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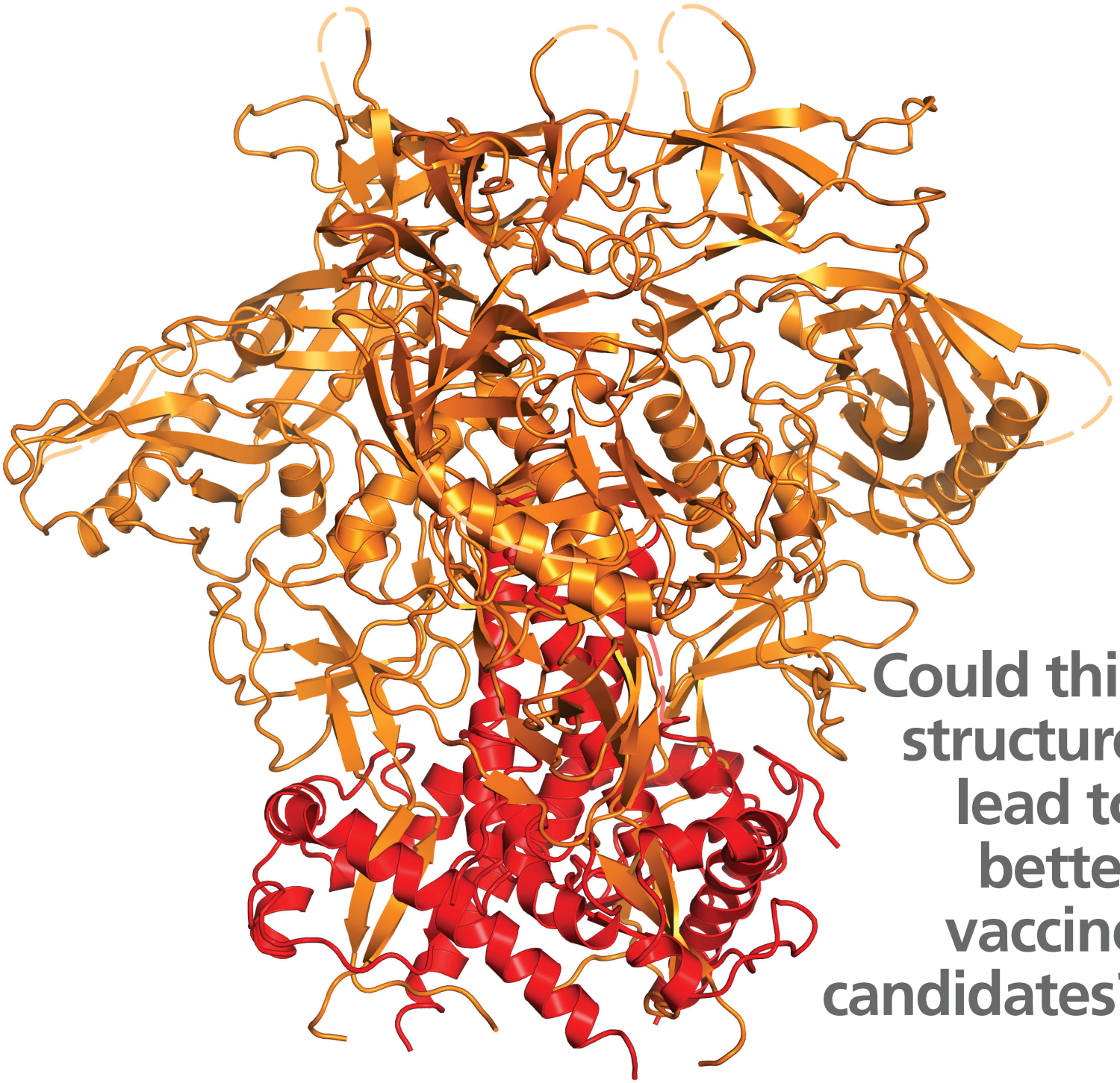
IAVIReport

The Publication on AIDS Vaccine Research



Could this structure lead to better vaccine candidates?

Plus: Ebola vaccine progress

EDITOR'S LETTER

As the year comes to a close there is, as always, much to reflect upon.

Vaccine research was brought once again to the forefront of people's minds as the world, and particularly the West African nations of Liberia, Guinea, and Sierra Leone, grappled with the deadliest outbreak of the Ebola virus since it was discovered in 1976. The epidemic, which started in earnest in March of this year, has already left thousands dead in its wake, and as this issue went to press the situation in Sierra Leone seemed to be worsening rapidly.

This outbreak jump-started efforts to develop a vaccine, inspiring renewed collaboration among government and private company researchers. As a result, the first human trials of an Ebola vaccine were recently completed and efficacy trials should begin as early as the end of this month in Liberia. I spoke with Dr. Anthony Fauci, head of the US National Institute of Allergy and Infectious Diseases at the country's largest research institution, the National Institutes of Health, about the vaccine research efforts at his Institute as well as how he tried to combat the fear that accompanied the Ebola outbreak far beyond its epicenter (see page 11).

Fauci also shared his opinions on the current status of HIV prevention research, which was of course the focus of the first HIV Research for Prevention (R4P) conference, held in Cape Town, South Africa, at the end of October. Our detailed coverage from the conference (see page 4) captures the major themes emerging in HIV prevention research and provides a preview of new results to expect in 2015.

Our final feature of this issue delves into the recently rejuvenated field of therapeutic vaccine research (see page 14). A renewed interest in curing HIV is revitalizing efforts to develop therapeutic vaccine candidates, many of which are testing similar strategies to those in the preventive vaccine field.

Just a week ago, the world commemorated World AIDS Day and the US government's theme for this day was Focus, Partner, Achieve: An AIDS Free Generation. If anything, the message of the inaugural HIV R4P conference was that the efforts to treat, prevent, and cure HIV infection are now intertwined and that it is only through a combination approach that an AIDS-free generation will truly be reached.

When I wrote my first letter here as Contributing Editor back in March, I didn't anticipate being involved through the year, but it has been my sincere pleasure to be back in the fold, working alongside a great team of staff and freelance contributors to bring this unique publication to you, our loyal readers. I hope you've enjoyed this year's issues as much as I have.

Happy New Year!

– KRISTEN JILL KRESGE

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.

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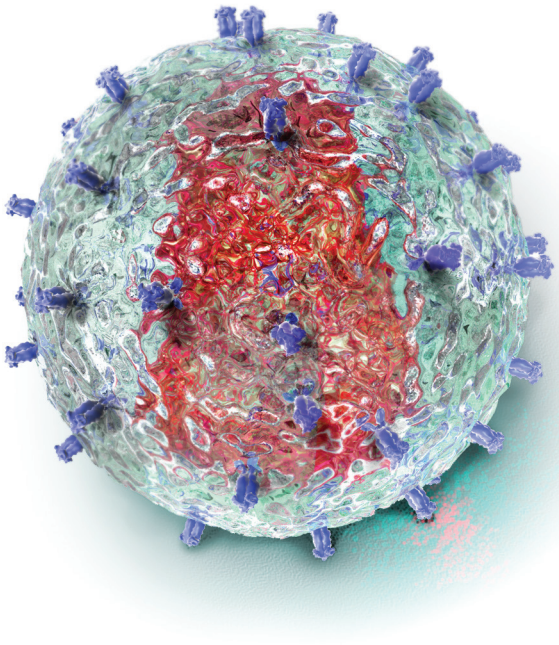

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SENIOR PRODUCTION MANAGER
Nicole Sender

CONTRIBUTING EDITOR
Kristen Jill Kresge

CONTRIBUTING WRITERS
Michael Dumiak
Mary Rushton

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[ON THE COVER]

Structure of the HIV-1 Env trimer: Ribbon representation of the HIV-1 Env trimer in the pre-fusion closed conformation with gp120 (receptor-binding subunit) shown in orange, gp41 ectodomain (fusion subunit) shown in red, and with dashed lines indicating disordered regions. In this orientation the viral membrane would be located towards the bottom of the page.

Image courtesy of Peter D. Kwong, Marie Pancera, and Jonathan Stuckey, National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIAID)/Vaccine Research Center.

Cape Town CONNECTIONS

Inaugural HIV R4P conference in Cape Town merges minds and efforts, blurs lines in HIV prevention—and, perhaps, encourages cross-pollination.

By Michael Dumiak

From her base in Johannesburg, South Africa, Glenda Gray is getting ready for a new round of clinical trials. Kicking off a month or so after the new year, one study is testing the only vaccine regimen to date that is effective in preventing HIV infection. The tests build on promising results she delivered in Cape Town at the end of October during the debut of HIV R4P—the R4P means Research for Prevention—which is billed as the first-ever conference dedicated to every aspect of biomedical HIV prevention research. Gray is not alone in looking for the new year to nurture green shoots brought to bear in Cape Town: as HIV R4P fades in the distance, its themes hint at what's expected to happen across a number of HIV research fields in coming months.

