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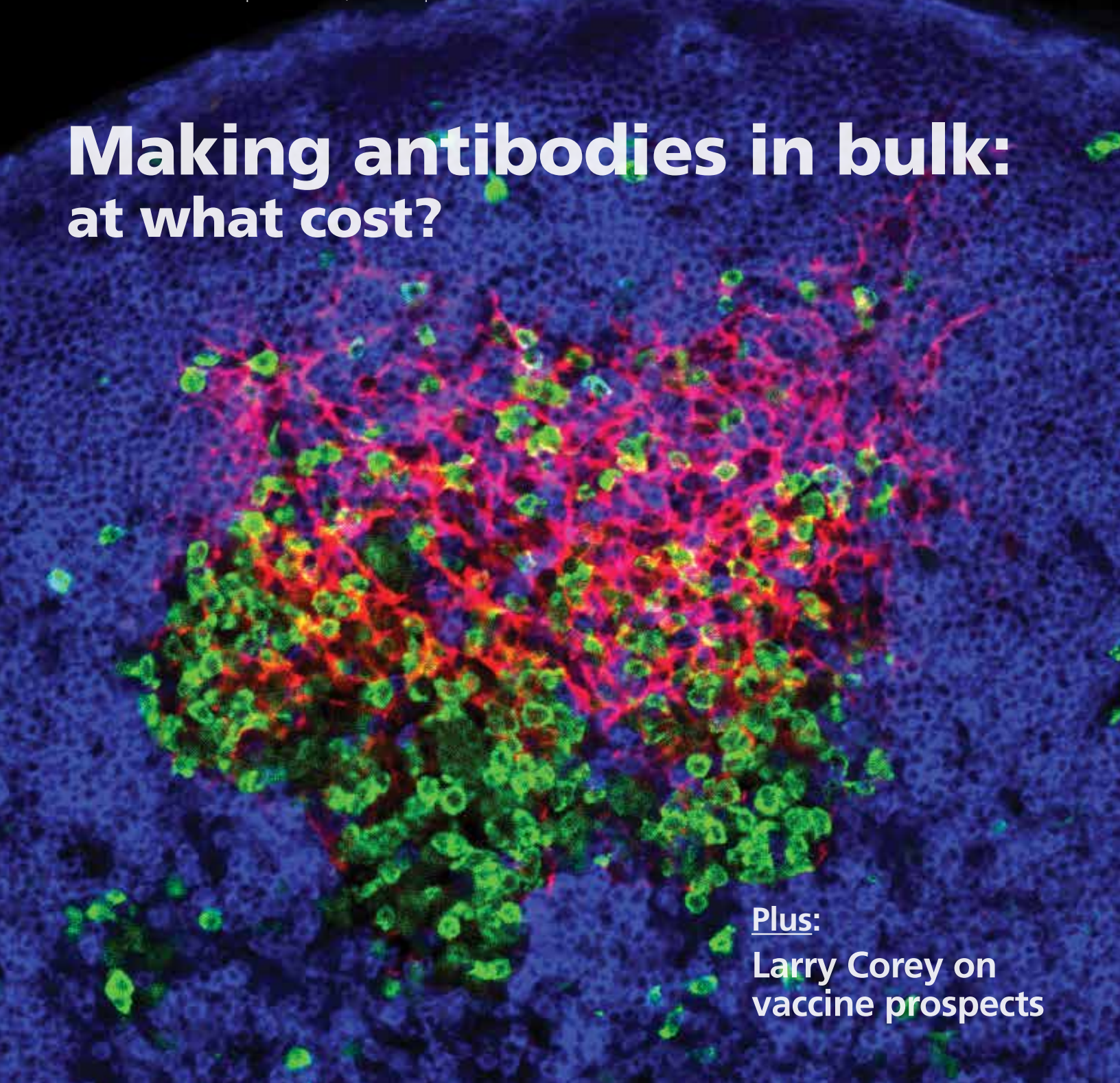
Making antibodies in bulk: at what cost?



Plus:

Larry Corey on vaccine prospects

EDITOR'S LETTER

If you're a regular reader of *IAVI Report*, it is no surprise that the HIV vaccine field is in the midst of an antibody renaissance. Following the first isolation of new, more potent broadly neutralizing antibodies (bNAbs) five years ago, researchers have been busily characterizing these antibodies, identifying their targets on the virus, and using this information to advance the arduous task of designing vaccine immunogens capable of inducing such bNAbs.

Some advances in immunogen design were discussed at the recent full group meeting of the HIV Vaccine Trials Network (HVTN), which we provide a brief update on in this issue (see page 12). Larry Corey, a principal investigator of the HVTN who recently decided to step down as president and director of the Fred Hutchinson Cancer Research Center, provided opening remarks at the HVTN meeting on June 3, and spoke to *IAVI Report* about his return to research and what he sees as the most promising avenues in vaccine research today (see page 15).

In addition to being of interest to vaccine researchers, the spate of new bNAbs is also garnering attention as a potential therapeutic to augment antiretroviral-based therapy, as well as a directly administered preventive measure, for example in the setting of mother-to-child HIV transmission (see page 18). Should any or all of these potential uses for bNAbs prove successful, the need to manufacture them on an industrial scale could become necessary. And as discussed in a feature article in this issue (see page 4), this could be prohibitively expensive, which is why the Bill & Melinda Gates Foundation, among others, is exploring feasibility of monoclonal antibody production for HIV treatment and prevention.

Finally, in a departure from antibodies, this issue provides a sobering snapshot of the raging HIV epidemic among black men who have sex with men in the US and how public health agencies are attempting to fan its flames (see page 8).

Next month, thousands of researchers, activists, and affected individuals will attend the 20th International AIDS Conference in Melbourne, Australia. After two decades of these meetings, it is clear that ending AIDS is a priority, but still a distant goal—one that we will continue to track and report on each step of the way.

– 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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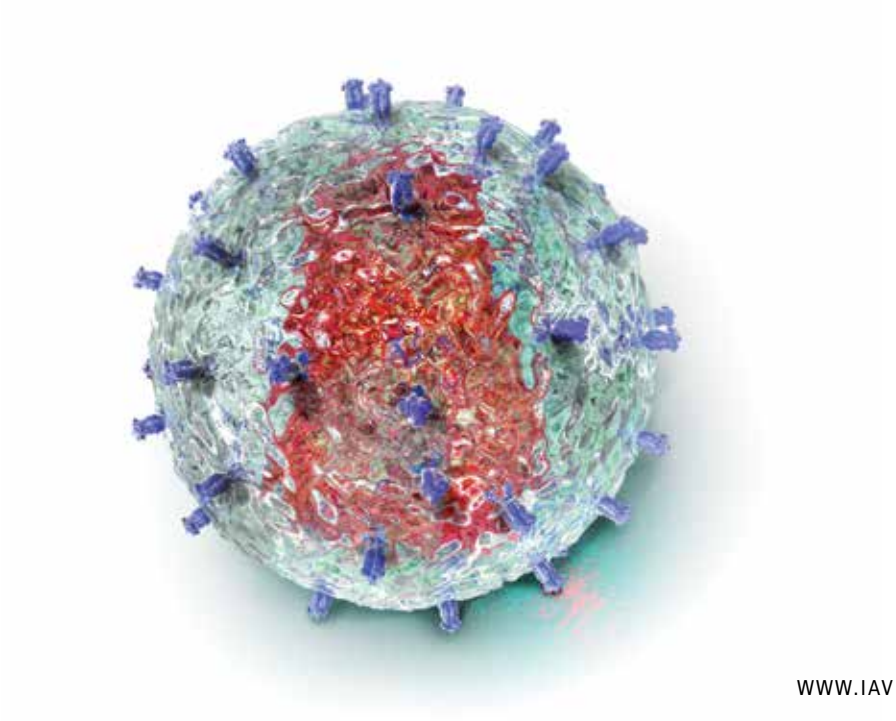

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SENIOR PRODUCTION MANAGER
Nicole Sender

CONTRIBUTING EDITOR
Kristen Jill Kresge

CONTRIBUTING WRITERS
Seema H. Bajaria
Michael Dumiak
Alexandra Morris
Mary Rushton

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[ON THE COVER]

The germinal centers of a mouse popliteal lymph node following immunization with NP-OVA. The blue represents Immunoglobulin D of naive B cells; the red represents CD35 on follicular dendritic cells; and the green represents green fluorescent protein staining of 50% of germinal center B cells.

Image courtesy of Gabriel D. Victora at the Whitehead Institute for Biomedical Science in Cambridge, Massachusetts.

