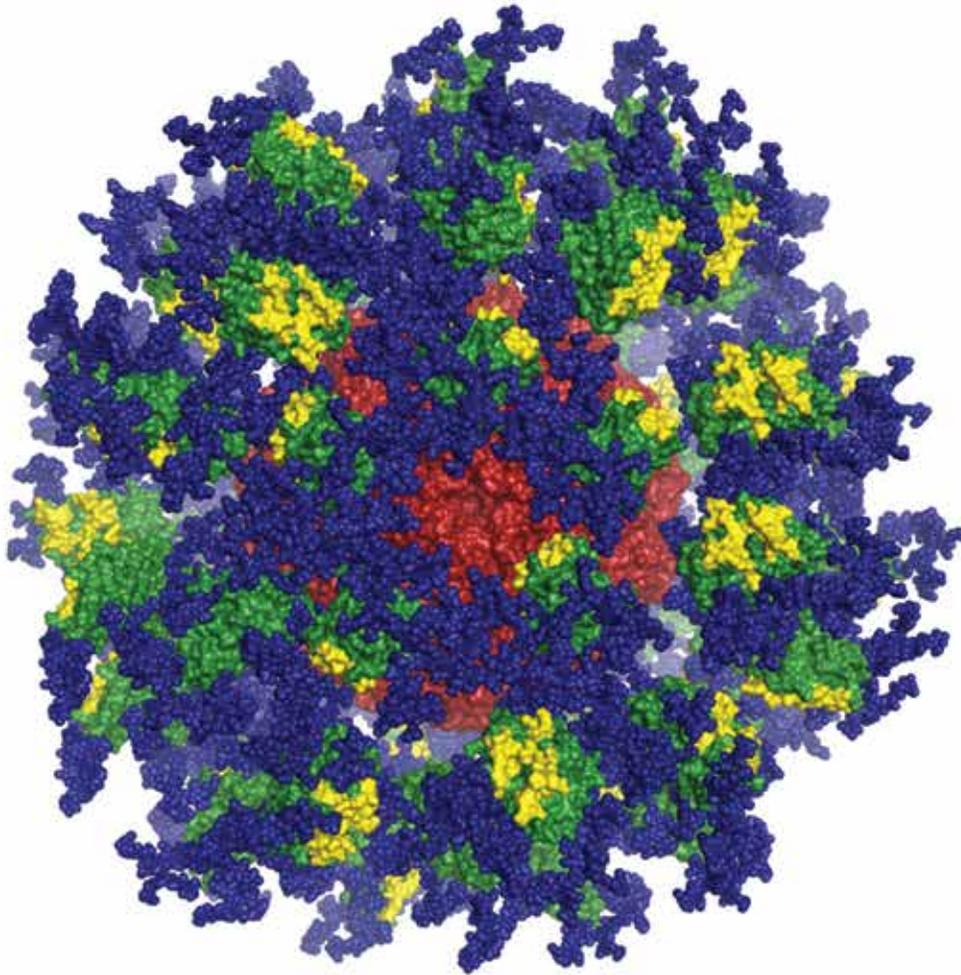
VOLUME 19, ISSUE 2

# IAVIReport

The Publication on AIDS Vaccine Research

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## HIV's Persistence



Plus: Advances in  
developing vaccine  
immunogens

## EDITOR'S LETTER

Every time I sit down to write an Editor's Letter, I think of food. It isn't that I'm hungry. It is because the only "letters from the editor" that I ever read are in food magazines.

These letters often mention some amazing dinner party or exotic locale and almost always feature some lust-worthy dishes that make me immediately want to start cooking—or at least eating. I don't have any stories of great meals to entice you with, but the articles in this issue provide a smorgasbord of scientific advances and personal stories that you will surely want to devour.

In one feature we interview six prominent women scientists involved in everything from vaccine research to implementing global HIV treatment programs. We ask what drives them to continue working in HIV/AIDS more than 30 years after the pandemic began (see page 9). Their answers are inspiring and in some cases surprising.

In another article on advances in HIV cure research, we follow esteemed researcher Steve Deeks from the University of California in San Francisco as he navigates the recently held Keystone Symposium on *HIV Persistence: Implications for a Cure* (see page 4). His broad history in and knowledge of the HIV treatment and cure fields are on full display, as is his droll sense of humor.

Finally, in this issue we report on advances in developing vaccine immunogens designed to induce broad and potent neutralizing antibody responses against HIV (see page 17). A trio of recently published research papers showcase promising first steps in developing and testing these new immunogens in animal models.

Bon appétit!

– KRISTEN JILL KRESGE



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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. IAVI supports a comprehensive approach to addressing HIV and AIDS that balances the expansion and strengthening of existing HIV-prevention and treatment programs with targeted investments in the design and development of new tools to prevent HIV. IAVI is dedicated to ensuring that a future AIDS vaccine will be available and accessible to all who need it. IAVI relies on the generous donations from governments, private individuals, corporations and foundations to carry out its mission. For more information, see [www.iavi.org](http://www.iavi.org).

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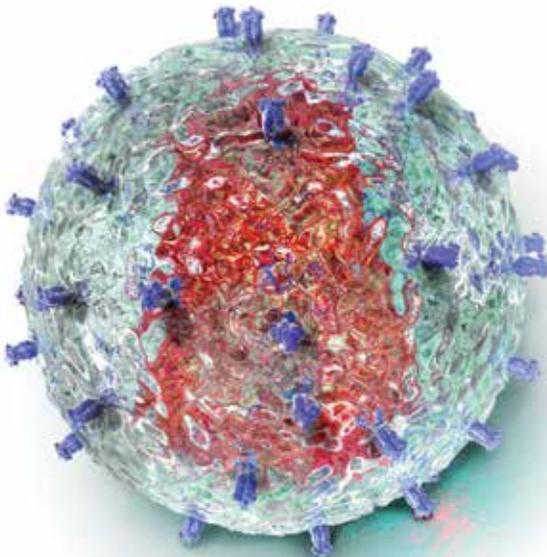
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## [ ON THE COVER ]

An outer domain of HIV gp120 engineered to form self-assembling 60-subunit nanoparticles (eOD-GT8 60mer) that was developed as a priming immunogen by researchers at The Scripps Research Institute (TSRI) in La Jolla and IAVI. The base particle is shown in red, the eOD-GT8 protein is shown in green with the CD4 binding site highlighted in yellow, and the glycans are represented as blue spheres.

Image courtesy of Joseph Jardine, Sergey Mennis, and William Schief of TSRI/IAVI.

# Roundabout TO REMISSION

Steve Deeks is peripatetic by nature: probably a good quality, given the challenges in HIV cure research. The biggest is HIV persistence, the subject of a recent symposium.

By Michael Dumiak

We would like treatment that we can stop.

This is the answer Institut Pasteur scientist and Nobel Laureate Françoise Barré-Sinoussi hears when she asks people living with HIV what they expect from scientists today. Barré-Sinoussi raised a smoldering question in HIV cure research as keynote speaker at the Keystone Symposium, *Mechanisms of Persistence: Implications for a Cure*, held in Boston April 24-May 1. What will it take for HIV-infected individuals to be able to interrupt antiretroviral (ARV) therapy and achieve a sustained remission from the virus?

This long-lived remission may be the field's best hope. But getting to that point may require understanding a vast amount of unsurveyed territory, starting with where to find and how to measure the reservoir of latent HIV-infected cells that forms soon after infection and persists even during suppressive ARV treatment.

Scientists are experimenting with ways to activate and ultimately flush out this viral reservoir. They are also hunting for markers they can use to measure the success of these efforts and are deciding when to study cure strategies in clinical trials that will require volunteers to temporarily stop treatment.

Interrupting ARV at this point in cure research is controversial. "We are not curing people. So why stop therapy?" asks Steve Deeks, a clinician at the University of California in San Francisco (UCSF) and an expert in the role of chronic inflammation. He concedes that this is a reasonable objection, but says the counter-argument is that a cure will never happen unless

researchers test multiple strategies. "Many of us believe the best way to do this is in the context of treatment interruption studies," he says. "The interruptions can be done safely, if people are monitored carefully."

Deeks is an early riser, awake by 4:30 or 5AM. By mid-morning he is ready to hit the gym and work out some of the boundless energy he's shown over the last week as co-host of the Keystone Symposium. He has a unique perspective on and long history in HIV research. He is a manager of one of the largest and long-lived cohorts of HIV-infected men and women in the world—a highly valuable living and breathing data set. Barré-Sinoussi tapped Deeks five years ago to help create a strategic framework for HIV cure research.