HIV RESEARCH FOR PREVENTION
HIV R4P
Shaping the Science of Prevention
Cape Town, South Africa 28–31 October 2014

As executive director of the Perinatal HIV Research Unit in Soweto, Gray has a broad perspective of what's happening in HIV science and in society. Organizers planned HIV R4P to make a similarly broad statement: while researchers tend to work in narrowly circumscribed fields because of how science is funded, the need for specialized expertise, and the difficulty of the work, emerging results are blurring the divisions between HIV prevention, treatment, and cure efforts.

HIV R4P reflected this trend. Data was presented by scientists experimenting with new microbicides that would employ the same broadly neutralizing antibodies (bNAbs) that serve as a crux of vaccine research and studying the idea of combining partially effective topical microbicides with partially effective vaccines. There was also a strong showing from molecular biologists, who are developing new images of atomic-scale protein crystal structures—and perhaps new ways of using them to develop immunogens—and ongoing discussions about developing improved animal models with which to conduct research and how to apply those results to humans. Carl Dieffenbach, director of the Division of AIDS at the US National Institute of Allergy and Infectious Diseases (NIAID), sees opportunity in HIV R4P's inclusive spirit. "All the fields now are mixing and mingling based on scientific opportunity. That's what you would hope would bring the innovation that we all need."

Activists were also among the 1,300 who attended HIV R4P from Oct. 28-31, and they, along with behavioral and social science researchers, brought their own concerns about how people might—or, if careful attention isn't paid, might not—in the end use the products resulting from these decades of research.

Cape Town, long a melting pot and trade center with aspects of endurance and elegance, bound to freedom and brutal history alike, seemed like the right place to begin with HIV R4P. In South Africa the HIV epidemic is very real. Two million people globally are infected with HIV every year; the virus still kills a million a year. Two thirds of those deaths are in sub-Saharan Africa. South Africa alone is home to the largest population of people in the world living with HIV. Amid strong calls for a boost in research efforts from African teams, a third of presenters selected by HIV R4P organizers came from the continent, with organizers granting 300 full and partial scholarships to researchers and advocates who otherwise wouldn't have attended.

Bigger might be better

One of the top priorities in HIV vaccine research remains figuring out and following up on the partial efficacy provided by the vaccine regimen tested in the now landmark RV144 trial in Thailand conducted by the US Army and Thai Health Ministry. This trial showed that a prime-boost combination of two experimental vaccine candidates reduced the risk of HIV infection from clade B and recombinant E/A virus (the types of HIV most commonly circulating in southeast Asia) by a modest 31% among 16,000 volunteers. The vaccine candidates were ALVAC HIV, a live, recombinant, non-replicating canarypox vector encoding clade B *gag/pro* and clade E *env*, and AIDSVAX B/E, a genetically modified version of HIV gp120 from clade B and E.

In Cape Town Gray reported on her team's local follow-up study, named HVTN 097, which tested the same regimen as RV144 even though the most common circulating virus in South Africa is clade C. Gray and colleagues tested the vaccine candidates at research centers in Cape Town, Klerksdorp, and Johannesburg. Preliminary results show that the immune responses induced by the vaccine regimen among the South African volunteers are equally expansive to those induced in Thai volunteers—if not more so—even given that the vaccine regimen was not designed using clade C HIV. Researchers worried because prior studies with DNA and replication-defective pox and adenoviral vectors showed larger people—specifically larger women—had weaker immune responses to the vaccine candidate and obesity rates are on the rise in South Africa. The population is also distinctly different, genetically speaking, from Thais. Researchers enrolled 100 South African volunteers: 51 men and 49 women, with 28 of the women and six men either overweight or obese.

During seven months of trial follow-up, immune responses to the vaccine candidates among the South African volunteers were even better than their Thai counterparts—for example, 69.2% of the South Africans had a peak $CD4^+$ T-cell response to a specific HIV protein, 92TH023-Env, versus 50.3% of the Thai volunteers. The non-neutralizing antibody concentrations (a specific type of which is associated with protection against infection in the RV144 trial) following vaccination are also similar to that seen in the RV144 participants, Gray said. So far there are no significant differences in responses between the two studies given body mass, gender, or age, but Gray and colleagues are still conducting a formal statistical comparison of the antibody responses among the volunteers.

It remains to be seen, however, if the good cross-clade immunogenicity observed in HVTN 097 implies an equivalent efficacy. Researchers have long wondered about cross-clade immune responses and whether vaccine candidates need to be strain-specific. More trials are needed for that, and more are coming. By February 2015 Gray expects to begin a Phase I trial in South Africa testing a version of the vaccine candidates tested in the RV144 trial that are based particularly on clade C HIV.

The Phase I HVTN 100 study will evaluate a clade C version of the Thai vaccine, vCP2438, delivered along with a new adjuvant—the Novartis-made squalene adjuvant, MF59, a proprietary compound originally developed for and still used to boost immune responses to the company's flu vaccine. Researchers hope this adjuvant will boost the potency of the vaccine candidates and the durability of the immune responses they induce. Researchers plan to eventually conduct efficacy trials of this regimen involving as many as 7,000 volunteers.

Just how much better the vaccine candidate will need to be to potentially gain licensure was a recurring question at HIV R4P. The experimental vaccine regimen tested in RV144 was only 31% effective at reducing HIV infection risk. Researchers are hopeful that modifications to the regimen,

such as a better adjuvant or additional boosts, might improve the efficacy to the point that it might be considered for licensure. Asked about it several times at HIV R4P, Gray pointed out that government licensure of a vaccine might be within reach at a level as low as 40% to 50% efficacy. "A partially efficacious intervention to prevent HIV acquisition would have public health benefit," she said during a press conference. Such a vaccine could be combined with other partially effective prevention strategies—such as a microbicide, use of female condoms, or male circumcision—one of the main messages of HIV R4P. Not only does this reflect the urgency of the situation—the kitchen-sink approach over the one-shot solution—it pointed to the field's mantra that the epidemic will not come to an end with a single 'home run.'

To that end, combining vaccines with antiretroviral-based microbicides is a new effort that received attention in Cape Town. Robin Shattock, a virologist at Imperial College London, collaborated with French immunovirologist Roger LeGrand and colleagues to test a vaccine candidate combined with a 1% tenofovir gel—an antiretroviral-based microbicide—in three groups of rhesus macaques, all compared to a group of untreated control monkeys.

Pharmaceutical company Novartis provided a nasally-delivered vaccine candidate derived from two HIV proteins—gp140 TV1 (clade C) and SF162 (clade B) that researchers administered to the monkeys along with an adjuvant, R848 (a toll-like receptor 7/8 agonist). This was followed by two booster injections of MF59. Although the vaccine on its own failed to provide protection, when used together with the microbicide the combination provided a higher level of protection than the microbicide alone. "Can we get more out of putting vaccines and microbicides together?" Shattock asked, a question at the heart of HIV R4P.

Shattock's findings will gain a boost if the tenofovir microbicide gel gains regulatory approval following an ongoing Phase III trial in South Africa expected to produce results next year.

Another combination approach is using the broadly neutralizing monoclonal antibody VRC01, isolated by researchers at the Vaccine Research Center (VRC) at NIAID, in a vaginal microbicide film or ring. Deborah Anderson, a Boston University obstetrics professor and microbiologist, is exploring this concept in collaboration with Kevin Whaley of Mapp Biopharmaceuticals and others. Together they are working with Kentucky Bioprocessing, a company that uses tobacco plants to make genetically-modified lots of human monoclonal antibodies, or "plantibodies." They expect to start human safety trials this spring. "It's the first plantibody in a human study in North America," Anderson says. Mapp is growing pilot lots of VRC01 in tobacco plants, and will test it in combination with an antibody that prevents herpes simplex virus (HSV) infection. "If we prevent HSV, we might prevent HIV. That's the idea behind the cocktail. You'll have a lot of different antibodies against different mechanisms that might work better together," Anderson says. Another goal is to add sperm-directed antibodies to the microbicide cocktail for contraceptive use.

Anderson's group will test the antibody cocktail in a film substrate for topical use. The idea is to deliver the antibodies using a device that is a combination diaphragm and microbicide provider. The diaphragm contains a ring with holes in it; these holes can contain pods carrying film or other material that would release the antibody-containing microbicide.

Antibody infusions

Researchers also continue to study whether directly injecting bNAbs into people—passive immunization, as it's called—will be an effective means of HIV prevention or as a treatment for those already infected. Barney Graham, deputy director of the VRC, presented initial results in Cape Town of passive immunization safety trials with VRC01.

Two Phase I studies of passive immunization are currently ongoing, one involving a group of 25 HIV-infected volunteers (VRC 601) and the other a group of 24 uninfected volunteers (VRC 602). Researchers are administering VRC01 at different dosage levels, both intravenously and subcutaneously, ranging from one milligram per kilo to 40 milligrams per kilo in different subgroups. So far there are no serious adverse events after more than 80 doses, Graham says. Early data for five-milligram doses show intravenous delivery produces peak concentrations in the HIV-uninfected group of up to 100 micrograms of the antibody per milliliter blood within a few hours following administration, with similar kinetics at four weeks. The 20-milligram doses produce much higher antibody concentrations, of up to close to 1,000 micrograms per milliliter. At the higher dose, antibody concentrations remain in the body at what Graham calls a "meaningful" level—40 micrograms per milliliter—for a month.

