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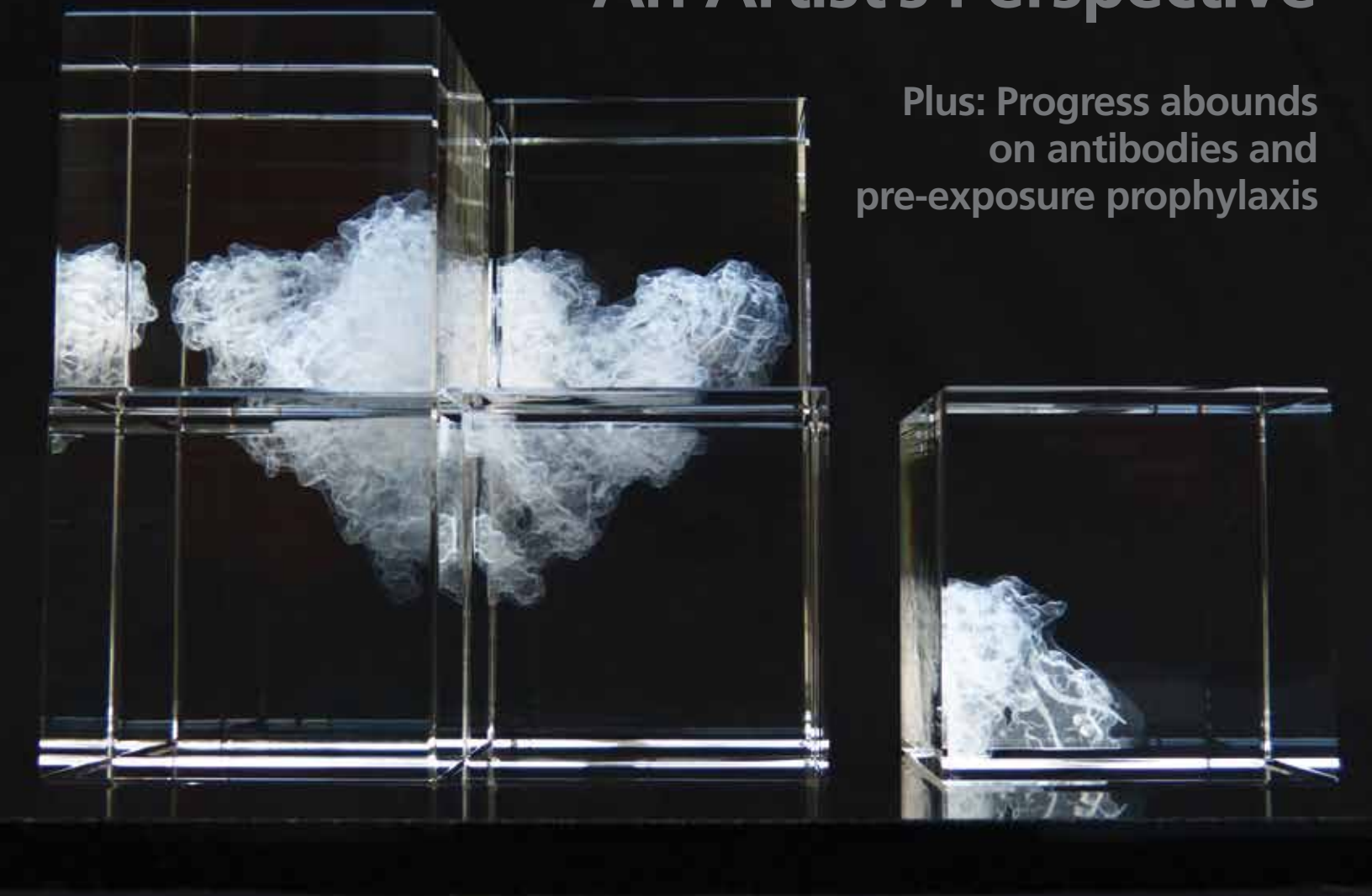
Tackling the Trimer: An Artist's Perspective

Plus: Progress abounds on antibodies and pre-exposure prophylaxis



EDITOR'S LETTER

We talk about science all the time in the pages of *IAVI Report*. We don't often have the opportunity to talk about art. But increasingly the worlds of art and science are colliding, and so in this issue we explore the work of several artists who are at the forefront of this trend. These artists are culling laboratories for inspiration and collaborating closely with scientists to make some arresting artwork (see page 11).