Making it to *MANUFACTURING*

The potential success of broadly neutralizing monoclonal antibodies for HIV prevention, treatment, and possibly even a cure could come at a cost

By Michael Dumiak

As researchers scrutinize the scores of antibodies isolated recently that can neutralize a wide variety of HIV strains, others are thinking about the potential price of success for using these antibodies as a possible means of preventing or treating HIV. Making these antibodies in mass quantities, at going rates, will not be cheap.

There are currently about two dozen bioproduction facilities that are capable of producing monoclonal broadly neutralizing antibodies (bNAbs) in bulk, commercial-sized batches. Whether the metric-ton sized lots can be made for the millions who would benefit from a successful HIV antibody-based therapy or preventive isn't really the question. The difficulty won't, in all likelihood, be with capacity or technology. It will be cost. Innovation in protein production methods and new technologies may be able to bring potential prices down. Given this rather serious constraint in antibody manufacturing, right now the lab seems to have a little more momentum than the factory.

However, the Bill & Melinda Gates Foundation and a few other institutions are now analyzing the costs, timelines, and best ways to potentially manufacture these monoclonal antibodies. Should these antibodies be proven successful in treating or preventing HIV infection, perhaps then manufacturing won't be such an obstacle.

Genetic broth in steel tanks

In 1986, the same year the International Committee on Taxonomy of Viruses ruled that human immunodeficiency virus should be the term for the etiological agent of AIDS, the US Food and Drug Administration approved Muromonab-CD3 (OKT3) as a drug for human use. Marketed by Janssen-Cilag and used until 2010 to fight rejection of transplanted organs, the immunosuppressant OKT3 became the first licensed monoclonal antibody.

The researchers who first created Muromonab-CD3 used genetic material cloned in hybrid cell lines—hybridomas—fused from mouse spleen B cells and myeloma, or cancer cells. The fused hybridoma divides perpetually, which is a property of the myeloma cell, and it produces antibodies, which is something the B cell does. Later OKT3 was produced in the ascites (abdominal fluid) in the peritoneal cavity of mice. Hermann Katinger, an Austrian microbiologist and founder of biopharmaceutical manufacturer Polymun Scientific, recalls that in the early days of antibody production and using animal cell technology that hybridomas, and specifically human-mouse hybridomas, were quite difficult to work with for mass cultures.

The switch to using cells lines from Chinese hamster ovaries (CHO) for cloning and expression of specific heavy and light chain antibody genes by the late 1980s—heavy chains and light chains being types of amino acid sequences that make up the basic units of a protein—solved some of these problems. Katinger's group went on to make some of the first broadly neutralizing monoclonal antibodies against HIV, including 2G12, 2F5, and 4E10.

From that point on, bulk production of antibodies in bioreactors became more and more standardized. Brian Kelley, now vice president of bioprocess development at Genentech, wrote in 2009 that the move over to CHO as an early stage incubator for bulk protein production allowed produc-

ers to take advantage of common technologies already used for recombinant products (*mAbs* **5**, 443, 2009). Over time, using CHO for this purpose also gained the confidence of the drug regulators enforcing good manufacturing practices (GMP), and of the manufacturers themselves, such as Lonza and Boehringer Ingelheim, who built large production plants based on this method.

Looking at antibody production in bulk now, sources say, is something akin to air travel: everything's changed and nothing's changed. A whale of an Airbus can take more than 500 people at a time from Singapore to London, but it still relies on velocity and lift.

Contemporary industrial production of monoclonal antibodies—once the arduous early upstream work of isolating the genetic material, cultivating it, and placing it into cell lines is completed—would be broadly recognizable to counterparts doing this work in the early 90s. The cell lines expressing the antibodies are placed into large stainless steel bioreactor tanks, ranging anywhere from 1,000-liter to 20,000-liter capacity for very large batches. This cellular 'broth' is then purified, generally using a licensed medium or resin containing Protein A, which binds immunoglobulins and can fish out antibodies from a 15,000-liter harvest to 95% purity in a single step, yielding titers of one to five grams of protein per liter. Further purification comes from filtering, straining, and column chromatography, leading to the end product, which is then put into vials or syringe format for administering to a patient.

During the late '90s and early aughts, antibody production capacities were barely able to keep pace with demand and analysts predicted manufacturing shortfalls. That worry was unfounded. As bioreactor production capacity increased, so did expression levels and cell densities, with harvest titers increasing as a result. Kelley outlines in his 2009 *mAbs* paper what a 'model' plant would be: it would run six 15,000-liter bioreactors, processing titers of five grams per liter with no purification limitations. Such a plant, running at full capacity, could produce 10 tons of monoclonal antibody a year.

New research, new possibilities

Meanwhile, antibody research in the HIV field is going through a remarkable renaissance, spurred by the 2009 discovery of the bNAbs PG9 and PG16 (*Science* **326**, 285, 2009). Researchers went on to isolate scores of other antibodies (*Nature* **447**, 466, 2011). These potent proteins have unusual shapes and characteristics that make them particularly good at neutralizing many strains of this notoriously difficult to tame virus.

Researchers are pursuing a number of different directions in learning how best to use the bNAbs against HIV. "The research discovery effort was originally aimed at isolating neutralizing antibodies so we can understand how they bind to HIV, to solve crystal structures, and to inform how to design vaccine initiatives," says John Mascola, director of the National Institute of Allergy and Infectious Disease's Vaccine Research Center (VRC). This is still a pursuit, though it's a challenging one.

But when scientists observed the potency of bNAbs, they also started thinking about how to use them in other ways, including clinically in what's called passive transfer or passive administration, when the antibody is directly injected in an effort to prevent HIV transmission. This could be particularly useful in preventing mother-to-child HIV transmission by administering the bNAbs to pregnant or breastfeeding women (*PLOS Med* 2014, doi:10.1371/journal.pmed.1001616; see page 18). Antibody-based prophylaxis could also be a replacement for antiretroviral (ARV)-based pre-exposure prophylaxis (PrEP)—using ARVs to prevent HIV infection. Adherence to daily PrEP drugs has shown in some cases to be inconsistent. Replacing the need for daily pills with a monthly or quarterly injection of bNAbs may in certain settings be a more successful alternative. Studies in non-human primates also suggest that administering the HIV bNAbs along with ARV therapy could potentially lead to a functional cure (*PNAS* **10**, 1073, 2013).

Passive administration of bNAbs is already being tested in the clinic. These antibodies are manufactured at the VRC's pilot plant, located on the outskirts of Frederick, Maryland. This plant can make vaccines and antibody products under GMP conditions and has small and medium-scale capacities to produce monoclonal antibodies: enough for Phase I and II trials, but not enough for widespread commercial use. This plant is supplying the bNAb VRC01, isolated by researchers at the VRC, for the institute's two ongoing Phase I clinical trials (VRC 601 and VRC 602) testing the safety and pharmacokinetics of the antibody when administered directly to humans, and can also supply subsequent Phase I and II studies, Mascola says.

The pilot plant uses stable CHO expression lines and stainless steel bioreactors, presumably for less than the US$8 million or so it would have cost, according to a presentation delivered by the VRC's Vaccine Production Program

Chief Richard Schwartz at a 2012 advisory committee meeting, to hire a contract manufacturing organization to produce enough VRC01 monoclonal antibody for a partial Phase I study.

VRC 601 and VRC 602, the first results of which are due in a matter of months, involve groups of 15 to 25 people—one a group of healthy volunteers, the other a group of HIV-infected volunteers. "Both are pretty standard dose escalation studies, starting at a low dose and going up to a standard dose," Mascola says. In this case, the doses range from one milligram of antibody per kilo of body weight to 40 milligrams per kilo. If an average person weighs 75 kilos, and a standard dose of 20 mg per kilogram of antibody is used, the necessary dose of antibody could be as much as 1,500 milligrams a person. That's just for a small Phase I trial. Mascola says researchers are already planning ahead for larger and more ambitious trials for HIV therapy, and possibly for prevention, and he expects these could get started as soon as next year.

"We really are a long way from where we need to be from a cost perspective."
–Steve Hadley

"One approach would be to treat patients who are on successful antiretroviral therapy and to look with sophisticated assays to see if there's any beneficial effect in adding antibodies, for example on the viral reservoir or the amount of cells infected," he says. Another study might determine whether patients who are on ART and doing well—but who need daily treatments—might be able to take a break from the drugs, replacing them with antibody treatments delivered once a month or every two months for a period of several months. This might limit drug toxicity and limit the burden of treatment, at least for a time.

Should any of these approaches pan out, HIV antibody-based therapy would be joining a half dozen or so of existing monoclonal antibodies used to treat a variety of conditions, including cancer and arthritis. All existing monoclonal antibody therapies are backed by big pharma: Johnson & Johnson, Biogen, Roche, Genentech, Pfizer, Bristol-Meyers Squibb, Eli Lilly, and Merck. They are all manufactured using cell-line expression methods that would be similarly employed, broadly speaking, in making HIV bNAbs in bulk batches. These products show that industrial-scale antibody manufacturing is certainly possible, but the question is at what cost.