Meanwhile, the team is planning tests administering VRC01 shortly after birth to babies born

to HIV-infected mothers to prevent HIV transmission to the child. Monthly antibody injections would continue until the end of the breastfeeding period to prevent subsequent transmission through breast milk. That would be in addition to standard antiretroviral therapy, which is already proven to be up to 95% effective in preventing mother-to-child HIV transmission. The idea is that a long-acting antibody injection could cover the gaps in adherence to antiretroviral therapy. Whatever the method, the goal remains the same: to create long-lasting, low-maintenance, effective ways to stop HIV. "Can an antibody with a particular level of neutralizing activity prevent HIV infection, either in the setting of mother-to-child transmission or in the setting of high-risk adult exposures?" Graham asks.

Graham and his colleagues are also developing other variations of the VRC01 antibody by mutating the antibody's amino acid structure to make it more potent and longer lasting. One variation, an antibody billed VRC07-523LS, was made by inserting four amino acids in VRC01's CDR3 loop and deleting a few from the end of its light chain. A CDR loop, or complementary determining region loop, is a structure that a bNAb uses to bind to its target epitopes. "With those minor modifications," Graham says, "it has quite a bit more potency."

There are several bNAbs that are also candidates for passive immunization, all with different viral targets: the bNAbs PG9 and PG16 target the V1/V2 glycan; PGT121 and PGT128 bind to the N332 glycan supersite; 8ANC195, PGT151, and 35O22 all bind to the gp120/gp41 trimer; and 2F5 and 4E10 that target the gp41 membrane-proximal external region. "We have targets in at least six different areas of the glycoprotein," Graham says.

These targets also avail themselves to other uses. Structural biologists are using atomic-level analysis to build new models to aid in vaccine development. "We're excited about all these human monoclonal antibodies that help us to define the important structural features of the glycoprotein, and how that might also then lead to active vaccination," Graham says. The ultimate goal, what Graham refers to as active vaccination, is getting the body to produce these antibodies on its own rather than having to deliver regular infusions. The way to do that is to design a vaccine immunogen that can provoke the immune system to generate such powerful antibodies against HIV.

From structure to immunogen

At HIV R4P Peter Kwong, chief of the VRC's Structural Biology Section, presented his team's recently published 3.5-angstrom resolution structural model of the HIV envelope protein (*Nature* **514**, 455, 2014). The published structure, which specifically shows the long-sought pre-fusion closed form of HIV, was derived using a procedure he thinks could emerge as a template for the design of effective vaccines.

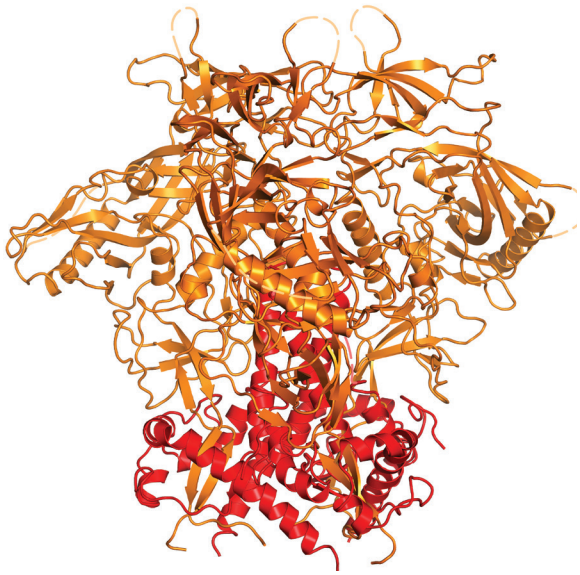
The group is applying structural techniques used to produce vaccine candidates against the pediatric respiratory syncytial virus (RSV), which causes severe respiratory tract infections in infants. Kwong and colleagues are also building on the work that Rogier Sanders and John Moore, both of Weill Medical College of Cornell; and Ian Wilson and Andrew Ward, both of The Scripps Research Institute (TSRI), did to produce a soluble trimeric complex called BG505-SOSIP.664, the first immunogenic mimic of the native HIV trimer (see *CROI: Progress on Prevention and Cure*, *IAVI Report*, Vol. 18, No. 1, 2014), a key discovery for vaccine researchers. As TSRI immunologist and the IAVI's Neutralizing Antibody Consortium Director Dennis Burton said in Barcelona last year, having a stable mimic of the HIV trimer was a holy grail of HIV vaccine research—for more years than anyone likes to remember.

What Kwong and colleagues at the VRC did with RSV was to examine in detail how a particular RSV glycoprotein, RSV-F (for fusion), undergoes a conformational change from its pre-fusion to post-fusion states. The pre-fusion RSV-F trimer changes quite a bit during this process, Kwong says. The VRC team used its understanding of these changes to engineer double cysteine mutations that form disulfides which keep the RSV-F trimer in its vulnerable pre-fusion state. For reasons that are not yet completely clear, humans make impressive neutralizing antibody responses to pre-fusion RSV-F. Kwong and his team were able to inject pre-fusion-stabilized RSV-F trimers into rhesus macaques and elicit very high titers of effective neutralizing antibodies, illustrating how atomic-level structural information can potentially lead to improved vaccine candidates. Human studies of this immunogen are slated to start in the next 10 months or so.

Kwong's group used neutralizing antibodies to help with stabilizing its RSV-F structural model and to guide their understanding of what happens during the fusion process. The Dutch pharmaceutical company AIMM Therapeutics identified two such neu-

tralizing antibodies, D25 and AM22, and Graham at the VRC isolated another, 5C4. These antibodies target the pre-fusion RSV-F, not the post-fusion version. By placing the D25 antibody in complex with RSV-F, the team knew they had a pre-fusion trimer structure on their hands. They were then able to experiment with nearly 100 mutations, Kwong estimates, which allowed them to fix it in this state before settling on cavity-filling alterations and a disulfide that seems to be most effective in keeping RSV in its pre-fusion state. Ergo the new antigen.

HIV, like RSV, is a fusion engine, changing its properties while binding to its target cell. But whether an approach similar to that used to identify immunogens against RSV will work against a more complex and cagey virus like HIV remains to be seen. "There's so much evasion that occurs with HIV, so much glycosylation," Kwong says. But he's hopeful, and the structural biology team is already racing ahead with a new mutated form of the HIV Env trimer structure. Kwong said he derived a structural working method from the RSV experience. The first step is to characterize—from natural infection—the most frequently elicited, effective neutralizing antibody responses. "If you want to make effective HIV-1 neutralizing antibodies," he says, "figure out how this happens naturally. I'm not saying natural infection is a precise model to follow, but rather follow the development of the antibodies."



The next step is to determine (through analyzing atomic-level and crystal structure information gained from assays, existing data, techniques such as measuring single-molecule fluorescence resonance energy transfer responses, and X-ray crystallization) the atomic-level characteristics of the prospective antigen. The third step is to create a matrix of physical properties, design, and structure in order to improve immunogenicity, and, *finally*, to use that information, according to Kwong, to recreate very specific B-cell ontogenies through vaccination.

Working with Ivelin Georgiev, a US National Institutes of Health (NIH) computational biologist, and John Mascola, the VRC's director, Kwong analyzed sera from HIV-infected cohorts, characterizing the ability of these sera to neutralize a panel of diverse HIV-1 isolates. This produced what he called 'neutralization fingerprints'. Kwong and colleagues then mapped these responses to the SOSIP HIV Env trimer in a mature closed state. The group's recently published trimer structure, crystallized and solved by Marie Pancera, a research fellow at the NIH, is BG505 SOSIP bound by two antibodies, PGT122 and 35O22 (see cover image and description, page 3). The antibodies were used to hold the HIV envelope in its closed shape, and Kwong's team thinks this shape could be vulnerable. "We could show the most prominent responses," he says. "These are the ones you want to go after. Those are the ones you find from natural infection."

Because HIV glycoproteins are unusually conformationally flexible, in Kwong's words, the immunogen is actually the structure of an unliganded, which is to say non-binding, mature envelope trimer. "That's the eliciting immunogen that you use when you immunize," Kwong says. "When you immunize, you don't immunize with the whole antibody-bound complex. You have to immunize with the naked molecule."

On its own, the immunogenicity of the SOSIP trimers looks good in rabbits but perhaps not so in non-human primates or humans, who have lots of CD4 which could potentially prompt a SOSIP-based immunogen to open from its closed state, making it ineffective. If a SOSIP antigen could be modified to stay in its pre-fusion closed form, however, perhaps that's a different story.

This is what the VRC team is now focused on. It's already used the Pancera structure to guide its effort to grow two fully unliganded SOSIP crystals.

"With that template we had a way to start analyzing," Kwong says. "We had the shape of the molecule that's seen by broadly neutralizing antibodies, and we could then say, aha—that's the exact atomic-level structure that we now need to fix." By using the SOSIP trimer containing Moore's and Wilson's stabilizing mutations and the new structural models, Kwong is experimenting further with disulfide fixers. Early results for one new mutation—a stabilizing disulfide, resulting in modified SOSIP trimer now called DS 201-433—seem promising.