At Keystone he eagerly jumped into discussions on the pros and cons of studies, encouraging some researchers to go further, challenging others on their findings. But he's reticent to discuss himself: Deeks says it's not in his phenotype to sit and talk, and he quickly gets bored and restless. "I don't do exciting things. I ride my bike," Deeks says. But in San Francisco, which is a very hilly place. "I have an electric bike," he counters. It is with this droll humor that Deeks steers *IAVI Report* through the current state of HIV cure research on display at the Keystone Symposium.

## Remission, rebound, reverberation

Timothy Brown is still the only person cured of HIV.

Brown, known as the "Berlin patient," was cured by two stem cell transplants, both from a

donor homozygous for a mutation that knocks out the CCR5 protein receptor from being expressed on cells. This matters because CCR5 is the primary receptor HIV uses to infect CD4+ T cells. The stem cell transplants were part of a panoply of other chemotherapies to treat Brown's acute myeloid leukemia. In other words, it is hardly a viable cure strategy. Last year Brown's physician, Gero Hütter of the University of Heidelberg, reported in the *New England Journal of Medicine* that six other patients from the US, Europe, and South America underwent treatments similar to Brown's for different types of cancer. None of these patients survived longer than a year. Researchers even wonder if one lesson from these cases is to continue ARV therapy during transplantation instead of stopping it.

Then there was the case of the Mississippi Baby, a newborn who received ARV therapy beginning just 30 hours after birth, even before medical staff had confirmed the baby's HIV infection status. After a month, researchers could not detect any virus in the infant and therefore stopped ARV therapy. After two years the child remained HIV-free, firing hopes that a cure was achieved. Unfortunately, last summer the child's virus rebounded and treatment was restarted.

There was also a study involving two HIV-infected individuals known as the "Boston patients" who received stem cell transplants for blood-borne tumors. In this case both patients had heterozygous mutations in their CCR5 genes, but received stem cells from a donor who lacked this mutation entirely. After stopping ARV therapy in the spring of 2013, their HIV levels remained undetectable for months. The virus, however, rebounded by the end of that year.

Deeks, who was one of the first to pick up on the significance of the early research on the Berlin patient, and who himself treated Brown, thinks these cases raise fundamental questions for cure researchers. Where is the viral reservoir located? What is the best way to detect and measure it? And is there a way to activate the reservoir and wipe it out?

For Barré-Sinoussi these questions may be so difficult to answer that a cure may, in the end, turn out to be more akin to a respite. "If you stop treatment, you have viral rebound," she says. This is because HIV infects cells just before they enter a latent or resting state. In this latent state, they are invisible to the immune system. Active viral replication is efficiently blocked by ARVs, but if therapy is stopped, the latent virus in the reservoir begins actively replicating, resulting in rebound of detect-

able viral loads. This means that to establish an actual cure, all latently infected cells must be eliminated, Barré-Sinoussi says. "It will be very, very difficult since we have this establishment of the reservoir early on," she adds. Research in monkeys suggests that the latent reservoir of HIV-infected cells may be established within a matter of days (*Nature*, doi:10.1038/nature1359). This suggests that while earlier initiation of ARV treatment may help reduce the size of the viral reservoir, it is unlikely to prevent the reservoirs from forming.

"In the case of HIV infection, or in general of retroviral infections which are latent, it is an almost impossible mission to totally eliminate latently-infected cells," Barré-Sinoussi says. She thinks this is still worth pursuing, but says strategies that allow for a sustained remission from ARV therapy are a more realistic goal. "Remission means to have a persistent reduction and control of the reservoir, without any antiretroviral treatment, and without risk of transmission to others. Remission, in my opinion, is possible."

But there are still many steps to achieve even this.

### The search for a biomarker

When Deeks sees Miles Davenport, leader of the Infection Analytics group at the University of New South Wales in Sydney, he is puzzled and intrigued by the Australian's new data. Davenport stood in front of a poster showing data from two methods he and his colleagues used to try and measure the frequency of viral rebound following the interruption of ARV treatment among HIV-infected individuals. Davenport aggregated data from four previous human studies of deliberate treatment interruption and used statistical models to analyze data from 100 volunteers. He says that while there have been larger studies, the team examined only those studies that employed regular sampling.

Davenport's team calculated that it takes an average of five to seven days for HIV to activate from latency following a treatment interruption. They also estimate that viral replication begins on average every six days or so—approximately 24 times more slowly than previously thought. While there may be 100 million actively HIV-infected cells present at any one time in an untreated HIV-infected person, Davenport's data suggests that a single cell becomes actively infected around once a week when an individual is on suppressive therapy. Prior studies predicted this occurred several times a day. According to Davenport's calculations, reducing the viral reservoir by 50- to 70-fold, instead of several thou-

sand fold, might therefore be enough to allow for prolonged treatment interruption.

“So do you think time to rebound is a legitimate, gold-standard measurement of the reservoir size?” Deeks asks Davenport. Deeks says he wants to develop a biomarker that will become a surrogate marker for the size of the reservoir during therapy. He isn’t the only one on this quest. If there were a biomarker for reservoir size, researchers wouldn’t have to find ways to measure the reservoir in every patient. This would be a good thing, since they don’t know where the reservoir is entirely anyway. “The gold standard is time to rebound, absolutely,” Davenport says, with Deeks chiming in, “A biomarker that can predict time to rebound: that’s the holy grail of cure research.”

### Tissue is the issue

As *Mechanisms of HIV Persistence: Implications for a Cure* implies, finding, assessing, and understanding the extent of the latent HIV reservoir is paramount to cure research. Deeks says he’s come to believe that relying on blood analysis alone will not accomplish even the first step of this gargantuan task, something he mentions several times during the symposium. “Where exactly is the virus?” he muses. “Where does it actually live during long-term therapy?”

He has one idea. “Many people believe this to be the final frontier, where HIV will actually persist the longest, and that is in the T follicular helper cells within lymph nodes,” Deeks says. T follicular helper (Tfh) cells are a special subset of immune cells found in the follicles of organs in the lymphatic system, such as the spleen and lymph nodes. They play many roles and could be a key component of HIV persistence.

And so tissue, not blood, is where Deeks thinks the hunt for the reservoir should focus. Many of his colleagues agree. “Everyone is looking at blood because it’s easy,” Deeks says. “Most people want to work in tissue, which is great, but it’s not easy. You’ve got to bite the bullet and go in there and do it.” Deeks says there are ethical hurdles to collecting tissue samples from study volunteers. “We have some brave volunteers in the community that are heavily motivated to stimulate cure research, so they participate.”

Olivier Lambotte, an infectious disease expert at the Hôpitaux de Paris who, like Deeks, was a co-organizer of the Boston meeting, and colleagues are studying abdominal subcutaneous and visceral adipose or fatty tissues and their long-term relationship with HIV. Over time, clinicians suspect the

body’s fatty tissues change as a result of long-term ARV treatment and the chronic immune activation that results from living with the virus. HIV-infected people can develop lipohypertrophy, which is an unusual fat buildup around the gut. They can also develop lipoatrophy, causing fat to decrease in their legs, arms, and face. Dorsocervical fat pads around the neck and shoulders (sometimes known as buffalo hump) can also accumulate.

Lambotte’s group figures adipose might also provide an ideal environment for HIV persistence, making fatty tissue another component of the viral reservoir. “Tissues are still a black box,” Lambotte says. “We don’t know exactly what happens inside. Most studies have been done in the gut. Other organs are rather badly investigated because it’s difficult to get access to these tissues.” Lambotte says it’s important to include more tissue sample study. “We are on the surface,” he says. “We don’t see what happens in the darkness of the sea. It is a major problem.”

Given the difficulty in obtaining human tissues, Lambotte and colleagues are studying how simian immunodeficiency virus (SIV), the monkey form of HIV, affects the abdominal subcutaneous and visceral adipose tissues in macaques. They found SIV infection increased adipocyte density and caused an enhanced inflammatory profile of adipose tissue immune cells. Lambotte’s group is also working with a small group of HIV-infected volunteers on ARV treatment, analyzing adipose for HIV DNA and RNA. Data from these experiments is pending publication.

Meanwhile, one of Deeks’ colleagues, Joseph Wong, a virologist at the San Francisco Veterans Affairs Medical Center, is showing just how variable, when finally quantified, the reservoir may be in tissues.