The cost of grams

Genentech's Kelley says that production costs for bulk monocolonal antibodies have come down since the late '90s from about $300 a gram to $100 gram—and could possibly be lowered to $20 a gram, using his model plant. But Steve Hadley, senior program officer for vaccine development at the Bill & Melinda Gates Foundation, says that if bNAbs are to reach their potential against HIV—as a general prophylaxis, as protection against mother-to-child transmission, or as a therapy taken in connection with ART—the price needs to come down to something more like $3 a gram to be feasible for the poorest places in the world where it is most needed. Right now there is no strategy to do this. So Hadley is in the early stages of figuring out what is needed to get bNAbs into the clinic at low cost and what the fully mature costs would be, particularly for passive immunization therapies for HIV.

"Our target profile is a quarterly subcutaneous injection," he says. "You come into the clinic, you get a subcutaneous injection of antibody, and there's nothing you need to do other than come back in three months and get another injection. There would be better compliance, as long as people came back in three months to get another injection. And the side effect profile would be much different, hopefully reduced to none."

He projects production using current manufacturing technologies and steel tank bioreactors at 15,000-20,000 liter scale to make HIV bNAbs will cost $30-$50 per gram at best. "Looking at the large number of people you'd have to dose to have a meaningful impact, we really are a long way from where we need to be from a cost perspective," he says.

While vaccine researchers are busy trying to identify immunogens that could get the immune system to do the difficult work of making these bNAbs, developing the ability to manufacture these human proteins in large scale could be required should antibodies be proven effective in HIV prevention or treatment. "The reason we need the large volumes is that when you start running the numbers, and figuring the actual metric tonnage of antibody you'd need to passively administer these antibodies in Sub-Saharan Africa…" Hadley says, trailing off. "You start looking at one to five to ten metric tons of antibody per year."

Appointing Hadley to the case is a sign the Gates Foundation, for one, is taking antibody manufacturing seriously. Hadley's knowledge of the industry and his background in recombinant protein development and production prompted the Foundation to bring him on last year. His team is considering how to bring together investigators and product development partners, Hadley says, to think through how the Foundation should be in investing in bioprocess research to support antibody development.

Improving manufacturing for bNAbs happens in a limited number of ways. One option is to increase the potency and half-life of the protein and thereby reduce the amount of antibody needed. Hadley argues that so far there has not been a consolidated effort to engineer the antibodies to improve their potencies in order to achieve a lower required dose. The other option is to make a better production line. While the methods haven't fundamentally changed for manufacturing using steel bioreactors, the equipment and materials have improved.

But that improvement doesn't necessarily solve the issue, either. Momentum and innovation in bioprocessing seems to be on the side of small- and medium-sized batch makers, and not in the big industrial-capacity production needed in order to consider an operation on the scale of introducing passive transfer of bNAbs through Sub-Saharan Africa.

Smaller batches mean producers have to use less Protein A to purify their antibodies. "That's a very expensive processing step," Hadley says. It can cost more than $10,000 a liter and it remains under license, at least in its modified form. There are other options for purification of antibodies, including updated precipitation or recrystallization technologies. While neither method would improve yields or is as powerful as using current protein A purification, they would cut costs.

Replacing CHO with a more optimal cell type is also an option, but the deep comfort regulators (and manufacturers) maintain with the hamster line, and the worry about contamination from bacteria, mycoplasma, or viruses when taking on new lines, is a big hurdle to establishing a new method.

Right now Lonza and Boehringer-Ingelheim continue to rule the roost in terms of large-scale bulk manufacturing of antibodies, but there are a number of small- to mid-size producers—Gallus, KBI Biopharma, Fuji Film Diosynth, CMC Biologics, Catalent, and Rentschler Biotechnologie—that are active in developing and incorporating new and recent upstream processing methods.

These producers are moving away from stainless steel toward single-use reactors that use disposable bags to perform the cell culture, saving on cleanup costs. Other recent developments include using a continuous bioprocessing or perfusion process, which uses a smaller bioreactor, under 1,000 liters or so, and allows for the continuous harvesting of protein. Particularly promising are the possibilities for doing this using simulated moving bed chromatography along with the continuous flow reactors. Simulated moving beds utilize an instrument—from Tarpon, or Novasep, for example—which highly regulates the movement of a column series of the automated feed and exit valves. This technique, also described as multicolumn continuous chromatography, switches the flowpath and tightly orchestrates the stream of purified product harvested from the reactor.

"It means you utilize a much smaller quantity of resins, but at the maximum capacity," says Stefan Schmidt, a vice president responsible for downstream production at Rentschler Biotechnologie near Ulm, Germany. "And this saves you a bit of material costs."

Whether these alternatives and innovations could ever make a real difference in bringing down costs for industrial production of HIV bNAbs, however, remains an open question.

Beyond bioreactors

Other potential ways to get around the costs associated with industrial-scale manufacturing of bNAbs might be to use plant cell lines to express the protein—or to use a viral vector to introduce the antibody gene and have the body itself take over production.

Yvonne Rosenberg, an Australian-born immunologist and chief executive of PlantVax, in Rockville, Maryland, can produce hundreds of milligrams per kilo expression of VRC01 using transient transfection in tobacco leaves. Plant-cell protein production has a relatively long history, but Rosenberg says she thinks it is finally picking up steam. June marked the first meeting in Berlin of a new group called the International Society for Plant Molecular Farming, drawing more than 100 participants. The PharmaPlanta project and its European partners completed Phase I human trials using 2G12. The trial proved that a monoclonal antibody produced and isolated in tobacco plants could be safe for use in humans. A group at St. George's University in London, citing the high bulk costs of industrial antibody production, are expressing VRC01 in tobacco plants in an ongoing effort to show it can be done effectively that way (*Plant Biotechnol. J.* doi: 10.1111/pbi.12137).

"If you make more than a ton a year, I don't think anything beats CHO right now," Rosenberg concedes, citing the purity of product, the efficiency of the process, and the comfort of regulators. "But plants have a big advantage because they are adaptable and you can make new antibodies and test them quickly."

Others wonder about its feasibility. Currently, production plants need to be kept in greenhouses in order to control the environment and

Continued on page 17

Sounding THE ALARM

Rates of HIV among black men who have sex with men in the US are skyrocketing, but what's being done?

By Mary Rushton

Thirty-three years ago on June 5, US public health officials issued a brief but haunting report describing an unusual cluster of *Pneumocystis carinii* pneumonia among five, otherwise healthy men, described as "homosexuals" in Los Angeles, CA.

Since then, much has changed regarding the HIV epidemic in the US, but one thing has remained disturbingly the same—men who have sex with men (MSM) still bear the greatest burden of HIV/AIDS in this country, accounting for nearly two-thirds of all new HIV infections in the US in 2010, and nearly three-quarters of the infections that occurred among men, according to the US Centers for Disease Control and Prevention (CDC; *HIV Surveillance Supplement Report* **17**, 4, 2011).

Moreover, the number of new infections among MSM rose every year from 2007-2010—evidence of a worrisome trend that runs counter to a declining HIV incidence among women and, notably, black women over the same time period.

These statistics are disconcerting enough, but there is another trend among black MSM in particular that has many researchers alarmed. While white MSM continue to represent the largest proportion of new HIV infections among MSM overall, and incidence is rising among MSM of all races (most rapidly among young MSM aged 13-24), the statistics among black MSM are even more dire. In 2012, black gay and bisexual men represented almost as many new HIV infections as white gay and bisexual men, despite significant differences in population size. According to the CDC, young, black MSM accounted for 45% of new HIV infections among black MSM overall, and 55% of new HIV infections among young MSM overall. This raging epidemic among black MSM has been borne out in many studies (see box, page 9).

But despite mounting evidence, the notion that epidemics might be different in sub-populations of MSM, and require different interventions, is remarkably still an understudied area, says Phill Wilson, president and chief executive officer of the Black AIDS Institute in Los Angeles.

"We have an unprecedented catastrophe among young black MSM," says Wilson. "We need a massive effort to raise attention to the magnitude of the HIV problem among black men. You can't put out a fire unless you sound the alarm."

But precisely what that effort should entail and who should deliver it is unclear. It's only within the last decade that surveillance experts have managed to deliver data that captures national HIV incidence from year to year, in this case using a serologic testing algorithm for recent HIV seroconversion (see *A Static Epidemic*, *IAVI Report*, May-June 2008). Prior to that, HIV surveillance registries were based on diagnoses that included a mixture of newer and older infections, making it difficult to pinpoint where and when new infections were occurring.