Updating animal models

Wayne Koff, IAVI's chief scientific officer, queried Kwong at HIV R4P on how immunogenicity studies might move the VRC's structural efforts ahead, specifically which animal models would be employed.

This is a subject that has riddled the HIV field for decades. The varying utility of different animal models is an increasingly important topic for researchers both because of recent advances in altering animal genetics to more closely resemble the distinctly human biological environment in which HIV operates—and the fact that it's difficult to ethically conduct experiments in humans.

Kwong weighed his options. Testing more broadly neutralizing antibodies requires the proteins involved to have long RNA loops, and mice don't have these. Guinea pigs might be a better possibility.

Oregon Health & Science University immunologist Louis Picker—for some years now—has generated interesting hypotheses using non-human primate (NHP) models. In the last 18 months the slow fine-tuning of creating antiretroviral therapies for monkeys that closely recapitulate the therapy effects in humans has helped his cause. In a parallel but separate effort, Picker's lab published research last year (*Nature* **502**, 100, 2013) showing that the use of a cytomegalovirus (CMV) vector-based vaccine candidate appeared to cure nine rhesus monkeys of simian immunodeficiency virus (SIV; see page 17), without antiretroviral treatment, in the context of a prophylactic vaccine given before SIV infection. This finding excited many researchers and is fueling efforts to develop this vector as both a preventive and therapeutic vaccine candidate, and there are many more than nine monkeys now. There may be only one Berlin patient, but there are 40 to 50 Portland monkeys who are now cured, Picker said at HIV R4P, and though it's a laugh line, Picker is serious about the monkeys.

"I think we should have 500 monkeys on antiretroviral therapy now that we can do experiments on," Picker said, which could fuel research on understanding how the viral reservoir is established—specifically where the virus goes to hide in sanctuaries—perhaps in the follicles of memory T cells—to escape destruction and enter a latent phase only to reappear again later. Picker's team showed in NHPs that even after reducing the viral reservoir from three to four logs—a factor ranging from 1,000 to 10,000—interruption of therapy allows the virus to come back. He says this means the reservoir needs to be reduced by five or six logs.

But from some corners the monkey models draw criticism because while SIV is similar to HIV, it is not the same; the same is true with monkeys and humans themselves. "I agree monkeys aren't humans. The clinical situation is different, but the biology is really similar," Picker says. "To ask intelligent questions and make sure our resources for clinical trials are used appropriately, it behooves us to invest in making monkey models as biologically relevant as we can." Picker argues that using monkeys speeds fundamental research. "You need to do things quickly and do experiments that make things worse as well as better," he says. "You can't do that in humans."

Anderson and LeGrand, immunovirology division coordinator at the French Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), say that improved animal models, especially NHP models, are needed to understand unresolved questions about cell-associated transmission of HIV. "Despite evidence for cell-associated transmission, most infection models used for screening vaccines and microbicides use cell-free HIV viral challenge," Anderson said in Cape Town. "The failure of HIV vaccines and microbicides to date could be in part due to the failure to address cell-associated HIV transmission."

Anderson and LeGrand are working with a small group of researchers to address this. In mid-December, the *Journal of Infectious Diseases* is publishing an 80-page supplement summarizing the group's work from a 2013 workshop; another supplement will come out next spring as a result of a workshop held in Cape Town at HIV R4P. In the *Infectious Diseases* supplement Le Grand calls for increased efforts to develop models recapitulating the complexity of natural sexual transmission, including mucosa and mucosal secretions, in order to improve the relevance of animal models for HIV prevention research. "The community needs to make efforts to try to improve the relevance of the models we are using," he said in Cape Town.

Shattock and Anderson say matching models to questions is the important—and sometimes

maddening—thing to consider. "Monkeys work for me for the right questions, but I see them not as a gate-keeper," Shattock says. "You can do a level of depth in monkeys that you can't do in humans, but you need to know what you're modeling is what you'd see in humans." Anderson says it's easy for research teams to develop vested interests in existing models, because producing ongoing results is important for funding. "The models you choose to work on kind of frames the answers you'll get," she says. "You have to be careful and know the shortcomings of the models you're using."

Realpolitik

For microbicides and pre-exposure prophylaxis (the use of antiretrovirals to prevent HIV infection), research is coming closer and closer to providing real-world interventions. At HIV R4P there was discussion about what to do with new products and approaches and how to get them to those who need them most. Helen Rees, virologist and director of the Wits Reproductive Health and HIV Institute, joined South African science minister Naledi Pandor in arguing for social science research to help with this. Gray says if there is a breakthrough, there needs to be follow-through. That's why another focus of HIV R4P was the social science needed to turn study results into meaningful interventions for those at risk of HIV infection.

Thinking ahead to a time when an HIV vaccine may come, Rees pointed to the country's vaccine rollout against the sexually transmitted human papillomavirus, which causes genital warts and can lead to cervical cancer, as a potential model for delivery. Even with something as intractable as HIV vaccine research, Rees says, advance planning and an understanding of the environments where it might be used are vital to making a vaccine effective. "Can we introduce vaccine service delivery in schools?" Rees says. "How do we reach nine- to 13-year-olds not targeted for immunization?"

Attitudes toward these products can greatly influence their real-world effectiveness, as Makarere University's Teopista Nakyanzi pointed out in showing why Ugandan women didn't join an otherwise promising study on topical HIV prophylaxis. Her studies suggest one main reason women didn't enroll was the fear of knowing their HIV status; another is that many did not have any financial income and feared losing support from their partners if they were involved in the trial. Ariane van der Straten, an expert in female-initiated HIV prevention at RTI International in San Francisco, says more broadly that there's potential stigma involved because there is a view that women who use HIV prevention products are promiscuous—which then implies that pre-exposure prophylaxis, for instance, is only appropriate for promiscuous women.

Dieffenbach sees an effective vaccine that could act both prophylactically and therapeutically as the ultimate way to deal with stigma. "If you had a safe and affordable and durable HIV vaccine which also worked therapeutically, you would not have to test people for HIV at the time you gave it. You could vaccinate your whole population," he says. "Talk about a way of de-stigmatizing."

Rees cites the World Bank President Jim Kim in trying to bring attention to these concerns. "I am just asking that we bring the same kind of rigorous approach and scientific thinking," she quotes the former physician and anthropologist, "to actually delivering these tools for health that we bring to creating them." ■

Michael Dumiak reports on global science, technology, and public health and is based in Berlin.

Learning from baby

Deborah Anderson, a Boston University obstetrics professor and microbiologist, might've said it best: HIV R4P wasn't a cure meeting, but cure is the ultimate prevention. Cure research, a booming area of study, was the focus at a satellite session in Cape Town. Much of the talk was on the complex and shadowy subject of the HIV reservoir—the pool of latently HIV-infected cells or virus hideouts in the body that allow viral replication to continue in full force if effective antiretroviral therapy is interrupted.

Carl Dieffenbach, director of the Division of AIDS at the US National Institute of Allergy and Infectious Diseases, focused specifically on the case of a Mississippi infant who received antiretroviral therapy beginning 30 hours after birth, starting with treatment even before medical staff had confirmed the baby's HIV status. After a month, the Mississippi baby had no detectable virus. After two years, the child remained HIV-free, firing hopes that a cure was achieved. Unfortunately, the child's virus eventually rebounded after discontinuing antiretroviral therapy. For Dieffenbach, the case of the Mississippi baby exemplifies the significance of understanding and quantifying the latent reservoir. "We are still in the stone age when it comes to assays on the reservoir," she says. "There's a range of tools. They're all challenged." To address this, NIAID recently approved a set of grants to seven labs searching for new and better assays to detect the latent reservoir.

More broadly speaking, early treatment for infants is still a tantalizing prospect; researchers want to see if antiretroviral treatment given in the first two days to HIV-infected babies at birth can lead to viral remission, allowing the children to eventually stop treatment for an extended period. Pediatrics professor Yvonne Bryson at the University of California, Los Angeles, will lead such a study, called IMPAACT P1115, which will enroll nearly 500 volunteers. "We're trying to develop a new cohort of infants treated in a similar way to the Mississippi baby," she says. It's a unique set of circumstances however, as the child's mother was not on antiretrovirals during her pregnancy. Dieffenbach says they're screening mothers coming into delivery rooms around the world, looking for volunteers who fit the profile. —*MD*

Battling Ebola:
THE VIRUS AND THE FEAR

By Kristen Jill Kresge

As 2014 draws to a close, one of the biggest and most tragic stories of the year is the unprecedented Ebola outbreak in West Africa—the deadliest since the virus was discovered in 1976. Recent estimates put the total number of Ebola deaths at close to 6,000, which means it will leave more dead than all other outbreaks of the virus combined. The majority of these deaths occurred in the West African countries of Liberia, Sierra Leone, and Guinea.

Only two people died from Ebola in the US after contracting the disease in West Africa, yet the threat of the virus seemed to dominate news coverage here over the past few months. During this time it was Anthony Fauci, the long-time, unflappable head of the US National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH), who spoke out in an effort to stoke Ebola fears and explain current efforts to develop a vaccine that could prevent future outbreaks like this one. As is often the case, Fauci was a voice of reason, and he is extremely confident a vaccine against this terrible disease will be a reality.