Wong found a wide variety of differences in HIV RNA expression levels in biopsies of gut-associated lymphoid tissue and lymph nodes from patients on suppressive ARV treatment. Zian Tseng, a UCSF cardiologist, is conducting a long-term study of sudden cardiac death in HIV patients. Wong was able to piggyback on this study and examine autopsy tissue from eight postmortem individuals who were receiving ARV at the time of death. His team looked at samples from the brain; a series of lymph nodes; the distal ileum, which is the point where small and large intestine intersect; and from the sigmoid colon. Even though it was a limited data set, Wong found a wide range of HIV infection frequency in these different tissues, with as yet no predict-

able patterns. They found uniformly detectable HIV DNA and RNA in lymphoid tissues, as well as measurable HIV DNA, but not RNA, in brain tissue. The DNA, Wong says, could represent both latent HIV and archived, defective virus. The RNA, however, indicates relatively recent and also possibly persistent viral replication.

Tissue is the issue, Wong says. “It’s not the only issue, but there’s a need to delve deeper into where the virus resides and better understand what are some of the consequences of HIV persistence.” The Keystone audience wanted to know more about the brain tissue, but so far Wong’s only examined one such sample. Not surprisingly these samples are hard to come by. “I know only one volunteer who’s had a brain biopsy,” Deeks says. “That’s Timothy Brown.”

The biochemist Janet Siliciano lights up when she spots Deeks. She and her husband Bob, leading cure researchers from Johns Hopkins University in Baltimore, carry on running debates with the San Franciscan during the symposium week. This time they are debating whether blood can be a true marker for the shadowy reservoir. But the conversation quickly turns to the recent cures that never were—the Mississippi Baby and the Boston patients. She and Bob authored a review about these cases last summer (*Science* 345, 6200, 1005). “Everyone was getting so discouraged. We wanted to say we’ve learned a lot from these cases, and that’s how we approach it,” Janet says.

What researchers have learned is just how hard this work will be. “These cases give us a really frightening picture of what we’re up against in trying to cure HIV infections,” Bob Siliciano says. “They prove that the virus persists in a latent state for months to years and then begins to replicate.”

Janet and Deeks get back to the blood versus tissue issue. “I am convinced that the blood is not a representative sample of the tissue,” Deeks says. Janet agrees that tissues need analysis. “I talked to Bob this morning,” Deeks replies. “I told him we’ll send you a lymph node tomorrow.”

“Really?” Janet is impressed. “We’re willing to do that,” Deeks says. Even though she thinks studying tissues is important, she’s not convinced that analyzing blood is a bad option. “I firmly believe,” Janet says, “that the blood *is* representative. Because those cells are circulating throughout the lymphoid system.”

Not T-follicular helper cells, Deeks counters.

“No. They don’t.” Siliciano concedes. “That’s where I agree with you.” And there’s a lot of virus

in there, Deeks nudges.

“I’m interested in exploring what’s going on with CD4<sup>+</sup> T cells in lymph nodes, and really interested to know if there are any latently-infected T follicular helper cells that survive and return back to a resting state,” Janet says. Deeks, as one of six directors of the 2,000-strong SCOPE cohort of HIV-infected volunteers in San Francisco, takes this recommendation seriously. “We’ll start working on it,” he says.

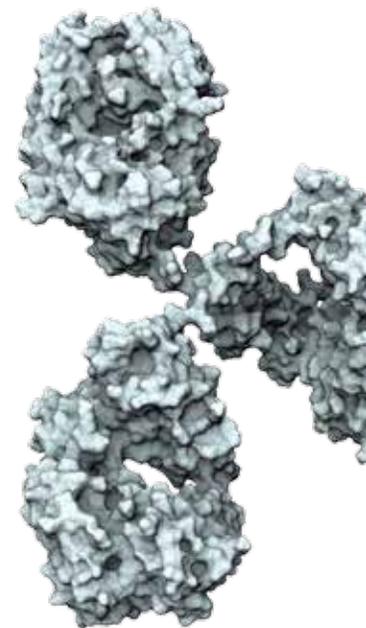
## Flushing out the reservoir

The Siliciano lab is interested in not only characterizing and understanding the viral reservoir, but also eliminating it. One current strategy involves stimulating the reservoir, wherever it is, by shocking the cells in which the virus lies, as Bob and Janet Siliciano write, transcriptionally silent. Once activated they can be killed. This is the basis of the aptly named “shock-and-kill” strategy.

“The dramatic stability of the reservoir is really a major problem that we face,” Bob Siliciano says in Boston. Which is why researchers must take drastic measures to try to rouse the virus from the reservoir and ultimately destroy it. Some shock strategies include using drugs, such as histone deacetylase (HDAC) inhibitors or toll-like receptor (TLR) agonists, to stimulate the reservoir. Some strategies to kill these newly activated cells include using therapeutic vaccines or broadly neutralizing antibodies. Other approaches could include dampening the expression of apoptosis inhibitor molecules, which defend against inflammatory molecules that promote cell death.

Thomas Rasmussen, a researcher in the Department of Infectious Diseases at the Aarhus University in Denmark, is part of a team evaluating different HDAC inhibitors for their potential to activate latent virus. His group is conducting small clinical trials testing panobinostat and romidepsin. One study involves combining romidepsin with a therapeutic vaccine candidate, Vacc-4x, developed by Bionor Pharma. “We would like to combine HIV latency reversal with a vaccine that would increase CD8 responses towards HIV antigens,” says Rasmussen, “to see if that combined approach would augment killing of infected cells stimulated into producing the virus.” He is hoping to present results of this combination study at next year’s Conference on Retroviruses and Opportunistic Infections.

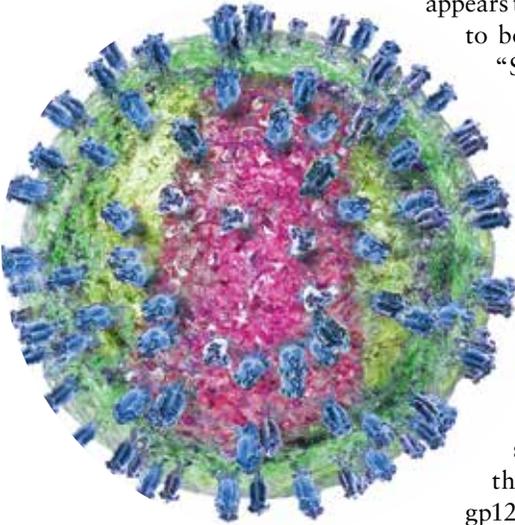
The California biotech Gilead Sciences is dedicating resources to a range of early-stage cure investigations. The company developed a TLR-7



agonist (GS-9620) and is currently conducting dosing studies in early human trials, one of which is currently enrolling HIV-infected volunteers on suppressive ARV treatment (see *PrEP Works, IAVI Report*, Vol. 19, Issue 1, 2015). Gilead's director of clinical virology, Romas Geleziunas, says that based on results from monkey studies, it appears this TLR-7 agonist has the potential to both shock latent HIV and kill it.

"Some patient cells might be more susceptible to the kick component of this, others the kill. We're not quite sure," he says.

Gilead is also experimenting with combining their TLR-7 agonist with the broadly neutralizing monoclonal antibody PGT 121. "We believe the TLR-7 agonist will expose latently infected cells by making them produce HIV proteins," Geleziunas says. That would include, they think, the surface envelope glycoprotein gp120. If the PGT121 antibody binds to gp120, then it can signal the immune system to kill off the cell. Geleziunas says the company obtained a license from Theraclone Sciences to develop these antibodies and is working on an enhanced version of PGT 121.



### A vacation from antiretrovirals

Meanwhile, researchers are mining data from a small but growing cohort of HIV-infected volunteers who have voluntarily stopped ARV therapy and seem to be in a state of prolonged remission. The Institut Pasteur's Asier Sáez-Cirión and his colleagues first reported on this cohort in 2013. Then there were 14 HIV-infected volunteers in the Visconti cohort, all of whom had started antiretroviral therapy during primary infection and maintained treatment for at least a year before voluntarily deciding to stop treatment. They also all effectively controlled their HIV for at least one year after treatment interruption. In this case control is defined as maintaining a viral load less than 400 copies/ml. Most of the cohort has been in this state for more than a decade now. Sáez-Cirión calls them post-treatment controllers. So far only one Visconti volunteer has rebounded and resumed ARV therapy.

Meanwhile the cohort is growing. Six new volunteers recently joined and the Visconti team is examining 25 other candidate volunteers from outside France and may start enrolling them into

the cohort. For Sáez-Cirión, these volunteers suggest that HIV remission is possible. Sáez-Cirión's team doesn't yet understand the mechanism responsible for viral control in these individuals. However, the presence of an allele in human leukocyte (HLA) antigen-B is a common thread among the cohort. This HLA-B-35 allele is present in three post-treatment controllers newly identified from Denmark. It is an unexpected observation: this allele is usually associated with high viral loads and rapid progression to disease in the absence of treatment, says Sáez-Cirión. "It's clearly something you don't expect in control of infection."