And before deciding how to combat the growing problem of HIV among young, black MSM, behavioral scientists and epidemiologists are exploring a litany of potential drivers that might explain the disproportionately high incidence among black MSM. Some of the key factors include higher poverty rates, complacency, higher rates of being uninsured or incarcerated, and less

access to clinics and doctors who might provide referrals to care and treatment for other sexually transmitted diseases (STDs) that increase the risk of HIV infection. The high prevalence of HIV/AIDS in poorer urban neighborhoods across the US, where mere geography puts one at risk for acquiring HIV (see *Why is HIV Ravaging DC?*, *IAVI Report*, Nov.-Dec. 2010), could also be driving a rising incidence, as well as the sexual networks that tend to flourish in these economically disadvantaged enclaves. However, different studies point to multiple, and different, factors that are the root cause of the surging epidemic among black MSM.

Perhaps most intriguing is what doesn't seem to be driving higher infection rates among black MSM. A meta-analysis of 600,000 MSM found black MSM are no more likely than other MSM to engage in serodiscordant, unprotected sex, yet are more likely to be HIV-infected *(Lancet* **380**, 9839, 341, 2012).

This paradox, researchers found, could partly be explained by the low rates of ARV use among the HIV-infected partners of black MSM. Greg Millett, the CDC behavioral scientist who led this study, says HIV-infected black MSM in the US were *less* likely to have health insurance, a high $CD4^+$ T-cell count, adhere to ARV treatment, or have their virus levels fully suppressed by ARV therapy. In other words, even if black MSM were having serodiscordant sex at the same rates as other MSM groups, the risk of acquiring HIV was greater because the viral loads of their infected partners were higher than those in other MSM groups. These low rates of successful treatment among black MSM are driving new HIV infections in black MSM networks and communities, Millett and colleagues concluded in *The Lancet* article.

Behavioral risk factors also could not explain racial disparities in HIV infection rates found in an earlier meta-analysis, also led by Millett, of 53 studies stretching from 1980-2006 that looked at unprotected anal intercourse. Black MSM reported less overall substance abuse, fewer sex partners, less gay identity, and less disclosure of same-sex behavior, compared to white MSM, and there were no statistically different differences by race in reports of unprotected anal intercourse, commercial sex work, sex with a known HIV-infected partner, or HIV testing history (*AIDS* **21**, 2083, 2007).

Instead, this meta-analysis linked the high HIV infection rates in black MSM with higher rates of gonorrhea, syphilis, and other STDs; less ARV use and undiagnosed HIV infection; and high rates of unprotected anal intercourse (UAI) early in the epidemic. "Since black MSM tend to have sex with other black partners, greater rates of UAI early in the epidemic may have increased the background prevalence of HIV among black MSM, which has continued to rise to the dispro-

Tracking HIV Among Black Men Who Have Sex With Men

The 2007-2010 surveillance report from the US Centers for Disease Control and Prevention (*see main story*) contains the most recent data on HIV incidence trends in the US among subgroups of men who have sex with men (MSM). But it is hardly the only evidence that HIV infection rates are higher among black MSM, and rising precipitously.

- A meta-analysis of 600,000 MSM from the UK, US, and Canada showed that black MSM in the US and UK were more likely to be HIV infected than other MSM, and young, black MSM in the US were five times more likely to be HIV infected compared with other MSM, despite engaging in similarly risky behaviors (*Lancet* **380**, 9839, 341, 2012).
- The recent HPTN061 study, run by the HIV Prevention Trials Network, is the largest longitudinal cohort of black MSM in the US. This study showed high HIV incidence in a cohort of 1,553 black MSM from six US cities: Atlanta, Boston, New York, San Francisco, Los Angeles, and Washington, DC. The study—also known as the Brothers study—was designed to evaluate the feasibility of a multi-component intervention to reduce HIV incidence among black MSM. Toward that end, the study used longitudinal data to estimate HIV incidence, determine correlates of infection, and describe changes in sexual risk over time. Of the 1,553 black MSM enrolled, 1,164 were HIV-uninfected at the time of enrollment. After 12 months of follow up, the estimated HIV incidence within this subset of 1,164 men was 2.8%—nearly 50% higher than the incidence among white MSM in the US. Among black MSM under age 30 within this cohort, HIV incidence was an astounding 5.9%, three times the rate among white MSM in the US (*PLoS One*, doi: 10.1371/journal.pone.0070413, 2013).
- In a sub-analysis of HPTN061, the study team also found alarmingly high patterns of antiretroviral (ARV) drug-resistant virus among HIV-infected black MSM. In three of the six cities, more than 40% of the men had some drug-resistant HIV and resistance was present in 11.3% of the cohort overall. Furthermore, 25% of the newly infected had some degree of drug-resistant virus, most likely reflecting transmission of drug-resistant HIV (see 21st Conference on Retroviruses and Opportunistic Infections abstract http://croiconference.org/sites/all/abstracts/581.pdf).
- A study that included an HIV-uninfected cohort of 260 black MSM and 302 white MSM from Atlanta, known as the InvolveMENt Study, also showed a stark difference in HIV incidence: 6.6 per 100 person-years among black MSM, compared to 1.7 per 100 person-years among white MSM over an average period of 1.5 years (see *Progress on Prevention and Cure*, *IAVI Report*, Vol. 18, Issue 1, 2014). The differential was even more notable in the 18-24 year old age group: 12.1 per 100 person-years (representing 16 infections) among black MSM, versus one per 100 person-years (one infection) among white MSM. *—MR*

portionately high HIV rates observed today in spite of comparable rates of UAI as white MSM since the 1990s," the study's authors suggest.

Indeed, the researchers who conducted the InvolveMENt Study (see box, page 9)—a multivariate analysis of HIV incidence among 562 black and white HIV-uninfected men from Atlanta—associated racial disparities in HIV incidence to a range of socioeconomic factors associated, with poverty and higher HIV prevalence of sexual networks, but found no significant differences in incarceration rates between white and black MSM. Black MSM in the study reported higher rates of homelessness, unemployment, and co-infection with other STDs, and lower rates of having health insurance (*PLoS One*, doi: 10.1371/journal.pone.0090514 2014).

"We have an unprecedented catastrophe among young black MSM. You can't put out a fire unless you sound the alarm." –Phill Wilson

Eli Rosenberg, an epidemiology professor at Emory University who led this study, says eliminating these structural determinants—particularly sexual networks and unemployment—would significantly reduce if not eliminate the racial disparities in HIV incidence. "Structural and community factors seem to be driving this," says Rosenberg. "How we deal with it is the challenge."

In HPTN061 (see box, page 9), co-infection with other STDs, having multiple sex partners, engaging in unprotected receptive anal intercourse with an HIV-infected partner, or having partners of unknown HIV status were the primary factors associated with a higher HIV risk. Geography also mattered. Living in Los Angeles, where 10 new HIV infections were diagnosed among the cohort, carried a much higher risk than living in Boston, where only one new infection was reported.

However, factors that were linked with an increased risk of HIV in other studies were not associated with increased incidence in the HPTN061 cohort. Nor did the HPTN061 study team find an association between incarceration and increased risk of HIV infection. While 24% of the 1,278 black MSM included in the follow-up reported a new incarceration within a year after enrolling in the study, the study found no association between jail time and HIV incidence (*J. Acquir. Immune Defic. Syndr.* **65**, 2, 218, 2014).

What about PrEP?

While there is an unprecedented amount of data emerging from the HPTN061 study team, which published nine studies in the past two years, as well as other studies that offer fresh insights into what is driving the rising incidence among black MSM, none of this research has translated into interventions that seem to be working to halt this alarming spread of HIV. One tool that clearly isn't being utilized extensively enough by MSM, and black MSM in particular, is pre-exposure prophylaxis (PrEP), the administration of antiretroviral drugs to HIV-uninfected individuals prior to exposure to reduce the risk of infection.

Nearly two years ago, the US Food and Drug Administration granted the California-based pharmaceutical company Gilead Sciences a license to market the once-daily, two-ARV (tenofovir/emtricitabine) combo Truvada to high-risk HIV-uninfected adults after the drug was shown to reduce HIV infection among MSM by 42% (see *FDA approves Truvada for use in PrEP*, *IAVI Report Blog*, July 16, 2012). The CDC has been recommending PrEP for MSM since 2011.

Yet PrEP use outside the context of research studies is sparse, according to a survey conducted by Gilead that used nationally representative anonymous patient data from over half the retail pharmacies in the US. The survey found 1,774 men and women had been prescribed PrEP between January 2011 and March 2013. Gilead wasn't able to break down PrEP use by race or transmission risk, but the drug maker did find that nearly half the PrEP prescriptions were for women, a group that accounts for only 20% of new infections. They also found PrEP use was less common in the young. Only 13% of those taking PrEP were under age 24.