Anthony Fauci

NIAID, which Fauci has led since 1984, and other groups at the NIH are involved in developing and testing some of the leading Ebola vaccine candidates, as well as conducting a portfolio of research activities on AIDS vaccines.

Fauci needs no introduction to anyone familiar with HIV/AIDS. He's remained at the forefront of the pandemic since the earliest days, as a physician, scientist, and administrator. He became an ally of AIDS activists fighting for treatment and is one of the most vocal proponents for AIDS vaccine and cure research. He oversaw a budget of US$4.5 billion at NIAID last year.

In November, just after the close of the HIV R4P conference in Cape Town at which his talk was delivered by video due to his need to remain in the US to deal with the Ebola response, *IAVI Report* Contributing Editor Kristen Jill Kresge spoke with Fauci about the similarities and differences between Ebola and HIV and the efforts to develop vaccines to target both of these deadly diseases.

Following our talk, researchers from the Vaccine Research Center at NIAID and their colleagues published the first data from a Phase I study of an Ebola vaccine in human volunteers (*NEJM* doi:10.1056/NEJMMoa1410863). This trial showed that a replication-defective chimpanzee adenovirus serotype-3 vectored vaccine candidate encoding two Ebola proteins isolated from previous outbreaks in Zaire and Sudan was safe at both doses tested and induced Ebola glycoprotein-specific antibodies in all 20 volunteers. Those who received the highest dose developed a transient fever following vaccination, but also had higher antibody titers and were more likely to have $CD4^+$ and $CD8^+$ T-cell responses than volunteers who received the lower dose. It remains to be seen if these immune responses will be sufficient to actually protect against Ebola infection. But Fauci said the current outbreak provides an unexpected opportunity to study the efficacy of these vaccine candidates in Liberian volunteers in efficacy trials that could start as early as this month.

On December 2, US President Barack Obama visited the NIH in Bethesda, Maryland, where he highlighted advances in Ebola vaccine research and congratulated NIH director Francis Collins and Fauci for their work.

In news coverage some public officials, including Thomas Frieden, director of the US Centers for Disease Control and Prevention, drew comparisons between the current Ebola epidemic in West Africa and the early days of HIV. How are these two epidemics similar in your view?

I don't make any analogy between Ebola and HIV. They are entirely different. The only thing that is possibly similar in an entire arena of dissimilarities is the somewhat understandable, but inappropriate, fear associated with transmission and contracting each of these infections. There was unreasonable fear early on in the AIDS pandemic about how you might get it. There were people talking about getting it from a gay waiter that was waiting on you in a Greenwich Village restaurant, or from a mosquito, or by sitting next to an infected child in school. Those are all fears, understandable as they may be, that were not based on any scientific evidence.

Somewhat similar, is the unrealistic association of what's going on with Ebola in West Africa and the possibility of there being an outbreak here. People being afraid to get on planes even though there's no indication at all that anyone who had Ebola was anywhere near the plane.

Those are the only similarities. Once you get beyond that, they are completely different diseases in their mode of transmission and in their impact. Thousands and thousands and thousands of Americans were getting infected with HIV and there wasn't a lot of attention paid to it in the 1980s. With Ebola, there have been only two infections and both have been in very courageous nurses who have deliberately, of their own volition, put themselves in harm's way by taking care of an Ebola patient. The amount of fear and sometimes hysteria we see here in the United States about the threat of Ebola is dramatically out of proportion to the risk. With AIDS, people were truly getting infected and the world wasn't paying attention to it. Here, the United States is intensively focusing on the possibility of Ebola and only two people got infected and we knew exactly *how* they got infected. They were taking care of a sick person. There are so many differences between these two diseases that I wouldn't equate them under any circumstances.

Does the fact that there are many people who are cured of Ebola make it more likely that a vaccine will be developed?

That is the most critical point and is why I am extremely confident that we will develop a successful vaccine against Ebola. As I have said many times in my discussions about an HIV vaccine, one of the real scientific obstacles to the development of a successful HIV vaccine is that unlike in classic vaccinology, when you look at the response to natural infection—be it against polio, smallpox, hepatitis, or measles—even though there are degrees of morbidity and mortality associated with those infections, at the end of the day, in most cases the body spontaneously and naturally recovers. And that leaves you with lifelong immunity against a similar strain. So nature has already provided you with a proof of concept in these infections that the body is capable of making an adequate immune response.

We don't have that with HIV because in an unprecedented way the body does not seem to make an adequate immune response, at least not very often or not very consistently. Whereas with Ebola, given the number of people who recover and have developed an immune response that ultimately protects them in subsequent infections with the same strain, the proof of concept has already been provided. Nature tells us it is quite possible if not highly likely that we will develop a successful vaccine against Ebola.

Which of the Ebola vaccine candidates are furthest along in development?

There are two already in Phase I trials and getting ready to move into more advanced trials. They are the NIH/GlaxoSmithKline vaccine, which is a chimpanzee Adenovirus serotype-3 that has already enrolled all Phase I volunteers, and we are now designing a randomized, controlled trial to be conducted in Liberia to determine efficacy [see page 11 for update].

The other candidate is a vesicular stomatitis virus (VSV) vector vaccine expressing the glycoprotein gene of Ebola and that is in a Phase I clinical trial here at the NIH and at Walter Reed in Silver Springs [Maryland]. Both of those are in the middle of completing Phase I trials.

There are several other vaccine candidates that are not yet at that level but soon will be.

Interestingly, both of those are vectors also being investigated for HIV vaccine research, as well as for other vaccines. Do you think the development of an Ebola vaccine could provide information useful to developing future HIV vaccine candidates?

I think so. I think any time you accumulate knowledge about a particular platform, in this case the adenovirus or VSV vectors, it's always helpful when you use that same platform for

other vaccines for other diseases. That's exactly what's going on with Ebola *vis a vis* HIV. I hope we gain information that is helpful and I think it's likely that we will.

Has the devastating epidemic that is ongoing in West Africa sparked more interest among the major pharmaceutical companies to develop Ebola vaccines? Were programs sidelined because there wasn't an urgent need?

We partnered with some companies, such as GlaxoSmithKline, well before the outbreak in West Africa and we were heading toward getting the vaccine approved by the two-animal rule of the FDA [US Food and Drug Administration] because we didn't anticipate that there would be an outbreak of such a size that you could actually make an attempt to prove efficacy. I think that the lack of an Ebola vaccine up to this point was more related to the lack of industrial partners than the fact that there were not outbreaks. But the fact that there were not major outbreaks may be the reason why industrial partners were not that enthusiastic. So they're probably indirectly related.

So when might the first Ebola vaccine be ready for efficacy testing?

Probably it would be ready for testing at the end of December or early January. Hopefully by the beginning of next year an Ebola vaccine will be in efficacy trials.

Do you foresee an Ebola vaccine only being used when an outbreak occurs?

Yes. Prior to the West African outbreak there were 24 Ebola outbreaks that occurred over 38 years between 1976 and 2014 and you had a total of about 2,500 cases, so that's not a reason to trigger massive vaccination. Also, when you make an Ebola vaccine you want to make it strain specific so that it induces optimal specificity.

How would you characterize the US government's response to the Ebola epidemic?

As a scientist, I'm trying to make sure we focus on the evidence. And the science tells us that we have a major epidemic in West Africa and that we know how to contain it through public health measures—isolation, identification, contact tracing, and protection of healthcare workers, among other things. We know how to do that and if we do it at a level that's appropriate to the size and level of the epidemic, we will ultimately get it under control in Africa. In the United States, you have an epidemic of fear. We've had two cases that were contracted in the United States. Nobody has gotten infected from somebody in the environment. So you have to continually focus on what the science tells us about how it is transmitted, and how it's not transmitted. I've spent a considerable amount of time in the public and the press trying to get that point across.

The Ebola Virus

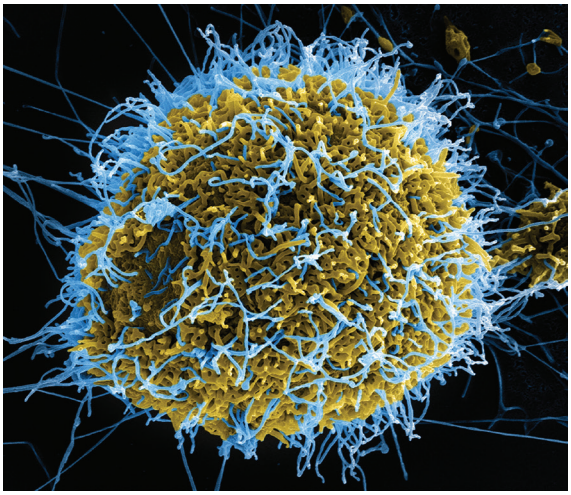


Image courtesy of the National Institutes of Health

HIV has proven an intractable target for vaccine researchers. Do you think it is possible to end AIDS without a vaccine?