Sáez-Cirión says results from a study conducted by the Agence Nationale de Recherche sur le Sida convinced him that a small viral reservoir is the likely starting point in creating post-treatment controllers. But if a single HIV-infected cell might be enough to initiate viral rebound, why does the size of the reservoir matter? "If you have one infected cell, the chances of this cell arriving to a place where there are all of the elements for rebound to occur will be extremely rare," says Sáez-Cirión. His group thinks the fewer infected cells, the easier it is for the host to control.

### Going straight to the genes

One bustling evening at Keystone outside the main hall Deeks spots Pam Skinner, a pathobiologist at the University of Minnesota, whom he doesn't know well. She and Liz Connick, an immunologist at the University of Colorado, are studying the behavior of killer T cells in lymphoid tissue, specifically within lymphoid follicles, where viral replication is concentrated. Her latest data from untreated macaques chronically infected with SIV shows that there are relatively low levels of cytotoxic T lymphocytes (CTL), or killer T cells, in B-cell follicles in lymphoid tissue.

"Here's the lymphoid follicle. There's six red cells in there," Skinner says, pointing to the few bright specks in dark space. "These are the virus-specific CD8<sup>+</sup> T cells." By contrast there was a field of red cells well outside the follicle. "There are too few in here," she says pointing inside the circle. Skinner speculates that increasing the number of virus-specific killer T cells in lymph nodes would allow these killer cells to target any latent cells that are hiding out in the lymphoid tissue should they begin actively replicating. Instead of shocking the reservoir inside the body, they'll use genetic engineering outside of it.

To test this, Skinner plans to treat SIV-infected

*Continued on page 19*

# Six Prominent *WOMEN SCIENTISTS* Making a Difference in the AIDS Fight

By Mary Rushton

It is no surprise that the AIDS pandemic, which began 34 years ago, altered the career paths of female scientists. The pathogen was mysterious, inscrutable, and killing millions of people around the world. Before long dozens of scientists, male and female alike, began studying this new human virus. Their efforts are transforming the fields of virology and immunology.

“The minute I started working in infectious diseases, to me there was no other infection I wanted to work on more than HIV,” says Sharon Lewin, director of the Doherty Institute for Infection and Immunity in Melbourne, Australia, and a prominent HIV cure researcher.

French virologist Françoise Barré-Sinoussi is arguably the most famous female in HIV research. She and her colleague Luc Montagnier of the Institut Pasteur in Paris received the Nobel Prize in Physiology or Medicine for co-discovering HIV, along with US researcher Robert Gallo. Barré-Sinoussi is now one of the most influential scientists directing HIV cure research.

Women are also now steering global AIDS treatment programs. Deborah Birx was appointed Ambassador at Large and US Global AIDS coordinator in 2014, putting her in charge of all US government international HIV/AIDS efforts. This includes overseeing the US President’s Emergency Plan for AIDS Relief (PEPFAR)—considered the biggest humanitarian effort since the Marshall Plan. Her prior posts include serving as director of the Department of

Defense’s US Military HIV Research Program (MHRP). While at MHRP Birx oversaw the launch of the RV144 vaccine trial in Thailand, the first and thus far only trial to show vaccine-induced protection against HIV.

Precisely how many women are studying HIV/AIDS and how this compares to other diseases is difficult to say, although men certainly outnumber women no matter what the scientific discipline. A more sobering statistic that resonates with AIDS researchers is the toll the epidemic takes on vulnerable populations, particularly women. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), 64% of new adolescent infections in 2013 were among young women and more than half of people living with HIV/AIDS are now women. In sub-Saharan Africa, young women aged 15 to 24 are almost twice as likely to become infected with HIV as their male counterparts, according to UNAIDS data. HIV/AIDS is also the leading cause of death among women in their reproductive years (ages 15-49). Advances in HIV prevention, including the use of antiretrovirals to prevent HIV infection, together with development in AIDS vaccine and cure research may reverse these trends.

We talked with six leading women scientists from the US, Australia, and Africa, to learn more about their careers and what inspires them to continue battling HIV/AIDS more than 30 years into the pandemic.

**“Women are the largest untapped reservoir of talent in the world.”**

*– Hillary Clinton, former US Secretary of State and former First Lady*



Linda-Gail Bekker and her husband  
Robin Wood

## LINDA-GAIL BEKKER

With more than six million people living with HIV/AIDS—around 3.5 million of them women—South Africa is ground zero of the HIV pandemic. South Africa continues to shoulder the biggest burden of HIV/AIDS in the world even though the number of new infections has been declining since 2000 and the number of AIDS deaths has been dropping since 2010. It is in this context that Linda-Gail Bekker works as principal investigator and chief operating officer of the Desmond Tutu HIV Centre. Bekker planned to become a geriatrician, but a clinical rotation in KwaZulu-Natal in the 1980s pushed her toward HIV and tuberculosis (TB) research. In Cape Town she works alongside her husband, Robin Wood, who is director of the Desmond Tutu Centre. She is also the President-Elect of the International AIDS Society (IAS). Bekker will be the first female African to hold this position when she takes office at the 21st International AIDS Conference in Durban in 2016.

### *Are the prevention and treatment programs for HIV and TB succeeding in South Africa?*

**Linda-Gail Bekker:** South Africa has carried an enormous burden of HIV since the 1990s and now the biggest worldwide. Unfortunately, a period of AIDS denialism slowed down access to antiretroviral treatment [ART], but attitudes have shifted and the new health administration is now grappling with the day-to-day challenges of getting more people into treatment. There is a real sense of urgency, although health systems are groaning under the load. With TB, I'm afraid we haven't had epidemic control for more than 100 years. Unfortunately, we don't fully understand what is needed to interrupt transmission so more research is the way to go.

### *How did you deal with the issue of AIDS denialism in South Africa professionally and personally?*

**LB:** On a certain level it was embarrassing hearing these dreadfully wrong statements. And when asked why these notions existed in government, to this day I don't really have a good answer. Researchers and clinicians did a fantastic job of working around the barriers and obstructions. Academic researchers haven't always agreed with activists in the history of the AIDS epidemic, but in this case civil society formed an alliance and took on the government, providing incontrovertible evidence that, amongst other things, maternal-to-child transmission of HIV can be prevented with antiretrovirals. On other occasions we joined the government and took pharmaceutical companies to court to drive down the cost of antiretrovirals (ARVs) and other critical medications. Thankfully, with the start of PEPFAR there were other ways to fund ARVs and now South Africa has the biggest treatment program in the world. This is something to celebrate!

### *What are some of the innovative ways your centre is tackling HIV?*

**LB:** One of the challenges we face in the region is tracking people who move between clinics and are lost to follow-up, so we are testing a biometric system that captures patient fingerprints electronically, along with their medical history, and stores the information in a confidential website. Names are often interchangeable and hard to track—biometric identifiers such as fingerprints are not. Long ago, we also realized the merits of task shifting. We trained community care workers living with HIV to be adherence counselors in order to ensure that people with HIV remain in care. Many of these participants now are the cornerstones of our treatment programs.

### *Last time the IAS Conference was held in Durban in 2000, the major theme was expanding access to treatment in developing countries. What are the key issues on the agenda for 2016?*

**LB:** I think we are at a critical crossroads. The prevention revolution is truly underway. We have tools to help end the epidemic but we're going to have to take bold steps. Now is the time for full investment and a worldwide concerted and courageous effort. I also think with the converging of global health issues, there are critical lessons we have learned from the HIV/AIDS response that can and must be brought to bear to change the way we do business in public health throughout the world, particularly in those areas where there are still significant healthcare disparities.

## DEBORAH BIRX

Ambassador at large and US Representative for Global Health Diplomacy Deborah L. Birx is the fourth Global AIDS Coordinator in charge of the PEPFAR—a US\$6.6 billion program in 65 countries that supports HIV/AIDS treatment and prevention efforts through bilateral and regional programs. This includes supporting life-saving antiretroviral treatment for 7.7 million people. She also oversaw the launch of the RV144 trial in Thailand as director of MHRP and served as the director of the CDC's Division of Global HIV/AIDS. Not bad for someone who seemed headed for a career making a better green dye.

### *How did you become involved in HIV/AIDS?*

**Deborah Birx:** In 1983-84, I was a clinical immunologist doing a joint fellowship at the NIH [US National Institutes of Health] and Walter Reed Army Medical Center. I was working on primary immunodeficiency—stuff like the “boy in the bubble”—when we started getting consulted about patients with this mysterious immunodeficiency. We didn't know what it was at first so we started analyzing their B cells. I was more compelled by the patients, their generosity and human spirit, than I had been with any other disease that I had worked on and I just never left.