"We think the numbers are artificially low, though," says Gilead's director of HIV medical affairs Keith Rawlings. "Don't forget that thousands of MSM are already receiving PrEP through demonstration projects and as they roll off the studies many may continue [with PrEP]," Rawlings contends. There are over a dozen demonstration projects, pilot studies, and rollout studies looking at ways to make the delivery of PrEP fea-

sible within MSM communities, including several that focus exclusively on black MSM and young MSM (see *Preparing for PrEP*, *IAVI Report*, Fall 2013).

But preliminary data from one of the earliest demonstration projects, known as The Demo Project, only managed to enroll a handful of black MSM at its study sites in San Francisco, Miami, and Washington, DC. Most of the 600 HIV-uninfected MSM and transgender women being offered a daily pill to protect them against HIV are white. Enrollment figures reported at the 21st Conference on Retroviruses and Opportunistic Infections, held earlier this year in Boston, showed 48% of enrollees were white, 35% were Latino, and only 8% were black.

These numbers are a stark contrast to the goal laid out in the Black AIDS Institute's five-year action plan, which calls for a major initiative to deliver PrEP to black MSM and high-risk heterosexual women by 2015. Wilson acknowledges the goal is ambitious, but he also thinks it's achievable. "This is a matter of investment and resources," he says. "We have the tools in our hands. I think the problem is money and political will."

A federal response

The passage of the Affordable Care Act (ACA) in March 2010, which extended health coverage to millions of uninsured Americans, may also provide opportunities for expanding prevention and treatment services for people at risk of HIV, policy makers say.

The ACA got off to a rough start last fall when the website created to sign up millions of uninsured Americans kept crashing, turning the program into a punch line rather than a lifeline. But the rollout gradually improved and there are now over eight million people receiving health benefits under the ACA. Groups like the Black AIDS Institute have been promoting the benefits of the new law on their website and urging young men in particular to sign up for coverage.

Unfortunately, the new law continues to face stiff resistance from many lawmakers, which could make it difficult for some low-income individuals to access services under the new legislation. According to the Kaiser Family Foundation, about 45% of HIV-infected individuals live in states unwilling to use the ACA to expand their Medicaid programs for the poor, including many states from the South, a region disproportionately affected by HIV.

Implementation of the US's first National HIV/AIDS Strategy four months after the ACA was enacted provides another opportunity to reduce the number of new HIV infections, increase access to care, improve health outcomes, and reduce HIV-related health disparities.

The White House recently appointed Douglas Brooks, a gay, black man living with HIV, to lead the Office of National AIDS Policy, the third person to hold this position in the past six years. Brooks, who grew up in Georgia and lives in Boston, has deep roots in AIDS advocacy. "Douglas's policy expertise combined with his extensive experience working in the community makes him uniquely suited to the task of helping to achieve the goal of an AIDS-free generation, which is within our reach," remarked US President Barack Obama when he made the appointment on March 25.

Jeff Crowley, who led the development of the National HIV/AIDS Strategy when he was the Obama Administration's director of the Office of National AIDS Policy from 2009-2011 and is now program director of the National HIV/AIDS Initiative at the O'Neill Institute for National and Global Health Law at Georgetown Law School, says young gay men have distinct needs that have rarely been the focus of attention of health systems.

"We know that young gay men are less likely to be aware of their HIV status and less engaged in care," says Crowley. "And they have less access to adequate health care services."

Crowley recently co-authored a 29-page report published by the Trust for America's Health that offers guidance on how to make the ACA more applicable for young, gay men. Crowley says state and local agencies also need to educate health care providers about the CDC's testing recommendations for young gay men and to identify clinicians experienced in working with the lesbian, bisexual, gay, transgendered (LBGT) community. "Young people should be able to go to a medical provider where they feel safe and where they receive quality care."

One program trying to raise awareness and reduce the risk of infection among MSM is Act Against AIDS, a CDC project that uses mass media to deliver HIV prevention messages. A major component of Act Against AIDS includes public-private partnerships with about a dozen organizations such as the Congressional Black Caucus Foundation, the National Association for the Advancement of Colored People, and a leadership initiative that focuses on organizations that

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The Path to PROTECTION

Clinical trials both past and present are contributing to the design of new and improved HIV vaccine immunogens

By Seema H. Bajaria

Larry Corey, who stepped down as president and director of the Fred Hutchinson Cancer Research Center (FHCRC) in June (see page 15), set the tone for the opening day of this year's Full Group Meeting of the HIV Vaccine Trials Network (HVTN), which was held in Washington, DC, from June 3-5, when he deemed the general mood of vaccine researchers as "optimistic." Five years have passed since the surprising results of the RV144 trial—the first to provide any evidence of vaccine-induced protection against HIV. And while researchers are still interpreting its aftermath, other areas of investigation are also advancing.

The first day of the HVTN meeting centered on four plenary sessions that covered research highlights in the areas of immune correlates, nonhuman primate (NHP) models, broadly neutralizing antibodies (bNAbs), and ongoing Phase I clinical trials.

Immune correlates

The ongoing immune correlates analysis of the RV144 trial, the subject of the first plenary, has so far centered largely on antibodies. Researchers have shown that plasma immunoglobulin (Ig)A antibodies to HIV Env in vaccine recipients are directly correlated with an increased risk of HIV infection, making them what researchers refer to as a correlate of risk; whereas IgG antibodies directed to the V1/V2 regions of HIV Env have been inversely correlated with infection risk (*NEJM* 2012, doi: 10.1056/NEJMoa1113425).

While it did not appear that the total frequency of antigen-specific T cells had a significant effect on HIV infection risk, researchers hypothesized that there were functional characteristics of the T cells induced by the RV144 vaccine that were protective for certain individuals. And it turns out this is the case. Greg Finak, staff scientist at FHCRC, presented data from a combinatorial polyfunctionality analysis (COMPASS) method that indicates polyfunctional T-cell responses are indeed independent correlates of infection risk in RV144. Further work is required to better understand this data, but one possible hypothesis Finak suggests is that multifaceted T cells could be the "help" that B cells require to be effective.

Researchers are also conducting sieve analyses of RV144, comparing the breakthrough virus sequences in vaccine and placebo recipients. This helps determine what effect immune pressure exerted by the vaccine had on selectively blocking, or sieving, the infecting virus, as well as on evolution of the virus after acquisition. Paul Edlefsen, a biostatistician at the Vaccine and Infectious Disease Division of the FHCRC, followed up on prior reported results of the significance of antibody-mediated immune pressure at amino-acid positions 169 and 181 (signature sites) in the V2 region of HIV Env (*Nature* 2012, doi: 10.1038/nature11519).

Researchers have also used a more expansive analysis to identify evidence of T-cell mediated pressure in regions of Gag and Pro that were included in the vaccine, and provide data that genetic differences occur outside of those immunogen regions. Volunteers who became HIV infected despite vaccination tended to have a higher level of phylogenetic divergence in Gag, a

finding consistent with what was seen in volunteers in the Phambili (HVTN 503) trial, and reminiscent of the T-cell epitope divergences seen in volunteers in the STEP (HVTN 502) trial, both of which tested Merck's MRKAd5 HIV *gag/pol/nef* trivalent vaccine candidate that failed to provide any protection.

Susan Zolla-Pazner, professor of pathology at New York University, compared two signature sites that Edlefsen and others identified in the V3 region of Env, with the two previously identified V2 signature sites. She found that position 307 in V3 and position 169 in V2 are similar in that both indicate the vaccine was able to protect against viruses with amino acids matching those in the immunogen. Zolla-Pazner presented evidence that these signature sites are both contact residues for vaccine-induced antibodies.

By contrast, position 317 in V3, which is similar to position 181 in V2, are both sites at which immunogen-matching residues are more frequently conserved in vaccine-recipient breakthrough viruses. Zolla-Pazner hypothesized that rather than directly impacting antibody binding, mutations in these residues reduced viral fitness and infectivity, and were thus more conserved in HIV sequences that were able to infect vaccine recipients despite the presence of antibodies targeting sites such as position 307 in V3 and 169 in V2.

NHP models

The second session of the day focused on identifying what immune responses might be required for protection using NHP studies. Dan Barouch, director of the Center for Virology and Vaccine Research at Beth Israel Deaconess Medical Center, is examining the earliest events during the so-called eclipse phase of infection, which is the time between virus inoculation and the first appearance of virus in plasma, including the pathways of virus spread and the host response, which are still poorly understood following intravaginal (IVAG) infection in rhesus macaques.