I think together the combination of non-vaccine prevention modalities with a modestly, or rather moderately—I don't think modestly is good enough, we will have to do better than the results of the RV144 trial in Thailand—protective vaccine is going to be the answer to putting an end to the AIDS pandemic.

Do you think improvements to the vaccine regimen tested in RV144 could ultimately lead to the first licensable vaccine against HIV?

It's possible. I think when you have different vectors that might be better, multiple boosts, and when you add another adjuvant to it, you can amplify the breadth, depth, and duration of immune responses induced in the original RV144 trial.

Are you confident that researchers will also be able to identify a strategy for curing HIV? If so, what might it involve?

I certainly think it's possible, otherwise we wouldn't be making investments to pursue an HIV cure. I think a cure is going to be very difficult and I think it's still so much in the discovery phase that it is almost impossible to make any predictions about how we're going to cure anybody. We still don't know enough about the nature of the HIV reservoir—its kinetics, distribution in different cell types, its durability. There are so many unanswered questions. We are very early in the quest toward a cure, so there's not much you can say other than it's very difficult to predict. ■

A Shot in the Arm for THERAPEUTIC VACCINES

Recent strides in HIV cure research have rekindled interest in therapeutic vaccines, but will they ever earn the field's respect?

By Mary Rushton

Therapeutic vaccination has long been considered the "Rodney Dangerfield" of HIV research, for somewhat good reason. None of the vaccine candidates tested so far has demonstrated efficacy, and the tremendous success of treating and controlling HIV with antiretroviral therapy (ART) stymied enthusiasm for this approach. Until four years ago when regulators approved a vaccine for metastatic prostate cancer, it was questionable whether therapeutic vaccination was useful in the treatment and control of any disease.

Therapeutic vaccine research faces several of the same hurdles as preventive vaccine research. Both strategies require induction of immune responses that are qualitatively different from those induced during natural infection because in all but rare cases those are insufficient in controlling the virus. Both also suffer from lack of exact nonhuman primate or mouse models. But could therapeutic vaccines at last be earning some respect?

Italian immunologist Barbara Ensoli, who has been working on therapeutic vaccine strategies since the late 1990s, certainly thinks so. "It's very dynamic and promising, as a number of different therapeutic approaches are in clinical testing," says Ensoli, director of the National AIDS Center's Istituto Superiore di Sanità in Rome. "It is quite probable that major advancements will first come from the therapeutic setting rather than the preventative side."

The therapeutic candidates tested in clinical trials in recent years have used a variety of platforms and approaches, including DNA, viral vectors, dendritic cells, and peptides. A few induced transitory reductions in viral load in the context of treatment interruption and modest delays in the time to viral load rebound. Although the clinical benefit of this type of response is unknown at this point, recent findings suggest therapeutic vaccines might have a role in an HIV cure strategy, according to findings presented at a two-day meeting on the topic held last year in Bethesda, Maryland, and recently published (*Vaccine* **32**, 5540, 2014). This is in large part what is fueling the resurgence in therapeutic vaccine research.

"There is definitely more interest in therapeutic vaccines than in the past," says Yegor Voronin, senior science officer at the Global HIV Vaccine Enterprise, the New York City-based group that organized the meeting in partnership with the HIV prevention advocacy group AVAC and Treatment Action Group.

However, financial support for the present-day pipeline of therapeutic vaccine candidates is still pretty dismal. Data compiled by the HIV Vaccines and Microbicides Resource Tracking Working Group shows only US$11.5 million was spent on therapeutic vaccine research in 2013, a 45% decrease from 2011 and a fraction of the $818 million spent on preventive AIDS vaccine research in 2013. But this could all change quickly. "A lot will depend on the results of some of the trials that are now underway. People are hedging bets on what they think will likely work," says Voronin.

Most cure strategies are combining therapeutic vaccine candidates with other immune-modulatory drugs primarily used in cancer therapy. Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases (NIAID) and a driving force behind the HIV cure renaissance, sug-

gested at the AIDS 2014 meeting in Melbourne that therapeutic vaccination would likely need to be a component of any successful cure strategy.

Aside from their potential role in combination cure strategies, therapeutic vaccines could also be a stand-alone approach to suppress HIV in the absence of ongoing ART. Broadly neutralizing antibodies (bNAbs), which are a main focus of preventive vaccine research these days, may also be useful in therapeutic vaccination based on encouraging animal studies that suggest infusion of a single bNAb or cocktails of different bNAbs can suppress HIV replication for short periods of time in untreated animals. Two studies are also underway at NIAID and Rockefeller University exploring passive transfer, or direct injection of bNAbs in both HIV-infected and uninfected volunteers. The use of adeno-associated virus (AAV) vectors as a vehicle to deliver antibody genes rather than directly injecting the antibodies—a strategy being studied in animals and humans as a way of preventing HIV acquisition—may be another way to harness the power of the antibodies in therapeutic vaccines.

Hitching on to a cure

One of the biggest obstacles to an HIV cure is the pool of virus in latently HIV-infected cells that constitute, at least in part, what remains the largely unchartered territory of the HIV reservoir. Antiretroviral therapy suppresses viral load but does not deplete the viral reservoir. If at any point antiretroviral therapy is interrupted, this latent virus often resurfaces, resulting in ongoing viral replication and progressive disease (see *CROI: Progress on Prevention and Cure*, *IAVI Report*, Vol. 18, No. 1, 2014).

Attempts to force this latent virus out of hiding using a variety of substances remains an area of intense investigation. Now, researchers are evaluating whether combining this approach with a therapeutic vaccine designed to boost the immune responses against the awakened virus, a so-called "Shock and Kill" strategy, could reduce or even eliminate the viral reservoir.

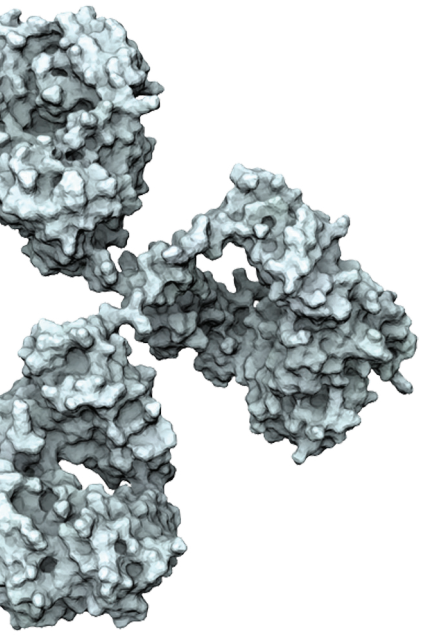
Six months ago, Danish researchers began testing a peptide-based vaccine candidate called Vacc-4x with Celdene's cancer drug romidepsin in a small study involving 20 HIV-infected volunteers. Romidepsin is among a handful of histone deacetylase (HDAC) inhibitors undergoing testing to assess their ability to roust HIV from the latently infected T cells that make up at least part of the viral reservoir. Vacc-4x, composed of four synthetic peptide sequences from within the highly conserved HIV p24 core protein, was tested alone in a Phase IIb trial but did not decrease viral loads among vaccinated HIV-infected volunteers as compared to placebo recipients after they discontinued highly active antiretroviral therapy (HAART). There was also no difference in $CD4^+$ T-cell counts at the end of the HAART-free period between the two groups.

However, further analysis indicated Vacc-4x reached one of its secondary endpoints—the virus levels in participants who received Vacc-4x never returned to pre-treatment levels, which is what typically happens when treatment is interrupted. There was a statistically significant difference in viral load in volunteers who received the therapeutic vaccine candidate as compared to placebo recipients. Now, the hope is that the vaccine candidate in combination with an HDAC inhibitor could effectively deplete the viral reservoir, and possibly even be a step toward an HIV cure.

Cure research is growing dramatically. While funding for most categories of HIV prevention research declined in recent years, HIV cure research grew a whopping 421% over the last three years, according to the HIV Vaccines and Microbicides Resource Tracking Working Group. US investment in this area is expected to increase even more with President Barack Obama's announcement that $100 million in US National Institute of Health (NIH) funds will be re-prioritized to launch a new HIV Cure Initiative.

In the six years since Timothy Brown, the so-called "Berlin patient," was the first to be considered cured of HIV, an increase in cure-related studies is generating a wealth of data that are helping to characterize the viral reservoir and develop strategies to combat it. Along with therapeutic vaccination and transcriptional activators (like HDAC inhibitors), scientists are also exploring how bNAbs, epigenetic agents that can induce changes in the genes controlling behavior of HIV provirus, immune-modulators like the checkpoint protein PD-1, and immune targeting might also contribute to a combination HIV cure strategy (see *Much Accomplished, Much More to Achieve*, *IAVI Report*, Vol. 18, No. 3, 2014).

Yet curing HIV is proving to be a huge challenge. Brown was treated for acute myelogenous leukemia, receiving two allogeneic bone marrow transplants from a donor who was homozygous for the CCR5Δ32 mutation, which renders cells resistant to CCR5-tropic HIV—not what you'd consider a widely replicable approach. And his case so far is unique.