### *And when did you decide on a career in immunology?*

**DB:** My two elder brothers were mathematicians and nuclear physicists, my father was an electrical engineer and mathematician, and my mother taught nursing. In our household, math and science were extraordinarily valued. So not to be the loser of the family, I went to college and majored in chemistry but soon realized that the best jobs in the mid-1970s were making a better green dye at Kodak so the photographic paper wouldn't turn yellow. I realized that I didn't want to spend the next three decades doing that, which is a good thing because when digital cameras came out that skill would have been completely worthless.

### *A cadre of leading scientists criticized the rationale for the RV144 trial, which you oversaw. Were you skeptical about this trial?*

**DB:** I like to believe I am always a skeptic about data and pushing the envelope to understand things in a deeper way. But that Thai trial was only possible, I think, because at the Department of Defense (DOD) you had the ability to fail spectacularly and yet still have a safety net underneath you. I mean, to have all these premier scientists write about how the DOD under the direction of Debbie Birx was probably doing one of the stupidest things on the planet! If we hadn't been supported by DOD and the NIH that trial could have been shut down before we got started. There was a casualty, however. We had written a companion protocol to the trial which would have put tissue, serum, and plasma samples from a subgroup of vaccines and placebo recipients away so if the [RV144] trial showed promise we would have the ability to do an in-depth immunological analysis of correlates of protection and correlates of immunity. That companion study was stopped. Roll forward 10 years when we found some evidence of vaccine efficacy and everyone is saying, where are the samples?

### *What was your experience like at the CDC?*

**DB:** It was clear from the amazing success that the Kingdom of Thailand had in controlling the epidemic that doing a series of vaccine trials there in the general population was going to become more and more difficult. So in 1998 we began setting up extra sites in Uganda, Kenya, and Tanzania. At that time, from 1998-2000, we wanted our investment in research to also support those areas in Africa with additional lab support and potential clinical support. Day after day, at the [Kenya] field site I was primarily associated with wheelbarrow after wheelbarrow of very sick children, Moms, and Dads were wheeled to the gates of the district hospital. The matron of the hospital, who was an extraordinarily dedicated woman, would turn them away saying, you know we don't have anything to treat them with so there really is no reason to bring an HIV-positive patient who is dying to the hospital because



Learning how to make peanut butter from a women's empowerment group in Malawi

it is just a drain on resources and nothing could be done to save them. This was not callous. They had witnessed thousands of deaths in the community. So here we were talking about doing molecular biology, spending millions of dollars on fundamental research and vaccine development, and you have an entire community dying outside your research walls. It was just too overwhelming and shocking.

And so when PEPFAR was announced by President Bush in the State of the Union address in January 2003, I flew back from Kenya to try and convince Joe O’Neill, the White House AIDS czar responsible for implementing PEPFAR, to include the broader DOD community in PEPFAR, especially the groups doing HIV research that were on the ground and could jumpstart the program with small amounts of funding. I knew if we continued to do research there and only treated a person who became HIV infected during a clinical trial, the very culture of Africa would require that the family split the pills because they believed fundamentally in the wholeness of the community. I’m still grateful to Joe for allowing the US Military HIV Research Program to be part of PEPFAR.



The ambassador with Dr. Danson Warui at Coptic Hope Center in Kenya

*What is one of the biggest challenges in your current role?*

**DB:** I come from bench-driven research where data is honored. It’s been challenging to figure out how to present data so it is understandable and actionable. We created the PEPFAR Dashboards to make our data accessible to all. It allows for transparency and accountability, which are priorities for us. We are taking slow steps forward but I have been witness to the last 30 years of the epidemic, the sheer magnitude of it—30 million people have died—and I guess I never think we do enough or move fast enough.



**SHARON LEWIN**

Sharon Lewin, Director of the Doherty Institute for Infection and Immunity in Melbourne, Australia, credits her post-doc training with David Ho at the Aaron Diamond AIDS Research Center in New York City with solidifying her interest in finding an HIV cure. Ho’s seminal findings that triple-combination therapy could so effectively and dramatically reduce the levels of HIV to the point where they were undetectable marked a turning point in the pandemic. However, initial theories that it might also eliminate the virus over time proved premature, prompting researchers like Lewin to try and figure out why.

*What drew you initially to HIV research?*

**Sharon Lewin:** The minute I started working in infectious diseases, to me there was no other infection I wanted to work on more than HIV. There were so many areas that were fascinating to me. The science was changing so quickly and there were all these challenging issues around consent, stigma, and patient inclusion. This was the late 1980s. There was no real treatment—largely, gay men were getting infected in Australia—and lots of people were dying. So there was this real urgency to do something.

*When did you start focusing on cure research in particular?*

**SL:** My PhD was actually quite relevant to HIV latency and persistence. In those early days, we were still trying to work out which cells HIV really infected. We always knew it infected T cells but there was this question about what other longer-lived cells there were. Then in 1997, I got an opportunity to do my post-doc with David Ho. At the time he and a colleague, Marty Markowitz, had some of the best-studied patients being treated with antiretroviral therapy. Some of the early modeling pre-

dicted that if you stayed on treatment for three years, the virus would decay to nothing. But that theory only lasted about three or four months when scientists discovered HIV latency—that pools of latently infected cells known as the reservoir were present from the beginning and persisted indefinitely on antiretroviral therapy. The first paper appeared in November 1997, two weeks before I arrived in New York, and so I became involved in that whole pursuit of can ARVs cure HIV and if they can't why and where is the virus sitting in people on ARVs.

***What was it like working with David Ho in the 1990s?***

SL: The whole environment at Aaron Diamond at the time was just really amazing. David was incredibly innovative—he had a million different ideas—and there were people there from all over the world getting training.



Sharon Lewin in her laboratory in Melbourne

***What is the focus of your cure research?***

SL: I've worked in three main areas. The first was how to mimic HIV latency in *in vitro* models. I had been involved in developing tools that allowed us to ask the question of how latency is established and how can we intercede. We were the first to describe that you don't have to fully activate the cell but just stimulate certain pathways to allow the virus to get in and integrate. That meant we could then look at drugs that reverse latency or activate latency, where you basically push the virus out of its hiding place in the hope it then will kill the cell or become visible to an immune response.

This is what is now called the “shock and kill” or “kick and kill” approach.

We were one of the first groups to look at these more potent histone deacetylase [HDAC] inhibitors like valproic acid. At the same time, in the mid-2000s, HDACs like vorinostat were exploding in the cancer world and they were 1,000 times more potent. And of course now we have HDACs that are 1,000 times more potent than vorinostat.

***Why did it take the HIV cure field another decade to really take off?***

SL: I think there were many other priorities in the 1990s regarding HIV care, such as toxicity and drug resistance, to sort out. There was a lot of interest in developing a vaccine, which remains of critical importance. I think those two issues really dominated research. I think the cure field as a whole really took off about five years ago with the Berlin patient [the only person to be cured of HIV] and leadership from people like Françoise Barré-Sinoussi and the IAS. Until then there was a lot of skepticism about whether we could cure HIV. By 2010, it had also become apparent that we had really good drugs, costs were lower, and we could get them into Africa. The question was how sustainable was this?

***Do you think a cure is possible?***

SL: There are a lot of challenges but I do think the field has moved a lot in the last five years. There are reports of people who have been able to safely stop treatment and achieve antiretroviral-free remission for a period of time. Plus, if you treat people early you can significantly reduce the amount of latency. There are also drugs that clearly activate the virus and push it out of its hiding place. I think we will find more defined ways to achieve antiretroviral-free remission, though how many people will be able to achieve that and for how long I'm not sure.

***What advice would you give to women considering a career in science?***

SL: I think women should follow their passion whatever it is. In the end you are judged by how good you are and that is often directly related to how passionate you are. I look at someone like Françoise [Barré-Sinoussi], who was trained and doing her major work in the early 1980s. It was a different time then and much has changed. But there are still immense challenges with families, kids, and finding success in research. I don't think that can be overlooked. I don't think it is the same for men. At the time when your scientific career is probably at its busiest, it is also when you are having kids. We need better systems in place to account for that.



## GALIT ALTER

You could say that Galit Alter, an immunologist and principal investigator at the Boston-based Ragon Institute of Massachusetts General Hospital, the Massachusetts Institute of Technology, and Harvard, plays the field. It's the goal that remains the same: finding an AIDS vaccine. Alter's earliest research focused on the role of T cells. At the time, hopes were riding on a T-cell based vaccine candidate being tested in an efficacy trial known as the STEP study. But when the STEP candidate was found to be ineffective, Alter switched her career focus. She is now studying sugar molecules that enhance the production of antibodies.