During IVAG infection with simian immunodeficiency virus (SIV)mac251, data show that the virus may breach the mucosal barrier as early as the first day following infection, followed quickly by systemic virus dissemination. While analyses are still underway, virologic data show that on the first day after infection, occasional lymphoid and gastrointestinal tissues tested positive for virus. Both $CD4^+$ and $CD8^+$ T-cell responses first appeared locally in the mucosa, but not until seven days post-infection, with systemic T-cell responses lagging even further behind, echoing earlier findings. Therefore, Barouch concluded that the eclipse phase is dynamic and complex, with virus broadcasting to distal tissues within the first few days of infection. His group now plans to use this NHP model to interrogate how neutralizing antibodies and vaccines protect and precisely when and where they might intercept the trajectory of virus spread following viral challenge.

Richard Koup, chief of the immunology laboratory at the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID), also spoke about using NHP models to understand the development of bNAbs. Koup showed that during chronic SIV/HIV chimeric virus (SHIV)-AD8 infection of eight monkeys, bNAbs develop slowly in the germinal centers and are associated with Env sequence diversity, both the quantity and quality of Env-specific TFH cells, and the quantity of Env-specific B cells in the lymph nodes.

Based on these observations, Koup concluded that antigenic changes will be required for Env immunogens to drive the degree of somatic hypermutation required for the development of bNAbs, and he thinks NHP studies can be effectively used to inform the design and efficacy testing of different Env immunogens.

Neutralizing antibodies

The focus on bNAbs continued with a presentation by John Moore, professor of microbiology and immunology at Weill Cornell Medical College. He described the work of a team that, as reported previously, has produced a stable trimer structure, BG505 SOSIP.664, that is able to bind multiple bNAbs (see *Progress on Prevention and Cure*, *IAVI Report*, Vol. 18, Issue 1, 2014). Pooled data from two rabbit studies shows that this trimer structure induces antibodies that neutralize the autologous tier-2 virus, as well as more sensitive, but less relevant, heterologous tier-1A and tier-1B viruses. In these studies, Moore found that induction of tier-1 NAbs, which are predominantly directed to the V3 region of gp120, is not predictive of the induction of tier-2 NAbs. Moore suggested it is possible that tier-1 NAbs may even distract from or interfere with the induction of antibody responses against tier-2 viruses, which should be the goal of vaccine design strategies.

Future experimentation on this point seems

justified, Moore said, while emphasizing that much work remains to be done to devise immunization strategies that might lead to the induction of cross-reactive tier-2 bNAbs with properties similar to those found in about 20% of HIV-infected individuals. New immunization and trimer design strategies could include cocktails of SOSIP trimers from the same or different subtypes, according to Moore, as well as the application of structure-guided improvements that are now being explored in the team's laboratories. A consortium including the International AIDS Vaccine Initiative, the Bill & Melinda Gates Foundation (BMGF), and the Center for HIV/AIDS Vaccine Immunology-Immunogen Design (CHAVI-ID) has been established to create BG505 SOSIP.664 trimer cell lines by the end of this year, with the aim of testing this trimer in humans. New and improved SOSIP trimer candidates will also yield additional immunogens for testing in animals in the coming year, Moore said.

Leonidas Stamatatos, scientific director at the Seattle Biomedical Research Institute, continued the discussion of Env immunogens as he described his efforts to elucidate why the generation of narrowly neutralizing antibodies (nNAbs) is more predominant than bNAbs. To understand how most recombinant Envs activate only those B-cell receptors (BCRs) that induce nNAbs, Stamatatos stressed the importance of understanding dynamics within the germinal centers where high affinity B cells are selected. As HIV has evolved to avoid detection by B cells that express BCRs giving rise to bNAbs, trimers that avoid recognition by nNAbs are necessary to elicit antibodies with broadly neutralizing capability, according to Stamatatos.

Phase I program

While immunogen development in the laboratory continues, others are already evaluating different antibodies, vectors, and immunogens in clinical trials.

Mark Connors, chief of the HIV-specific immunity section at NIAID, provided an update on the need for small, flexible clinical trials to evaluate which immunogens drive B-cell somatic hypermutation and T-cell cytotoxic capacity, including the testing of Ad4-based HIV vaccine candidates. In a Phase I clinical trial, an Ad4 vector-based H5N1 influenza vaccine was well tolerated and induced neutralizing antibodies and somatic hypermutation (*Lancet Infect. Dis.* 2013, doi: 10.1016/S1473-3099(12)70345-6; and other unpublished observations, according to Connors), making Connors optimistic about the potential for an Ad4 vector-based HIV vaccine to also induce neutralizing antibodies.

He and colleagues are now evaluating the immunogenicity of replicating Ad4 vectors in humans, including an Ad4-mGag (designed to induce T-cell responses) and an Ad4-EnvC150 (designed to induce antibody responses). This Ad4-HIV trial, which began in January, is part of a long-term collaboration between NIAID, NIAID's Division of AIDS (DAIDS), and the biotechnology company PaxVax, Inc.

Meanwhile, Marina Caskey, assistant professor of clinical investigation at Rockefeller University, and colleagues are evaluating the direct administration of two monoclonal antibodies, 3BNC117 (directed to the CD4 binding site) and 10-1074 (directed to the V3 region of HIV Env), discovered by Michel Nussenzweig's laboratory at Rockefeller. Based on the initial success of certain doses of 3BNC117 and 10-1074 to prevent virus acquisition in NHPs, as well as transiently decrease viremia in chronically infected macaques (*Nature* 2013, doi: 10.1038/nature12746), a Phase I dose-escalation study to evaluate the safety, pharmacokinetics, and antiretroviral activity of 3BNC117 began in February. Two other anticipated Phase I studies include a dose escalation, safety, and pharmacokinetics study of 10-1074 and a safety study of 3BNC177 and 10-1074 combined. Future studies could evaluate the efficacy of 3BNC117 or 10-1074 to prevent acquisition of HIV, as well as to treat chronically HIV-infected individuals during antiretroviral therapy interruption or in addition to traditional antiretroviral therapy.

Lastly, George Lewis, co-director of basic science and vaccine research at the Institute of Human Virology, discussed progress in developing full-length single chain gp120-CD4 complex Env immunogens. Data from five completed NHP protection studies will be published soon, according to Lewis, and a Phase I trial is slated to begin by early next year in collaboration with several partners, including Profectus Biosciences, the US Military HIV Research Program, DAIDS, Sanofi Pasteur, the National Institutes of Health, and BMGF. ■

Seema Bajaria reports on infectious diseases, systems biology, and public health and is based in New York City.

Back to the *BENCH*

Three-and-half years ago, immunologist Larry Corey left his laboratory at the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle, Washington, and his post as director of the HIV Vaccine Trials Network (HVTN), to become the president and director of the FHCRC. But as of June 1, Corey decided that at age 67 he was ready to trade the board room for the bench and return full-time to research focusing on cancer immunology, HIV vaccines, and herpes simplex virus.

During his tenure as director of the FHCRC, which many refer to simply as "the Hutch," Corey raised over US$100 million and bolstered the research profile of the institution, particularly in the areas of genomics and immunotherapy. In April, the FHCRC secured its largest gift ever—a $20 million donation from the family of Amazon founder and billionaire Jeff Bezos to develop novel cancer therapies, following recent advances in the use of genetically modified human T-cell therapies to fight leukemia and lymphoma. Under Corey's leadership, the FHCRC also collaborated with Memorial Sloan-Kettering Cancer Center in New York City to establish Juno Therapeutics Inc., a biotechnology company focused on bringing forward novel immunotherapies for cancer.

Corey's roots in Seattle and his history at the Hutch go back much further. He trained in infectious diseases at the University of Washington School of Medicine and joined the faculty there in 1978. He moved his laboratory to the FHCRC in 1997, and since then has been involved in, and in some instances headed, groundbreaking studies that led to the development of more effective antiviral therapies for hepatitis B virus, HIV, and herpes. At the direction of Anthony Fauci, the longtime director of the US National Institute of Allergy and Infectious Diseases, Corey also helped establish the HVTN in 1998. He remains a principal investigator there.

Corey was also a major driver in the creation of the Global HIV Vaccine Enterprise in 2004 and founder of the FHCRC-based Washington Vaccine Alliance (WAVA), a virtual biotechnology coalition of nonprofit research institutions dedicated to developing novel vaccines for the prevention of human diseases ranging from typhoid to syphilis.

"The Hutch's loss of Larry as president and director is a major gain for the field of HIV vaccine research," said Fauci, reacting to Corey's decision, which was announced in early May. "Dr. Corey is an extraordinary thought leader and practicing physician-scientist in this area and we welcome him back on a full-time basis to this critical area of biomedical research and global health."

IAVI Report caught up with Corey right after he attended the annual HVTN meeting, held June 3-5 in Washington, DC. We asked him about the meeting, his return to research, his take on the current state of AIDS vaccine and cure research, including the follow-up trials to RV144—the only HIV vaccine trial to demonstrate protection against HIV—and what he considers to be his most significant accomplishments as director of the Hutch.