A flurry of reports over the past year underscore how difficult it will be to achieve even a functional cure—defined as the lack of detectable viral replication in the absence of ongoing ART. The groundbreaking case of the "Mississippi baby," an infant considered potentially cured of HIV two years ago following early initiation of ARVs (see *A Toddler Stole the Show*, *IAVI Report*, Spring 2013), was reported to once again have detectable levels of virus following interruption of ART (see *Much Accomplished, Much More to Achieve*, *IAVI Report*, Vol. 18, No. 3, 2014). Similar relapses occurred in a three-year-old Italian boy whose virus rebounded weeks after treatment was suspended (*The Lancet* **384**, 1320, 2014), and two HIV-infected males from Boston who, like Brown, received stem cell transplants for cancers (see *CROI: Progress on Prevention and Cure*, *IAVI Report*, Vol. 18, No. 1, 2014), though unlike Brown their donors were not homozygous for the CCR5Δ32 mutation.

"These recent experiences drove home for me that we are going to need some way to survey what residual virus might persist," says Steven Deeks, a professor of medicine at the University of California, San Francisco, who treated Brown and is studying different HIV cure strategies. "The Mississippi and Boston cases were people we thought might be cured, but many months and years later the virus rebounded and did so without people knowing it was happening. What that says to me is that we are going to need something to maintain control of the virus and the best way, I think, is through a vaccine."

Finding therapeutic vaccine candidates that work effectively enough in HIV-infected people won't be easy though, says Stuart Shapiro, who leads a vaccine discovery team at NIAID's Division of AIDS. "We know that those already infected have been primed in a way for the immune responses to be inadequate. So we have to find a way of redirecting the immune response. That's much more difficult than figuring out how to direct it in the first place," says Shapiro. "Secondly, people who are infected have a large amount of virus in them so you need a much larger immune response than you would if you were simply trying to prevent people from becoming infected. The vaccine has to be much more potent."

The potency issue

Potency of therapeutic vaccine candidates does seem to be a problem. Two years ago, Spanish researchers found that stimulating dendritic cell (DC) function in HIV-infected individuals briefly controlled their viral load after discontinuing HAART. Unfortunately, the reprieve observed in the Phase I trial was short-lived (*Sci. Transl. Med.* **5**, 166ra2, 2013).

To make the vaccine candidate, the research team led by University of Barcelona scientist Felipe Garcia extracted autologous monocyte-derived dendritic cells (MD-DCs) along with HIV from the blood of 36 HIV-infected individuals on HAART, and used heat to inactivate the HIV in 22 of the 36 samples. They then vaccinated the 22 individuals three times over a six-week period with high doses of their own DCs and with their own intact HIV. The immunizations were given either before or immediately after interruption of ART. Twelve weeks after treatment interruption, researchers observed a 90% drop in setpoint viral load in 12 of the infected individuals who received DC cells pulsed with the inactivated HIV, compared to just one in the control arm. By week 45 the virus had rebounded, though. Nonetheless, the study was important in that it was the first randomized placebo-controlled study of a therapeutic vaccine candidate showing a statistically significant downward trend in viral load.

Because the logistics of developing a candidate like this for each patient is prohibitive, Garcia's team now plans to target the DCs *in vivo* with a rationally designed messenger RNA-based immunogen. The HIV antigen is based on viral targets of protective HIV-specific T-cell responses that were previously identified in three large cohorts of HIV-infected individuals. The candidate also includes a TriMix of three different immunostimulatory molecules that systematically alter the activation status of DCs and enhance the induction of antigen-specific T cells. This vaccine candidate is being developed as an alternative to ART.

Like the Spanish study, results were also fleeting in a randomized, placebo-controlled study of HIV-infected individuals on HAART who received a replication defective adenovirus serotype 5 (Ad5) *gag* vaccine candidate that was also tested prophylactically. The therapeutic study of this vaccine candidate showed a trend toward lower viral load following treatment interruption, but the results did not quite reach statistical significance (*J. Infect. Dis.* **(202)5**, 705, 2011).

One way around the potency problem, though, might be to use therapeutic vaccines to try to eliminate only those HIV-infected cells that are reactivated from the viral reservoir—a much smaller target. This is the approach that Ole Schmeltz Søgaard of Aarhus University is taking in the Danish trial of romidepsin and

Vacc-4x. Søgaard created a buzz at the International AIDS Society's two-day cure symposium in Melbourne this past July when he reported that the HDAC inhibitor romidepsin provoked such robust bursts of virus replication in people whose virus had been suppressed for years due to HAART that their HIV became detectable on standard blood tests. Other scientists were quoted in news reports hailing the tiny six-person study as one of the key cure-related findings of the meeting.

In the current trial, in which Søgaard and his team are evaluating the safety and tolerability of the "shock and kill" or "kick and kill" approach using romidepsin combined with Vacc-4x, the HIV peptides in the vaccine are injected with granulocyte macrophage-colony stimulating factor (GM-CSF), which sparks $CD4^+$ and $CD8^+$ T-cell responses to target the p24 proteins and hopefully control the virus. Three weeks after being immunized, volunteers will receive weekly infusions of romidepsin for three weeks and then discontinue HAART. The trial has many endpoints but a primary goal will be a proof-of-concept of the cure strategy's ability to deplete the viral reservoir.

How a vaccine candidate that did not perform with flying colors following treatment interruption can be expected to hold its own in a cure setting comes down to the breadth of the required response, says Søgaard. "When therapeutic vaccines have been tested as an alternative to HAART, the vaccine-induced immune responses required when treatment was interrupted needed to be broad and potent enough to control viral replication, including immune evasion and viral evolution. That is a pretty big task," he says. "In the kick-and-kill setting, HAART is continued during reactivation and the vaccine-induced immune response only needs to target a small number of reactivated cells. There is no or only very limited ongoing viral replication, and so it may not require as broad and as potent vaccine responses."

A delicate balance

Beyond treatment interruption, researchers are also using therapeutic vaccine candidates to address the damaging effects of immune activation in HIV-infected individuals. Ensoli's group, for instance, developed a subunit vaccine candidate made from recombinant HIV Clade B Tat protein that is designed to elicit antibodies against Tat epitopes.

The HIV Tat protein greatly increases the rate of viral transcription and replication, and therefore is considered a prominent player in both the establishment of infection and in the replenishment of the viral reservoir in chronic infection and under antiretroviral therapy. A recent animal study led by Ruth Ruprecht at Harvard Medical School suggests that antibodies to the HIV protein Tat might also be involved in protection against HIV (see *IAVI Report blog*, March 28, 2013).

The vaccine candidate used in Ensoli's study is intended in part to restore balance to the immune systems of the HIV-infected individuals, leaving them less prone to tumors, accelerated aging, atherosclerosis, and other diseases to which HIV-infected individuals are at an increased risk.

A Phase II safety and immunogenicity study conducted in Italy several years ago found Ensoli's Tat vaccine helped reduce immune activation and loss of regulatory T cells, and improve immune function in 168 HIV-infected individuals on HAART (*PLoS One* **5**(11), e13540, 2010). Ensoli says the open label study helped establish the most appropriate immunological and virological parameters to monitor in future trials. The biomarkers of note included early increases in $CD4^+$ T cells, which were associated with a reduction of effector memory cells, and an increase in central memory $CD4^+$ and $CD8^+$ T cells, and natural killer cells. Immune reconstitution increased progressively over time as well, says Ensoli.

Ensoli's group recently completed a randomized, double-blinded, placebo-controlled confirmatory study involving 200 HIV-infected individuals on HAART in South Africa, from which results are expected soon. While Ensoli and her team have been using the Tat candidate in combination with ART, they are also looking into whether it can be used to simplify or delay therapy, or as a substitute for ART.

The CMV story

Another challenge in therapeutic vaccination will be finding vectors and adjuvants that produce the broadest and most potent immune responses in HIV-infected individuals. A variety of vectors are undergoing testing in the preventive realm, but one vector is garnering great enthusiasm among both vaccine and cure researchers. The vector is based on the common cytomegalovirus (CMV).

Louis Picker, professor of pathology/molecular microbiology and immunology at Oregon Health & Sciences University, and his colleagues are studying the CMV-vector based HIV vaccine

in non-human primates. Their studies show the vector induces a remarkable pattern of viral control in about half of rhesus macaques vaccinated with a CMV-derived vector encoding simian immunodeficiency virus (SIV) genes. Not only did the animals suppress plasma SIV to undectable levels after repeat rectal challenge with SIV (*Nature* **473**, 523, 2011)—suggesting the likely induction of an unusual and broad effector memory T-cell response (*Science* **340**, 940, 2013)—they also suppressed plasma SIV to undectable levels following vaginal and intravenous challenge (*Nature* **502**, 100, 2013).

Although low level SIV RNA and DNA, and replication-competent SIV could be found in the tissues of protected monkeys early after challenge, its presence waned over time. By 70 weeks after challenge all evidence of the SIV infection was gone, despite extensive analysis using the most sensitive assays. Thus, for the first time, an AIDS-causing virus in these monkeys was cleared by immunologic mechanisms, says Picker.

Picker, who began working with the CMV vector over a decade ago, says he wasn't focused at first on its potential as a therapeutic tool. "That came after we found that CMV-vector vaccinated monkeys were able to clear infection."