One of these artists, Katharine Dowson, created the eerily beautiful sculpture of the HIV Envelope trimer etched in polished glass that graces the cover of this issue. This sculpture was commissioned by the Bill & Melinda Gates Foundation and was featured at a temporary exhibit in Berlin that accompanied Gavi's (The Vaccine Alliance) pledging conference in January.

Just as Dowson's sculpture indicates, this trimeric viral protein is somewhat shadowy and mysterious. But thanks to great progress in isolating and characterizing broadly neutralizing antibodies that target HIV Envelope, more is known about it than ever before. Much of this progress was showcased at the recent Keystone Symposium on HIV Vaccines, held this March (see page 16). The good news is that there are more conserved regions of Envelope than previously thought and this gives vaccine researchers more options to work with when developing immunogens designed to induce these powerful antibodies. Meanwhile, researchers are also developing new ways of tracking responses to vaccination that will also aid efforts to design better vaccine candidates.

This issue also features a full report from this year's Conference on Retroviruses and Opportunistic Infections (CROI), held in February (see page 4). Talks at CROI also focused on the progress in understanding antibodies and optimizing their functions, as well as the unquestionable evidence that pre-exposure prophylaxis (the use of antiretrovirals to prevent HIV infection) is a surprisingly effective means of HIV prevention, even when used on demand instead of on a daily basis.

This issue is the first in the 19th volume of *IAVI Report*. After 19 years there are still many vaccine-related stories to tell and our goal is to continue to bring you, our readers, a wide variety of opinions and perspectives on the ongoing quest for an AIDS vaccine.

– 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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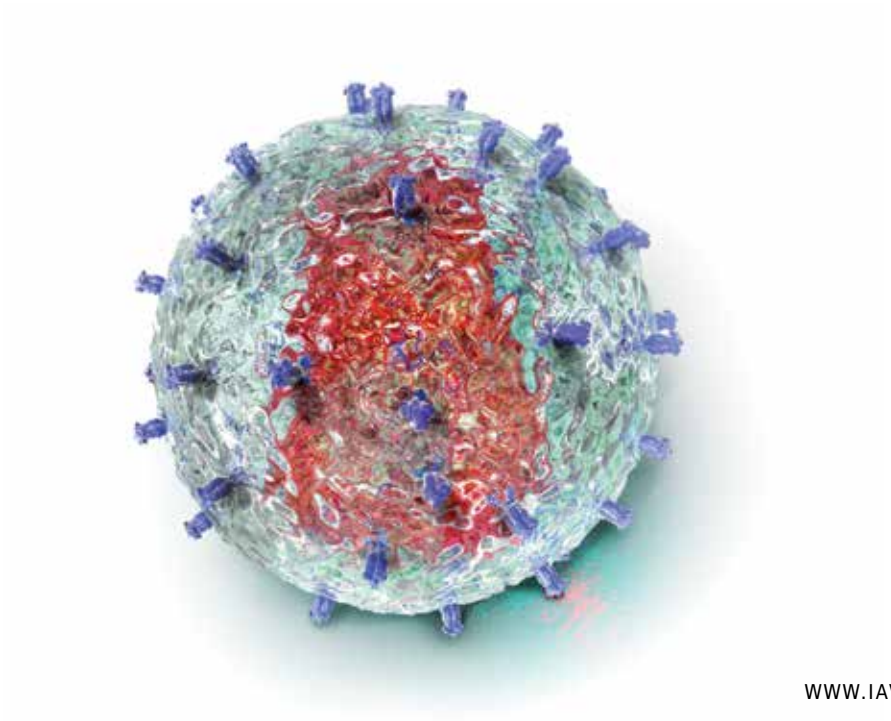

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[ON THE COVER]

UK artist Katharine Dowson's laser etching and glass sculpture, *A Window to the Future of an HIV Vaccine*. Dowson's work is a highlight of the Bill & Melinda Gates-sponsored project *The Art of Saving a Life*, which had a temporary exhibition in Berlin in January accompanying the Gavi (The Vaccine Alliance) donor conference.