Larry Corey

How did your colleagues react to your decision to return to research full time?

They gave me some smiles. And they didn't say, 'Gee, we don't want him back' (laughs). It was very gratifying to be able to have people say we have missed you and it's nice that you are spending more time on this and you can make a difference here. Whether they were pandering or not, I don't know.

What was it that you missed most about research?

I spent considerable energy looking at what I wanted to do for the next five to six years. I had

done a lot at the Hutch but there's a freedom with research and it came down to where I wanted to focus my energy and where can I make the most difference.

You have different research interests—HIV/AIDS, herpes, and cancer. What will be occupying most of your time now?

It's going to be a little bit of all three, to be honest. My lab discovered a new T cell that I think has given us some insights about how to rationally design a genital herpes vaccine. That is really exciting. With regard to the HVTN, I can see myself being able to devote more time to this now. I can think more, I can create partnerships or relationships that have some scientific insights, and develop leadership skills that will help move this thing forward. And now that we have the reagents to explore how to define correlates of protection or drive the immune response to a more diverse set of neutralizing antibodies, that kind of leadership skill becomes useful. And there is no question I will be involved with cancer as I am a scientific advisor to Juno, the immunotherapy company we founded at the Hutch. I am an immunologist by training, so helping Juno is also very gratifying.

What do you consider your biggest accomplishments as head of the FHCRC?

We totally improved the translational program, brought in new leadership in the public health sciences division, and built better relationships with the University of Washington and Children's Hospital. I raised more funds than any other Hutch president—there's the $20 million Bezos gift and another large gift that I am responsible for that will be announced fairly soon.

Importantly, we energized the faculty to think bigger and more collaboratively. The institution became less insular and more involved with our lay and scientific communities. We started an institute of health economics of cancer. We totally transformed the immunotherapy program and made that the signature program. We also raised the money to start Juno and spun it out of the Hutch in collaboration with Memorial Sloan Kettering Cancer Center. We raised $175 million for Juno and it is all going into cancer immunotherapy to fund the science and trials. I look at all the goals I set out to do in five years, and I did them in three and a half.

I read recently that you coined the phrase "Miracles start in the lab." What was the genesis of this?

That phrase, to be honest, took a while to evolve. Part of the job of being an administrator is that you are brought to dinners with potential donors and you become the evening's entertainment. You explain the science, what the Hutch is, what cancer is, and occasionally about HIV. It is a talent of mine that I can explain science to lay people. So referring to major discoveries, I think I said that making major cancer discoveries begins in the lab—that the lab is where cures start and where cures are developed. Later on, I was at a talk and Stuart Sloan, who is a donor and friend, turned it around and was talking to someone and said, 'Yeah miracles start in the lab.' That clicked and we started using it. Of course, when we started treating our first patients with immunotherapy, or when you see these cancer drugs that are cancer-specific and all of a sudden a person responds and their tumors melt away, it is a miracle. It's very cool.

What was the big news at the HVTN meeting this year?

I think the story is that the vaccine field, from a development point of view, is sort of emerging from what was a four or five year funk. There is now renewed optimism that we have some novel approaches and there is acknowledgment and maybe some new efforts on how to solve some of the structural problems in bringing a vaccine to fruition.

We are finally moving forward both with the post-RV144 program as well as the design of new immunogens for neutralizing antibodies. We are really starting to look at potentially more innovative approaches to vaccine development like vectored immune prophylaxis using adeno-associated virus vectors that express broadly neutralizing antibodies like PG9. There is finally a sense of looking forward rather than bemoaning the present.

What's happening now with the RV144 follow-up studies?

Well we are *finally* getting the timeline set. In January 2015, we will start the HVTN100 trial in South Africa [a Phase I trial of a canary pox prime (ALVAX), followed by a protein boost, with an MF59 adjuvant], which is a big one. We'll also be starting the DNA protein program in February

2015 in South Africa [a DNA clade C vaccine with Novartis' bivalent gp120 boost].

I think the hypothesis behind the post-RV144 work continues to be valid, and if anything continues to be strengthened. The data from the HVTN 505 trial show that the vaccine regimen [consisting of a DNA prime and an adenovirus serotype 5 vector that did not work] did not produce the same kind of antibody responses that were seen in RV144 vaccine recipients, and all the major correlates of protection issues associated with RV144 still hold up after HVTN505. The scientific underpinnings of RV144 actually are getting stronger over time. We have a hypothesis behind the post RV144 program that is stronger now than it was a year and a half ago. That's all good.

There have been significant developments on both the AIDS vaccine and HIV cure fronts. From your vantage point, what looks more promising right now?

I'll take vaccines. There are some things going on with cure, but to me, the best ideas for cure are the transplantations—making everybody CCR5-resistant through cord blood. But that technology is sort of a one-person-at-a-time approach.

I don't see the drug technology or the small molecule technology leading to a cure. The things I see leading to a cure are cellular and gene therapies, and that is going to take a while. At Juno we are investigating the link between cellular and gene therapy in the cancer therapy realm. I think that is what is going to be required for a cure at the moment, but we are going to have to perfect it in cancer first.

Are you more optimistic that researchers will be able to design a vaccine candidate that induces broadly neutralizing antibodies against HIV?

I am more optimistic. But first we'll need to show what the targets of neutralization are. I come from a field in which we developed a vaccine that induced neutralizing antibodies against herpes, yet it still failed. So what we measure in a standard neutralizing assay may not be the mechanistic basis of protection. Now, I'm hoping the nature of the antibodies against HIV are going to be better, but I think we are going to have to pursue vectored immune prophylaxis studies to tell us just how good the neutralization needs to be.

I think blocking HIV acquisition is an antibody-based phenomenon, or an interaction between T helper cells and antibodies. I think this is what is leading to the protection against acquisition seen in RV144. So, yes, I am optimistic, but it would be nice to also have a little luck. ■

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this affects cost. Supporters counter that the upfront and production costs for plants can be one-tenth the costs of that for CHO production using steel tanks, even though the downstream costs are higher. Greenhouses are becoming more sophisticated and plants grown in these conditions are more uniform and productive.

Another kind of delivery system is also being pursued by Philip Johnson, chief scientific officer and executive vice president at the Children's Hospital of Philadelphia. He and his colleagues are testing the idea of inserting the PG9 antibody gene in an adeno-associated virus vector and injecting this into humans. "The idea is that we make a vector that contains the gene that represents the antibody. The gene then gets into the cell—in our application, into muscle cells—the muscle cell then makes the antibody that gets into circulation and continues to make it," Johnson says.

Bioreactors are still in the picture here, but in this case they are used to make the vector instead of a protein. By only needing one administration per patient, successful vector delivery would brighten the prospect of endless manufacturing costs considerably. Johnson is working with the International AIDS Vaccine Initiative and the University of Surrey in the UK on a Phase I clinical trial investigating whether healthy volunteers injected with the PG9-encoded vector will then produce the antibody and at what levels. The California Institute of Technology's David Baltimore and his team are trying a similar approach using different antibodies and a different vector. So-called gene therapy, however, remains shadowed by safety concerns.

For every research question answered, basic production questions come closer. The Gates Foundation's Hadley responds by posing a simple equation. "Our interest is global access," he says. Hadley says the intent is to create partnerships with large pharmaceutical or biotechnology companies that can, and will, do commercial-scale antibody development. "We'd be thrilled if we get Phase I data that says this bNAb looks great, and a large company comes along and says, 'We'll take it from here.'" ■

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

In BRIEF

PEPFAR's New Leader Faces Challenges as Program Enters Second Decade

The US President's Emergency Plan for AIDS Relief (PEPFAR), which has provided life-saving antiretroviral therapy (ART) to more than 6.7 million HIV-infected people in developing countries since its launch in 2003, begins its second decade with a new leader and shifting strategies, and also faces new challenges abroad as countries pass laws criminalizing homosexual behavior that could potentially disrupt HIV/AIDS treatment and prevention efforts on the ground.

In April, the Obama administration appointed Deborah Birx, a US Army colonel and physician with deep roots in the global AIDS fight, as the new Ambassador at Large and US Global AIDS coordinator, placing her in charge of all international HIV/AIDS efforts, including PEPFAR, and making her the program's fourth leader. Prior to joining PEPFAR, Birx led the US Military HIV Research Program (MHRP) during the launch of the RV144 AIDS vaccine trial in Thailand, which drew intense criticism at the time from several prominent AIDS vaccine researchers, but eventually made history as the first and thus far only vaccine trial to demonstrate efficacy. Birx left MHRP in the midst of the trial to head the US Centers for Disease Control and Prevention's (CDC) global AIDS program.