Questions that arose out of Picker's original findings are now being addressed in a new round of studies. A large challenge study underway is comparing the efficacy of the original RhCMV vectors that elicited the unconventional $CD8^+$ T-cell responses with modified RhCMV vectors that elicit otherwise similar responses targeting conventional epitopes. The original vectors were genetically modified wild-type strains that lacked two genes (UL128 and UL130). These modifications are responsible for the unconventionally targeted $CD8^+$ T-cell responses, according to Picker. While the modified vectors—with the two genes repaired—have been shown to have conventional epitope targeting, they still demonstrated a higher breadth of responses than those elicited by other vectors, or SIV itself. Results due out in six to eight months will hopefully shed light on how the unusual responses induced by the original RhCMV vaccine candidate helped 50% of the vaccinated animals suppress SIV after vaginal, rectal, and even intravenous challenge. "We, at this point, don't know whether or not the unconventional responses are required for protection," says Picker. "That's the reason why we're testing the repaired vectors."

Picker and his colleagues are also getting closer to understanding the CMV genes responsible for differential $CD8^+$ T-cell targeting, but he says it is more difficult and time-consuming to determine the mechanisms by which these genes work. And it may not even be necessary, he says. "Mechanisms of protection have been defined for few licensed vaccines," he says. "It would be helpful to have a strong immune correlate of protection to guide clinical development, but ultimately vaccine efficacy in humans will have to be shown in human efficacy trials."

Picker is also vaccinating SIV-infected animals on ART to look at how well they control the virus following treatment interruption. Pending the manufacture of the prototype CMV vectors, completion of toxicology tests, and regulatory approval in 2015, a Phase I safety and immunogenicity trial in healthy, HIV-uninfected individuals could be launched in 2016, says Picker. But developing the CMV vaccine as a prophylactic candidate will take much longer, in part because of the populations in the trials. "The process of vaccine development will be slower for prevention than cure because giving a vaccine to millions of healthy people requires a higher safety bar than therapeutic use under medical supervision," says Picker. "Bottom line: I strongly believe our CMV vector approach has a shot at contributing to both, but it will be seven to ten years before we know for certain."

Other researchers are encouraged by Picker's work, but still have reservations. "I think CMV is one of the most exciting things that has happened to the field in the last dozen years," says Shapiro. "One of the very promising aspects of the vaccine is that it is a persistent vector, meaning that it if it works it may not need to be boosted. However, I'm not sure it's going to work in people. First of all, the vaccines we have tested thus far have worked much better in NHPs [non-human primates] than in people. To simply say that because this has worked so well in non-human primates it will work the same in people is like ignoring all of history."

Deeks likes the CMV vector as well but says the lack of an activator—something to awaken the latently infected cells—and a biomarker for the size of the reservoir complicates proof of concept studies to evaluate this strategy in the setting of cure research. Finding a quick method of measuring the size of the latent reservoir requires a biomarker that can distinguish cells in the reservoir from other infected cells (*eLife* **3**, e04742, 2014), and this remains elusive.

Other challenges

In addition to a lack of a biomarker for the viral reservoir, the poor predictive value of the animal models used to study HIV is another obstacle to studying therapeutic vaccines. Scientists have struggled to identify the immune responses that correlate best with viral control. There also isn't universal agreement on which assays are the most valid and reliable ones at gauging the immunogenicity of vaccine candidates.

The frequency of HIV-specific interferon-gamma producing T cells remains a widely used criterion because they are readily found in individuals with detectable virus, but the magnitude and breadth of the responses do not necessarily correlate with $CD4^+$ T-cell count or viral load, says Lucy Dorrell, a senior clinical lecturer at the University of Oxford's Jenner Institute who has been studying therapeutic vaccination for about a decade.

Dorrell says the quality of $CD8^+$ T cells may be a better predictor of disease progression. In a recent study she used a viral inhibition assay developed by Asier Sáez-Cirión, an HIV cure scientist at the Pasteur Institute. The test measured the capacity, *ex vivo*, of HIV-specific $CD8^+$ T cells to suppress HIV infection of autologous $CD4^+$ T cells in 50 HIV-infected individuals with diverse disease progression rates. The study found antiviral inhibitory capacity of $CD8^+$ T cells to be highly predictive of $CD4^+$ T-cell loss in early HIV infection (*J. Infec. Dis.* **206**, 552, 2012). The viral inhibition assay will also be used in two upcoming studies of HIV-infected individuals in Barcelona and London, says Dorrell.

Passive immuinization

While most of the current therapeutic vaccine candidates are focusing on the T-cell side, one of the most promising strategies in preventive vaccine research is inducing bNAbs. These antibodies may also have therapeutic value. In the last two years, studies in humanized mice and NHPs found that infusions of either single or combined bNAbs were capable of suppressing viral replication following infection (*Nature* **492**, 118, 2012; *Nature* **503**, 224, 2013). On the heels of those findings come several clinical studies that are looking at bNAbs as a therapeutic strategy.

An open label, dose-escalation study led by Rockefeller University is testing the safety, pharmacokinetics, and antiretroviral activity of 3BNC117, a potent monoclonal antibody that binds to the CD4 binding site, in both HIV-infected and uninfected volunteers. Participants will receive a single intravenous infusion in three increasing dose levels.

A separate open-label, dose-escalation trial is being conducted by NIAID's Vaccine Research Center (VRC). In this trial, up to 25 HIV-infected individuals will receive two infusions of the VRC's VRC01 bNAb either intravenously or subcutaneously. Samples will show if the antibody is detectable in mucosal secretions and blood of participants and how long VRC01 can be detected in the blood after dosing (see page 6).

The advantages of passive immunization are that one doesn't have to wait for development of a vaccine that is able to induce the highly affinity-matured bNAbs; a person is armed immediately to fight infection. But passive immunity is short-lived, which means that individuals would have to be boosted routinely to stay protected.

Georgia-based biotech GeoVax Labs is considering using bNAbs in combination with its T-cell based therapeutic vaccine candidate, GOVX-B11, and a latency reversing agent to try and cure HIV. In a recently completed Phase I study, the DNA/Modified Vaccinia Ankara (MVA) therapeutic vaccine candidate demonstrated mixed results in a study of nine HIV-infected individuals following treatment interruption. GOVX-B11 induced enhanced $CD8^+$ T-cell responses in almost all participants, but ultimately failed to prevent the virus from re-emerging or to remain at levels that minimize immune escape.

But GeoVax Chief Scientific Officer Harriet Robinson says by combining GOVX-B11 with the infusion of bNAbs (or perhaps protective non-neutralizing antibodies) you might be able to boost $CD8^+$ T-cell responses and prevent viral rebound after HAART is halted. "The vaccine [GOVX-B11] would add potentially protective T cells as well as boosting the host's antibody responses, and the passive antibodies would bring specificities that scientists had selected for protective efficacy," says Robinson.

Shapiro thinks the development of a monoclonal antibody cocktail that can be used therapeutically could be only five years away. But in all likelihood it will mean that HIV-infected individuals will need to be boosted once a month for the rest of their lives, he says. Still, he remains optimistic about both therapeutic and preventive vaccine development. "I am hopeful about both," he says. "I just think it is going to take a lot longer than people think." ■

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

Upcoming HIV-Related Meetings

JANUARY 2015

Keystone Symposia: Viral Immunity
January 11-16; Breckenridge, Colorado
More information: www.keystonesymposia.org/index.cfm?e=web.Meeting.Program&meetingid=1343

17th Bangkok International Symposium on HIV Medicine
January 14-16, 2015; Bangkok, Thailand
More information: www.hivnat.org/bangkoksymposium

FEBRUARY 2015

CROI 2015
February 23-26; Seattle, Washington
More information: www.croiconference.org/

MARCH 2015

Keystone Symposia: Co-Infection: A Global Challenge for Disease Control
March 15-20; Ouro Preto, MG, Brazil
More information: www.keystonesymposia.org/index.cfm?e=web.Meeting.Program&meetingid=1348

Keystone Symposia: HIV Vaccines
March 22-27; Banff, Alberta, Canada
More information: www.keystonesymposia.org/index.cfm?e=web.Meeting.Program&meetingid=1344

Keystone Symposia: The Golden Anniversary of B Cell Discovery
March 22-27; Banff, Alberta, Canada
More information: www.keystonesymposia.org/index.cfm?e=web.Meeting.Program&meetingid=1338

APRIL 2015

Keystone Symposia: Mechanisms of HIV Persistence: Implications for a Cure
April 26 - May 1; Boston, Massachusetts
More information: www.keystonesymposia.org/index.cfm?e=web.Meeting.Program&meetingid=1345

MAY 2015

Cold Spring Harbor Laboratory: Retroviruses
May 18-23; Cold Spring Harbor, New York
More information: meetings.cshl.edu/meetings/2015/retro15.shtml

JULY 2015

8th IAS Conference on HIV Pathogenesis, Treatment & Prevention
July 19-22; Vancouver, Canada
More information: www.ias2015.org

For a full list of meetings and their descriptions, go to www.iavireport.org/meetings.