Image courtesy of the Bill & Melinda Gates Foundation.

PrEP *WORKS*

The annual Conference on Retroviruses and Opportunistic Infections offered a broad survey of the state of HIV research, with new oral prevention results as the highlight

By Richard Jefferys

The 2015 Conference on Retroviruses and Opportunistic Infections (CROI) is likely to be remembered as a watershed moment for pre-exposure prophylaxis (PrEP)—the use of antiretrovirals to prevent HIV infection. Just as the 1996 International AIDS Conference launched highly active antiretroviral therapy into the mainstream, some attendees viewed this year's CROI, held February 23-26 in Seattle, as the one that proved once and for all that PrEP is an effective HIV prevention strategy. PROUD and IPERGAY, two studies that were designed to evaluate PrEP's effectiveness in men who have sex with men (MSM) in relatively real-world settings, reported reductions in HIV incidence that surprised even the investigators.

News from the vaccine research realm was not as dramatic, but an array of presentations illustrated recent progress, including advances in analyzing antibody functions and the development of a potential antibody simulacrum with unprecedented breadth and potency. Meanwhile, the pursuit of a cure remains at an early stage but is increasingly a focus at CROI, with several sessions addressing the state of the field, including areas of overlap with vaccine research such as the study of broadly neutralizing antibodies (bNAbs).

Oral PrEP results are easy to swallow

It isn't news that PrEP works. Back in 2010 investigators first showed that oral Truvada (a pill combining the antiretrovirals tenofovir and emtricitabine) was 44% effective at preventing HIV infection in MSM and transgender women. At CROI, Sheena McCormack, professor of clinical epidemiology at the Medical Research Council Clinical Trials Unit at University College London, debuted results from the PROUD study, conducted in the UK, which was designed to assess how PrEP would perform in routine practice. To that end, investigators recruited high-risk MSM over 18 years old at sexual health clinics. High risk in this study was defined as having anal sex within the last 90 days without a condom and anticipating it would occur again in the next 90 days. Participants were randomized to receive Truvada immediately or after one year, in addition to standard-of-care prevention services. Follow up occurred every three months, in accordance with normal clinic practice.

The initial phase of the trial was intended as a pilot to explore whether recruitment and retention would be sufficient to embark on a larger efficacy trial, but in April 2014 the Data Safety Monitoring Board (DSMB) recommended evaluating efficacy due to the high HIV infection risk observed in the cohort. The DSMB then intervened again in October 2014 because the divergence between the number of HIV infections in the immediate and deferred PrEP groups was so significant that it would have been unethical to continue. Truvada was then offered to all participants.

At that point, 545 individuals were enrolled. The effectiveness analysis was based on 267 volunteers in the group that received Truvada from the start and 256 volunteers in the deferred group. The difference was striking: only three HIV infections occurred in the group receiving Truvada from the start, while there were 19 in the deferred group. Expressed in terms of incidence per 100 person-years, the comparison was 1.3 versus 8.9, indicating an efficacy of 86%. McCormack noted that this equated to one HIV infection averted for every 13 individuals on PrEP (referred to as the "number needed to treat").

Of the three infections in the MSM randomized to receive Truvada immediately, two may have occurred just prior to enrollment (at which

time the individuals were still HIV antibody negative and therefore not kept from enrolling in the study) and the other was in a participant who defaulted from follow up after receiving an initial Truvada prescription. When he was later diagnosed with HIV infection, he informed medical staff that he'd stopped taking PrEP. Six of the infections in the deferred arm could also have represented infections prior to study entry that were undetectable by antibody-based tests, but if all such cases were excluded from the analysis the reduction in incidence was still highly significant.

Adverse events were uncommon: of 13 cases where Truvada was interrupted due to a side effect (mostly gastrointestinal related), 11 volunteers successfully restarted PrEP. A drug resistance mutation associated with emtricitabine use was observed in three of six individuals who appeared to have been seroconverting at the time of starting PrEP, but there was no evidence of resistance to tenofovir. Rates of other sexually transmitted infections (STIs) were not significantly different between the arms, and rates of reported condom use did not change over the course of the study. Reported adherence to Truvada in this study was high, and this was confirmed by measuring drug levels in a 57-person sub-study.