Nelson Michael, who worked for Birx during the early days of the RV144 trial and took over the directorship of MHRP when she left, said the US$105 million trial would probably have derailed were it not for her single-minded determination. "It was a rocky time, and there was obviously a lot of discussion in the scientific press," recalls Michael, referring to a policy forum in *Science* magazine in which leading scientists raised serious questions about the scientific rationale for the large trial. "But if she thinks something is right, nothing will deter her."

By the time the surprising results of RV144 were announced in 2009, Birx was already in place at the CDC. "She didn't get as much credit as she deserved," says Michael. "She deserves all the kudos."

Michael says Birx is politically adept and he was delighted when he first heard she had been nominated to lead the PEPFAR program.

PEPFAR, which was launched in the spring of 2003 by George W. Bush, does much more than provide access to ARVs. In 2013, it also supported HIV testing and counseling for more than 57.7 million people, providing a critical entry point to prevention, treatment, and care services. One challenge facing PEPFAR, and now Birx, is the process of shifting responsibility of PEPFAR-established programs to the host country's government. PEPFAR, which has already committed more than $52 billion to fight HIV/AIDS, malaria, and tuberculosis, is now trying to strengthen capacity in recipient countries so they can manage their own treatment and prevention programs. Eric Goosby, the previous head of PEPFAR, began the process of shifting responsibility to host countries before leaving PEPFAR late last year to lead a new center at the University of California in San Francisco on implementation sciences that will examine the practicalities of running public health programs and applying business-world efficiencies to improve them.

This process of shifting control over to the recipient countries and getting them to shoulder the costs may be more difficult given the increasing political tension over new anti-homosexuality laws passed in several countries, notably in Uganda, the largest recipient of PEPFAR funding.

The Uganda Legislature also passed a bill on May 13 that includes mandatory HIV testing for pregnant women and their partners, and allows medical providers to disclose a patient's HIV infection status to others. The bill, which Uganda President Yoweri Museveni is yet to sign into law, also criminalizes HIV transmission, attempted transmission, and behavior that might result in transmission by those who know their HIV status.

Birx responded quickly to the most recent legislation passed in Uganda. "I join with the many health practitioners, HIV/AIDS and human rights activists, multilateral institutions, and individuals everywhere—in Uganda and around the world—in calling for the people and the Government of Uganda to reject this regressive bill," she noted, in a May 14 release. ■

Exploring Antibodies to Prevent Mother-to-Child Transmission of HIV

Following the isolation and characterization of dozens of antibodies that can neutralize a broad swathe of HIV isolates, these so-called broadly neutralizing antibodies (bNAbs) are under study in several realms of HIV prevention research, including helping to further reduce rates of mother-to-child transmission (MTCT) of HIV.

Antiretroviral (ARV) prophylaxis during pregnancy, delivery, and breastfeeding is currently the most effective approach to prevent MTCT. But despite widespread efforts to eliminate MTCT, there were 210,000 new pediatric HIV infections in sub-Saharan Africa in 2012, nearly half of which occurred during the breastfeeding period.

The persistence of MTCT of HIV is due to a number of factors. One is identifying HIV-infected pregnant women and pre-

scribing ARV treatment. Another is that ARV prophylaxis requires women to adhere to a once-daily, three-drug suppressive treatment regimen. "The attraction of the antibodies is that they are so long lasting," said Barney Graham, of the US National Institute of Allergy and Infectious Diseases' Vaccine Research Center (VRC). "So if you can give it even once a month, it can cover some of the gaps that might occur in daily ARV treatment non-adherence." ARV prophylaxis during breastfeeding is also not foolproof—breakthrough infections can occur at the rate of 2%-5% by six months.

Studies have shown that some of the recently isolated bNAbs can protect against mucosal challenge in rhesus macaques. In a 2012 study, Brian Moldt of the Scripps Research Institute and colleagues showed that the bNAb known as PGT121 protected three out of five monkeys at the lowest of three antibody doses, and all five monkeys at the two higher doses (*PNAS* **109**, 18921, 2012). And enrollment in a Phase I trial testing the safety and pharmacokinetics of the bNAb VRC01, isolated by researchers at the VRC, is already underway in HIV-infected and uninfected adults. If the results of the Phase I trials are promising, researchers will begin enrollment for a Phase I study in infants born to HIV-infected mothers in the US.

Plans to evaluate the ability of VRC01 to prevent MTCT were outlined as a case study in a recent article, stemming in part from a meeting convened in Uganda in January 2013 by the Global HIV Vaccine Enterprise *(PLOS Med*. 2014, doi:10.1371/journal.pmed.1001616). The focus of the meeting was to develop recommendations on trial designs for evaluating the passive administration of VRC01 in infants, such as sample size and informed consent procedures, as well as to provide broader recommendations on the conduct of clinical trials to prevent HIV infection among breastfed infants in developing countries.

But there are a number of challenges associated with using bNAbs to prevent MTCT, including the high cost of manufacturing these antibodies (see page 4) and the logistics of delivering regular injections in the populations where MTCT occurs at the highest rates. In the Phase I trial of VRC01, researchers are evaluating a monthly injection of the antibody in adults by a qualified health professional. But such a regimen may be difficult to implement, said Pontiano Kaleebu of the Uganda Virus Research Institute and a co-author of the *PLOS Medicine* paper. "In the early stages, when this is being developed, I think that you may find that women are coming [to the clinic], but the question is when you roll out, what will happen?" asked Kaleebu.

Researchers hope that either more potent antibodies or antibodies engineered to have longer half lives will help address these issues. "We hope that we can have antibodies that can be longer lasting so that they don't have to go to the clinic so many times," said Kaleebu.

Graham believes that once more potent combinations of antibodies with longer half lives are developed, protection may extend to three months, which could allow mothers to link clinic visits with the World Health Organization's recommended schedule for childhood vaccinations. Ultimately, he said, it may be possible for the antibodies to last for six months of protection, which would further its practicality.

"They're at the very initial stages of testing this as a proof-of-concept," said Yegor Voronin of the Global HIV Vaccine Enterprise and lead author on the *PLOS Medicine* paper. "Once it works, then you can explore a variety of different ways to improve it." —*Alexandra Morris*

Alexandra Morris is a freelance science writer and graduate student at Massachusetts Institute of Technology.

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exclusively target black and Latino MSM, including 100 Black Men of America and Black Men's Xchange.

But Wilson is critical of these efforts. "There is not one [HIV] intervention developed by and for black MSM, almost no original work focused on the black MSM community. This is the definition of insanity."

Testing, testing

More widespread HIV testing is something almost everyone agrees would help deliver prevention messages to black MSM. According to a CDC analysis of 16,069 MSM, HIV testing rates among MSM rose from 2008 to 2011, with an even greater increase among black MSM. The authors of the CDC analysis, who presented their data at CROI in March, say increasing the number of MSM who are tested and linked to care will improve health outcomes and may reduce further HIV transmission.

David Purcell, the deputy director for Behavioral & Social Science in CDC's Division of HIV/AIDS Prevention, says the CDC is working with state and local health departments to identify and implement the most cost-effective and scalable interventions in the geographic areas hardest hit by HIV and among the most severely affected populations within those areas. The approach is referred to as High-Impact Prevention and he says it is one of the reasons why HIV testing rates are up among MSM, particularly black MSM.

CDC behavioral scientist Patrick Sullivan, in a special report about the HIV epidemic among MSM (*Lancet* **380**, 9839, 388, 2012), says no single HIV prevention approach will be enough to curtail HIV incidence among MSM. But a combination of structural, biomedical, and behavioral interventions that are evidence-based might avert a quarter of new infections in certain countries.

Purcell agrees and says the fight requires action on every level. "Action is needed not only from government agencies, but also from community organizations, and among gay and bisexual men themselves, to ensure all men know their HIV status and take appropriate steps to stop HIV. More prevention strategies are available now than ever before." ■

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

Upcoming HIV-Related Meetings

JULY 2014

AIDS 2014
July 20 - 25, 2014; Melbourne, Australia
More information: www.aids2014.org

SEPTEMBER 2014

Cold Spring Harbor Asia Conference on Frontiers of Immunology in Health and Diseases
September 2-6, 2014; Suzhou, China
More information: www.csh-asia.org/2014meetings/immune.html

16th Annual International Meeting – Institute of Human Virology
September 14 - 17, 2014; Baltimore, MD
More information: hivr4p.org

OCTOBER 2014

HIV Research for Prevention 2014 (HIV R4P)
October 28 - 31, 2014; Cape Town, South Africa
More information: medschool.umaryland.edu/ihvmeeting/default.html

NOVEMBER 2014

32nd Annual Symposium on Nonhuman Primate Models for AIDS
November 11-14, 2014 , Portland, Oregon
More information: www.nhp2014.com

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