### *How did you come to study HIV and antibodies?*

**Galit Alter:** I was working toward my PhD when people started recruiting HIV-positive people for acute infection studies. That was when there was a lot of momentum and excitement around T-cell biology. But then STEP failed and I didn't want to be in T cells anymore. My inclination has always been to do something a little bit different. If everyone is playing in the sandbox, I want to find a different sandbox. I was working in T cells before it got really popular. Then when everyone jumped on the bandwagon I switched to natural killer [NK] cells. Then when the NK cell field got too crowded, I jumped to antibodies. And now that antibodies have gotten popular, I have jumped to sugars. You have to keep pushing barriers. If you don't push yourself to explore new frontiers you end up not being funded because you keep doing the same old, same old.

### *What are you learning about sugars or glycans and their role in vaccination?*

**GA:** What we know is that these glycans change during inflammatory diseases. People with autoimmune diseases have different glycan profiles than people who don't have autoimmune diseases. Pregnant women and older people have different glycans and we know they change during different inflammatory states. Whether they are selected in different ways in vaccination is not totally clear. But these glycans are, selectively, probably being programmed by B cells under different kinds of inflammatory cues, and understanding how they are regulated is really a black box and sort of where my efforts are. What we're seeing is that different antigen-specific antibody populations all have their own antibody glycan signature that allows them to direct different kinds of functions. That suggests that B cells can learn this. The question is how do they learn this and how can you develop a vaccine that induces that in a selective way.

### *What tools are you using to answer these questions?*

**GA:** Well, glycans are not easy to study. They are kind of the overlooked molecule because the tools haven't been right, but better tools have been emerging over the last decade due to a massive investment from the NIH, which has basically been creating these centers to help develop newer approaches to analyzing glycosylation. With help from some of the gurus in glycomics we've been able to adapt high throughput techniques to study hundreds of thousands of antibody populations. It's being done all in house, which gives us the opportunity to tackle all kinds of cool questions.

### *Did you always know you wanted to be a scientist?*

**GA:** I had no idea and I think it was serendipity that I ended up in HIV research. I stumbled on microbiology and immunology and found viruses really interesting.



## NELLY MUGO

As the AIDS epidemic decimated sub-Saharan Africa, Kenyan Nelly Mugo was beginning her clinical career in obstetrics and gynecology. Nearly two decades later, the principal research scientist at the Kenya Medical Research Institute is part of an AIDS success story. Mugo helped conduct the Partners PrEP Study and the Partners Demonstration Projects that showed pre-exposure prophylaxis (PrEP)—the administration of ARVs to prevent HIV transmission—was effective in serodiscordant couples.

### *What convinced you to become a researcher?*

**Nelly Mugo:** I was working at a hospital in Kenya and we were seeing a lot of complications from

pelvic inflammatory disease, especially in HIV-positive women. I thought better research might help improve the clinical care and so I began working with Craig Cohen [a US researcher now at the University of California in San Francisco], who had helped establish the Research Care and Treatment Program in Nairobi. I later got my Masters of Public Health at the University of Washington specializing in epidemiology.

***What was it like being on the front lines of the epidemic before there was effective treatment?***

**NM:** It was a tragedy and a very fearful time. There was so much ignorance even among health care providers. There were some who were afraid to treat people and people died from the stigma associated with AIDS. It was a terrible time for our healthcare system.

***What is the status of PrEP in Kenya now?***

**NM:** The Partners Demonstration Project found that daily PrEP use among serodiscordant couples was even more effective than we thought. I'm very excited about that. There is still a lot of advocacy that we need to do around PrEP—who should receive it, how will they access it, and the role of providers in implementing PrEP. That's what we need to understand. Working with our colleagues from the University of Washington we are committed to working with the government to see PrEP move from research to practice.



Nelly Mugo with members of the Partners PrEP Demonstration Project in Thika, Kenya

## SUSAN ZOLLA-PAZNER

A decade into her career as a B-cell immunologist, Susan Zolla-Pazner was consulted about a handful of cases of Kaposi's sarcoma occurring among homosexual men in New York City. This thrust her into the forefront of an epidemic that has come to define her life's work. Zolla-Pazner has spent most of her career studying antibodies against HIV and using this information to design vaccine strategies, concentrating in particular on certain variable regions of the outermost viral protein known as HIV Envelope. Much of her work involves the second and third variable loops of HIV Envelope, referred to as V2 and V3 respectively. Zolla-Pazner was not involved with the conduct of the RV144 trial in Thailand, however, her subsequent work demonstrated that the modest protection against infection among vaccinated volunteers correlated with vaccine-induced antibodies targeting V2. After 45 years at New York University, Zolla-Pazner is moving across town this summer to join the Icahn School of Medicine at Mount Sinai as a professor of medicine in the Division of Infectious Diseases and the Department of Microbiology.



***How did you get involved in HIV research?***

**Susan Zolla-Pazner:** I finished a post-doc and started a faculty position at New York University in 1969 working on multiple myeloma and focusing on B-cell immunology. In 1981 I got a call from a physician who had had four male patients with an unusual cancer called Kaposi's sarcoma [KS], which I had never heard of before. Those were the first four patients with KS associated with what we later found out was HIV infection.

***That must have been quite an interesting time.***

**SZP:** It was like living in the middle of an Agatha Christie novel. There were only a handful of people who had an inkling of what was going on. When we realized that what we were seeing was occurring in gay men, we contacted a physician in New York who treated mainly gay men and we asked for blood specimens from 40 healthy gay men so we could match them to the first 20 patients we had with KS. We found that a third had the same immunologic abnormalities as the KS patients. I remem-

**“From a purely immunological point of view, the work we do is fascinating. From a humanitarian view, this epidemic is devastating. The combination of those things keeps driving us.”**

– Susan Zolla-Pazner

ber sitting down and pouring over the data and realizing that a third of the gay population was already suffering from this strange malady. It still gives me the shivers.

*Can you describe the early days of your work on AIDS?*

**SZP:** I was interested in antibodies and I was particularly focused on B-cell abnormalities in patients, which was pretty peculiar at the time because everybody was focused on T-cell responses. What we noticed right away was that all patients had elevated levels of Immunoglobulin G (IgG), and that their B cells were quite activated. By the late 1980s, early 1990s, Genentech (VaxGen) and Chiron developed their gp120 vaccine candidates. When it appeared that the vaccine-induced antibodies were not providing any virus neutralizing activity, the field turned to T cells. And for 15 or 20 years it was pretty lonely working on antibodies. You would go to meetings and antibodies would always be discussed on the morning of the last day.

*Were you surprised by the results of the RV144 trial?*

**SZP:** I was astonished. All of the work I had done up until then had suggested that the V2 and V3 regions of HIV Envelope were involved. It was hypothesis-driven and hypothesis-based on a good 20 years of research. What I was most astonished by was that V2 antibodies were the only correlate of reduced risk in RV144; that I never, never would have expected. I still don't think it is the only correlate. I think we just have to know what to look for and we'll find new correlates, but in RV144 it was the only one significantly associated with reduced risk and our findings were subsequently supported by many different streams of data produced by many different labs independently, so I don't think there is any question that it is real, even though there are still some naysayers.

*There is so much excitement centered on antibody-based strategies lately. How much of that is due to better tools and technology?*

**SZP:** The generation of monoclonal antibodies, the ease with which antibodies can be crystallized so you can view the epitopes, and the explosion in bioinformatics have been important, but again I think that the RV144 trial was the turning point. The previous large-scale efficacy trial was the STEP Trial. Being in the HIV field when the results of that trial were announced was like being at a wake. It was devastating. So the marginal protection observed in RV144 was something the field really needed.

*What is the status of your vaccine research efforts?*

**SZP:** I am always sort of an outlier. When the field was focused on T cells, I was interested in B cells. There is now a tremendous amount of interest in [Envelope] trimers as immunogens. The B-cell lineage approach is another hypothesis that deserves to be looked at very carefully. Both approaches are very different from one another but they are both aimed at inducing these incredibly potent neutralizing antibodies, which I refer to as “Michael Jordan antibodies.” They are very broad and very potent, but only a small proportion of HIV-infected individuals make them and they require extensive somatic hypermutation, so the probability of inducing those antibodies through vaccination is small. I hope I'm wrong, but that is how I feel. What RV144 showed us is that you don't need these exceptional antibodies for protection. There was no indication from that trial that anyone made them. The vaccine induced conventional antibodies to V2 and V3, as well as to other Envelope regions, which essentially every infected person makes. So our view, which is an outlier's view, is that if we can focus the immune response on the V2 and V3 regions of the molecule, we will induce antibodies that will not be as potent or as broad as the broadly neutralizing antibodies, but may be effective. Our approach is to design epitope-scaffold immunogens that direct antibodies to the V2 and V3 regions. Most of our work has been done in rabbits but we are in the midst of moving into monkeys. We are looking at both active and passive immunization approaches.