McCormack pointed out that the HIV incidence in the deferred PrEP arm of PROUD was three times higher than anticipated based on analysis of the overall MSM population attending the clinics, suggesting that those at the very highest risk had chosen to volunteer for the study. "People who needed it really came forward," McCormack said, and this emerged as a theme in PrEP presentations at CROI, raising the hope that if PrEP is made widely available, it will be used by populations that stand to benefit the most.

The resounding success of PROUD was duplicated—to the percentage point—in the results of the IPERGAY trial, which were presented by Jean-Michel Molina, chief of infectious diseases at the University of Paris Diderot. The purpose was to test whether "on demand" PrEP might be both efficacious and convenient in high-risk MSM from France and Canada. Participants were instructed to take two Truvada (or placebo) pills two to 24 hours prior to sex followed by two additional doses 24 and 48 hours afterward, in addition to receiving standard prevention counseling and condoms. The regimen was based on a study in macaques, which showed that intermittent Truvada could protect against an intra-rectal challenge with a hybrid simian immunodeficiency virus (SIV)/HIV (SHIV) challenge (*Sci. Transl. Med.* **2**, 14, 2010).

As was the case for PROUD, the IPERGAY DSMB ended the randomized comparison in October 2014 due to the observed difference in HIV infections between the arms, and at that time Truvada was offered to all volunteers. The efficacy analysis was based on 176 participants from the Truvada-treated group and 177 placebo recipients.

Molina revealed that two HIV infections occurred in participants taking Truvada while 14 occurred among placebo recipients, a comparative incidence of 0.94 versus 6.6 per 100 person-years. The estimated efficacy was therefore 86% and the number needed to treat to prevent one HIV infection was calculated to be 18. Incidence was higher than anticipated in the placebo group, echoing PROUD's suggestion that the MSM who enrolled were cognizant of their elevated risk of acquiring HIV. As with the primary results, the analyses of adherence, adverse events, STI rates, and sexual behavior over time all closely mirrored those of the PROUD study. The average number of Truvada pills consumed during the study was four per week, meaning monthly usage was close to half that of daily dosing. Molina concluded that "on demand" PrEP represents an attractive alternative to daily use for high-risk MSM.

Jared Baeten, professor of allergy and infectious diseases, epidemiology and global health at the University of Washington, addressed the use of both PrEP and antiretroviral therapy (ART) in a different population: heterosexual serodiscordant couples in Kenya and Uganda. Baeten's goal was to investigate whether a combined strategy of PrEP use by the HIV-uninfected partner and ART initiation in the HIV-infected partner was feasible and reduced HIV transmission. In this setting, PrEP was administered on a time-limited basis, as a "bridge" until the HIV-infected partner had been on ART for six months (unless there were adherence issues or the individual's viral load remained detectable despite treatment).

A total of 1,013 couples were enrolled, deemed to be at high risk of transmitting HIV based on a scoring system developed by Baeten and colleagues that takes into account risk factors such as younger age, lack of circumcision, and condomless sex in the prior month (*J. Acquir. Immune Defic. Syndr.* **62**, 339, 2013). Of the HIV-uninfected partners, 33% were female and 67% male, and over 95% initiated PrEP with good adherence. ART was started by 80% of the HIV-infected partners, with more than 90% achieving viral load suppression. ART was prescribed in accordance with new guidelines recommending treatment for all HIV-infected individuals in serodiscordant relationships. Rather than employing a control group, which was considered unethical, Baeten constructed a counterfactual simulation

model to predict likely HIV incidence in serodiscordant couples not using PrEP and in whom ART was prescribed based on previous guidelines (CD4+ T-cell count less than 350). The model was based on data from previous studies involving over 5,000 people.