*How many years away is an AIDS vaccine?*

**SZP:** I'm hoping we get lucky but I don't know how long it's going to take. From a purely immunological point of view, the work we do is fascinating. From a humanitarian view, this epidemic is devastating. The combination of those things keeps driving us.

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*Mary Rushton is a freelance writer based in Cambridge, Massachusetts.*

# In BRIEF

## Studies Showcase Advances in Designing Antibody-inducing Antigens

Antibodies are the reason most, if not all, licensed vaccines provide protection against disease. So it is not surprising that vaccine researchers long ago set their sights on inducing them against HIV. For many reasons, inducing antibodies that could neutralize the vast array of HIV variants in circulation is proving a difficult task. But researchers are now making strides in developing vaccine immunogens designed to induce broadly neutralizing antibodies (bNAbs). A trio of research studies published recently in the journals *Science* and *Cell* showcase promising first steps in developing immunogens that are capable of effectively stimulating the immune system and goading it to develop a desirable antibody response.

This progress is due in part to recent advances in stabilizing HIV's highly mutable and unstable trimeric Envelope (Env) protein that is the target of all antibodies, and in isolating and characterizing naturally occurring bNAb responses in HIV-infected individuals. For the first time, researchers are now developing trimeric proteins that closely mimic the natural structure of the HIV Env glycoprotein and testing them as vaccine immunogens. "This represents more than 10 years of hard work and good virology," says John Mascola, director of the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID), who was not involved directly in the new studies. Researchers are also engineering vaccine immunogens based on the conserved viral epitopes targeted by the slew of recently identified bNAbs and their precursors.

Both of these strategies are designed to induce the types of broad and potent antibody responses that develop rarely in natural infection—researchers estimate approximately only 20% of individuals develop such bNAbs—and which occur only after years of infection. Continuous exposure to the constantly mutating virus is what stimulates the process of antibody maturation, eventually giving rise to potent antibodies that are capable of neutralizing a wide swath of HIV variants. Although these antibodies do not help infected individuals control the virus, they can prevent infection in animal models and are now being tested in clinical trials of passive transfer to see if they can do the same in humans.

There are still many obstacles to developing an effective bNAb-based HIV vaccine, but this latest research makes some scientists optimistic. Mascola calls this trio of research papers a "major advance."

### Trying the trimer

After almost two decades of failed attempts to stabilize HIV's floppy Env protein, John Moore, professor of microbiology and immunology at Weill Cornell Medical College, and colleagues reported successfully stabilizing an HIV gp140 protein designated

BG505 SOSIP.664 in 2013 (see *Keystone in Rio: Breakthroughs, Predictions, and Surprises, IAVI Report*, Winter 2013). This trimeric Env protein adopts a native-like conformation and was based on a clade A virus isolated from a six-week-old Kenyan infant who developed a bNAb response after approximately two years of infection.

The earliest immunogenicity data generated by testing BG505 SOSIP.664 in rabbits was presented early last year (see *CROI: Progress on Prevention and Cure, IAVI Report*, Vol.18, Issue 1, 2014). Now Rogier Sanders, adjunct assistant research professor of microbiology and immunology at Weill Cornell Medical College and the University of Amsterdam, Moore, and colleagues have published more complete immunogenicity data from five experiments in rabbits and one in macaques testing the BG505 SOSIP.664 trimer, as well as the B41 SOSIP.664 trimer, which is based on a clade B founder virus from an HIV-infected adult (*Science* 2015, doi:10.1126/science.aac4223). Founder viruses are the transmitted viruses thought to be responsible for establishing an infection.

These data show that the soluble, native-like BG505 trimer protein generated cross-reactive neutralizing antibody responses in rabbits against a panel of viruses classified as Tier-1 viruses—a designation given to those viral strains that are easier to neutralize—and potent neutralizing antibody responses against only those Tier-2 viruses with sequences matching that of BG505. Tier-2 viruses are representative of the most commonly transmitted strains of HIV and are what a vaccine would ultimately need to protect against. In other words, the antibodies induced by BG505 were not able to broadly neutralize Tier-2 viruses. However, in some cases these Tier-2 neutralizing antibodies did target some of the same epitopes on HIV Env that are targeted by bNAbs.

Researchers also compared the neutralizing antibody responses in rabbits with those that developed in the Kenyan infant from whom the BG505 virus was isolated. This comparison shows that the recombinant trimers induce antibody responses similar to those occurring during the primary infection phase of the infant, but the BG505-induced antibodies were not nearly as broadly neutralizing as those detected in the infant after 27 months of HIV infection.

BG505 induced similar immune responses in macaques, however the antibody titers, as measured by ELISA assay, were approximately five-fold lower than in rabbits. This suggests a better adjuvant could be used to boost antibody titers in monkeys, researchers say.

Results with the B41 SOSIP.664 trimer were similar. This clade B protein induced heterologous Tier-1 neutralizing antibody responses in all rabbits studied, and induced autologous Tier-2 neutralizing antibody responses in eight of ten immunized rabbits.

Although BG505 did not induce bNAbs against Tier-2

viruses, the study's authors suggest that inducing neutralizing antibody responses against an autologous Tier-2 virus is an "excellent starting point for iterative vaccine design." While almost all Env protein immunogens induce antibodies against Tier-1 viruses, identifying an immunogen that could induce antibodies against an autologous Tier-2 virus was previously a challenge. "Previous Env immunogens did not consistently induce potent Tier-2 neutralization against heterologous Tier-2 viruses, but not even autologous Tier-2 viruses, indicating they are inadequate immunogens when the aim is to induce broadly neutralizing antibodies," says Sanders, lead author on the paper.

Mascola agrees. "Before this we haven't had the ability to express a protein that really has the antigenic structure of the viral trimer. Now we can do that," he says.

Still, there is a long way to go. "It's now clear to us that native-like trimers are the best route to neutralization breadth," says Moore, "so we are going to do all we can to refine their design and learn how to use them better."

Researchers are exploring several strategies to rationally improve the immunogenicity of the SOSIP.664 trimers, or other soluble, stable trimeric Env proteins. Strategies include removing non-neutralizing antibody epitopes from the proteins to avoid distracting the immune response, immunizing with sequential SOSIP.664 trimers, or immunizing with cocktails of different trimers, among several others. Simply increasing the titer of neutralizing antibodies against easier to neutralize Tier-1 isolates is unlikely to improve the antibody response to Tier-2 viruses, according to the rabbit studies with BG505. These data indicate that the antibodies mediating neutralization of Tier-1 and Tier-2 viruses have different specificities and most likely arise from different B cells.

"Our goals now are to devise ways to broaden the neutralizing antibody response to eventually counter a wide range of heterologous Tier-2 viruses," says Moore. "Only if we can succeed in doing this will we have a chance of coming up with a practical vaccine that might confer a meaningful degree of protection from infection."

## Engineering a better immunogen

Another approach to inducing bNAbs that is gaining traction is designing vaccine immunogens based on the epitopes of Env that are targeted by these antibodies. Thanks to advances in B-cell isolation techniques, over the past six years researchers have isolated scores of bNAbs from HIV-infected individuals. Many of these antibodies were then fully characterized, and through that process it became clear that there are multiple highly conserved regions of Env that are targeted by antibodies. This is welcome news for vaccine researchers.

One class of bNAbs that is widely studied is those targeting the CD4 binding site on Env—a crucial epitope where the virus binds to CD4 receptors, allowing it to infect CD4<sup>+</sup> cells. The antibodies that target the CD4 binding site mimic the way HIV binds to cells. Researchers at the VRC identified the first antibody from this class, known as VRC01. Since then, VRC01-like bNAbs were identified in at least seven different HIV-infected donors.

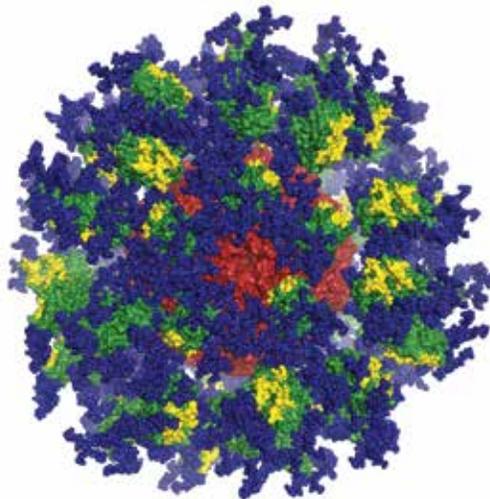
VRC01, like many of the bNAbs recently identified, has several unique characteristics. One is that this antibody, and others that are similar, are heavily somatically mutated. This means the B cells from which these antibodies are derived have undergone multiple rounds of mutation and selection in the germinal centers in response to chronic exposure to the ever-mutating virus. It is through this process of somatic hypermutation that antibodies mature and develop a higher affinity for HIV. While an average

antibody may have a degree of somatic hypermutation in the range of 3%-5%, the VRC01 class of antibodies are 30% mutated. It has been shown that not all of these mutations are required for these antibodies to neutralize HIV so effectively. However, the amount of mutation required is still much higher than what is typically achieved by vaccination. While the germline precursor of VRC01 is unknown, researchers are able to work backwards from this antibody and formulate their best guess of what the germline or immature antibody looks like. This hypothetical germline version does not bind well to native HIV Envs.

Which is why researchers from The Scripps Research Institute (TSRI) in La Jolla, IAVI's Neutralizing Antibody Center, and the Ragon Institute

designed what they call "germline-targeting" vaccine immunogens. These immunogens are capable of binding germline VRC01-class B-cell receptor and initiate immune response, setting off what these researchers hope is the initial step in shepherding the immune system to induce broadly neutralizing VRC01-like antibodies.

Joseph Jardine, a postdoctoral research fellow in Bill Schief's laboratory at TSRI, and colleagues developed one of these immunogens composed of a stripped down outer domain (eOD) of HIV gp120 that can react with germline VRC01-class antibodies. This eOD was then engineered to form self-assembling 60-subunit nanoparticles that mimic the size and shape of a typical virus. Jardine and colleagues tested this engineered immunogen, referred to as eOD-GT8 60mer, as a priming immunization in a transgenic mouse model developed by David Nemazee of TSRI that allows the mice to develop human antibodies. The first immunogenicity data for the eOD-GT8 60mer were presented earlier this year at the Keystone Symposium on HIV Vaccines (see *Opening*



An image of the eOD-GT8 60mer. Image courtesy of Joseph Jardine, Sergey Mennis, and William Schief of TSRI/IAVI.

*the Envelope, IAVI Report*, Vol.19, Issue 1, 2015), and published recently (*Science* 2015 doi:10.1126/science/aac5894).

The results are encouraging. Mice immunized with a single injection of eOD-GT8 60mers developed antibodies with characteristics similar to that of the VRC01 class of antibodies, whereas neither a previous version of an engineered “germline-targeting” immunogen or the native-like BG505 SOSIP.D664 trimer did. By 42 days after immunization, many of the antibodies in eOD-GT8 60mer-vaccinated mice had accrued mutations that resulted in more than a 1,000-fold increase in their binding activity to HIV Env, as measured by surface plasmon resonance. And importantly, these antibodies started to bind to the CD4 binding sites of more native-like Envs. This suggests a second immunization with more native-like Env immunogens would be recognized by these antibodies and could further guide their development toward a VRC01-like bNAb response.

Jardine and colleagues also evaluated different adjuvants and found that selection of adjuvant greatly influenced the responses, resulting in differences in the antibody titers in serum, the mutations selected for by the B cells, and the generation of high-affinity antibodies in this mouse model. “The adjuvant makes a huge difference,” Jardine says, which is something researchers think should be considered when testing vaccine candidates in humans.

In a related study, researchers at The Rockefeller University collaborated with the TSRI researchers to test the same eOD-GT8 60mer immunogen in a different transgenic mouse model. This immunogen also binds the predicted un-mutated precursors of the 3BNC60 antibody—a VRC01 class antibody that was identified by researchers at Rockefeller. Like VRC01, reverting 3BNC60 to its presumed germline form results in complete loss of the antibody’s ability to bind to and neutralize HIV.

Immunization with eOD-GT8 60mer resulted in a higher number of B cells expressing antibodies with traits similar to that of 3BNC60 and other CD4 binding site-directed antibodies, and induced somatic hypermutation, providing additional support for this approach (*Cell* 2015, doi: 10.1016/j.cell.2015.08.011).

This is a promising first step. Researchers speculate that a series of immunizations with different immunogens that are increasingly similar to the structure of native HIV will be required to induce the type of broad neutralizing activity conferred by VRC01-like antibodies. Still, these results are exciting to those involved. “We’ve initiated this process and we think that’s a big step forward,” says Jardine, who is now plotting studies of this sequential immunization approach, which, if successful, could be used beyond HIV as a new paradigm for vaccine design and development. —*Kristen Jill Kresge*

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monkeys with ARVs until they have undetectable viral loads and then remove their T cells and transduce them with CXCR5; a type of gene therapy. CXCR5 is a chemokine receptor expressed on cells within lymphatic tissues. It directs the migration of B and T cells into lymphoid follicles of the spleen and lymph organs.

Skinner hopes that by transducing the monkey T cells with CXCR5 so that they express this receptor, it will direct them into lymphoid follicles where they could then kill any actively replicating, viral-infected cells. “Ongoing viral replication in the follicles that goes unchecked is the key to HIV/SIV pathogenesis,” says Skinner. “If we can eradicate or better control viral replication in follicles, this may well lead to lower to undetectable viral loads in patients, prevent disease progression to AIDS, and thus, lead to a functional cure.” All without even targeting the latent reservoir.

Deeks is a little leery of gene-manipulative approaches, calling it an extreme approach. Objections aside, Deeks tells Skinner he’s worked with gene-splicing or gene-therapeutic techniques with some 50

or 60 patients, by his own reckoning. It is just a very challenging field. “You have to get a company to do it,” Deeks says, citing Sangamo, the California biotech he’s worked with in the past. “Gene therapy is not for the weak. You need a lot of resources.”

Paula Cannon, a microbiologist at the University of Southern California, is already working with Sangamo. She is now preparing for early-phase clinical trials testing a gene therapy strategy intended to knock out the same CCR5 co-receptor gene that produced such an astounding result in Timothy Brown. The strategy is to remove hematopoietic stem cells from an HIV-infected volunteer and treat the cells outside the body with a zinc finger nuclease using messenger RNA as a vector. The treated cells are then delivered back into the volunteer after a mild dose of chemotherapy to promote re-engraftment of the engineered cells. While this is similar to trials run four years ago at the University of Pennsylvania, Cannon’s team will be treating stem cells instead of T cells. Their thinking is that those stem cells will give

rise to T cells in the body and be a longer-lasting treatment than engineering T cells.

Cannon is also working with an adeno-associated virus vector in combination with the zinc finger nuclease messenger RNA. “It allows us to not only knock out a gene, but make precise edits to that gene,” she says. It’s an approach that’s not yet ready for clinical testing, but may make it possible to make additional genetic changes that could confer HIV resistance.

This conversation will continue when cure researchers meet in Vancouver in July for the 2015 Towards an HIV Cure Symposium. After Boston, it seems that remission may be the first stop on the road toward a cure. “It’s an important point to show that remission, even if it is long-term remission, cannot be definitive,” Sáez-Cirión says. “We have one patient who is very clearly losing control of infection even after many years. Remission is remission. It does not imply that it is a cure.” ■

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# Upcoming HIV-Related Meetings



## JULY 2015

### *8th IAS Conference on HIV Pathogenesis, Treatment & Prevention*

July 19-22; Vancouver, Canada

More information: [www.ias2015.org](http://www.ias2015.org)

## SEPTEMBER 2015

### *US Conference on AIDS*

September 10-13; Washington, DC

More information: [nmac.org/2015usca](http://nmac.org/2015usca)

### *World STI & HIV Congress*

September 13-16; Brisbane, Australia

More information: [www.worldsti2015.com/ehome/index.php?eventid=91027&](http://www.worldsti2015.com/ehome/index.php?eventid=91027&)

## OCTOBER 2015

### *15th European AIDS Conference*

October 21-24; Barcelona, Spain

More information: [www.eacsociety.org/conferences/eacs-conference/conference.html](http://www.eacsociety.org/conferences/eacs-conference/conference.html)

## NOVEMBER 2015

### *International Conference on AIDS & STI in Africa (ICASA)*

November 22-27; Tunisia

More information: [icasa2015tunisia.org](http://icasa2015tunisia.org)

### *3rd International Conference on HIV/AIDS, STDs & STIs*

November 30-December 2; Atlanta, GA

More information: [hiv-aids-std.conferenceseries.com](http://hiv-aids-std.conferenceseries.com)

For a full list of meetings and their descriptions, go to [www.iavireport.org/meetings](http://www.iavireport.org/meetings).