In the current study, only two HIV infections were observed, an incidence of 0.2 per 100-person years. By contrast the model predicted 40 HIV infections, an incidence of 5.2 per 100-person years, therefore the estimated reduction compared to the model was 96%. The two individuals who seroconverted during the study reported breaks in PrEP use and had no Truvada detectable in plasma at the time their HIV infection was detected. In one case, the relationship with the HIV-infected partner had ended, suggesting a different transmission source, and in the other the partner had not started ART due to a relatively high CD4+ T-cell count. In response to a question from Glenda Gray, executive director of the Perinatal HIV Research Unit at the University of the Witwatersrand and current president of the South African Medical Research Council, Baeten stated that both the HIV infections were in women. Gray's point was that there may still be some uncertainty about oral PrEP efficacy in women compared to men (one concern is possible differential tenofovir penetration into the vaginal versus rectal mucosa, see *Sci. Transl. Med.* **3**, 112re4, 2011), but Baeten said that there appeared to be other explanations for the two seroconversions in this study.

Microbicide news disappoints

The encouraging news about oral PrEP that emerged at CROI was, unfortunately, accompanied by another blow to hopes that the antiretroviral tenofovir administered as a vaginal microbicide might be similarly efficacious. In 2010, the CAPRISA 004 trial reported a statistically significant 39% reduction in HIV incidence associated with use of 1% tenofovir gel by South African women at high risk of infection (*Science* **329**, 1168, 2010). The microbicide was used pericoitally, meaning study participants were instructed to apply the gel within 12 hours before and 12 hours after sex. However, a subsequent trial of daily tenofovir gel administration, the VOICE study, was unable to demonstrate efficacy (*N. Engl. J. Med.* **372**, 509, 2015). A confirmatory study known as FACTS 001, designed to evaluate the original CAPRISA 004 pericoital approach in a larger number of women in South Africa, also found no efficacy, as reported at CROI by Helen Rees, executive director of the Wits Reproductive Health and HIV Institute in Johannesburg.

The FACTS 001 trial was conducted at nine sites and enrolled 2,059 women aged 18-30 years. Because the results from VOICE indicated poor adherence contributed to the lack of efficacy, intensive adherence support was incorporated into the design. The efficacy analysis included 1,015 women in the tenofovir gel group and 1,014 who were randomized to receive a placebo gel. There was no significant difference in the number of HIV infections in the two groups, with 61 and 62 occurring in each group, respectively. Analyses of adherence bolstered previous findings that suggest the gel is not user-friendly—a paltry 13% of the participants applied it during more than 80% of sexual activity. There was some indication that a small proportion of women who had detectable tenofovir levels in cervical fluids and who reported having sex during the 10 days prior to sampling were protected. In this sub-group, HIV acquisition risk was reduced by 52%.

Rees stated that the results, while disappointing, represent important science that highlights the urgent need for prevention options that are easier to integrate into women's lives.

Striking an upbeat note for vaccines

In the self-described role of motivational speaker, Galit Alter, professor of medicine at the Ragon Institute of the Massachusetts Institute of Technology, Massachusetts General Hospital, and Harvard, delivered an upbeat overview of recent advances in HIV vaccine science at a workshop on the opening day of the conference. Alter delineated two approaches to successful vaccination: completely blocking HIV entry, or rapidly killing virus-infected cells at the site of exposure before systemic infection ensues. Citing recent data from Dan Barouch, associate professor of medicine and chief, Division of Vaccine Research in the Department of Medicine at Beth Israel Deaconess Medical Center and Harvard Medical School, Alter noted that the window of opportunity for extinguishing infection at the site of entry before a persistent HIV reservoir is established is very narrow, certainly less than three days in the SIV/macaque model (*Nature* **512**, 74, 2014).

Hopes for blocking HIV entry with bNAbs have been bolstered by the discovery that a substantial proportion of HIV-infected individuals develop antibodies with neutralizing capacity over a period of two to three years after seroconversion. The appearance of bNAbs is associated with high initial viral load and greater viral diversity. Alter emphasized that this demonstrates the human immune system is capable of generating bNAbs, and that it therefore should be possible to recapitulate the process with immunization.

Progress is now being made toward designing immunogens that engage and activate specific naïve germline B cells that have the capacity to differentiate into bNAb-producing memory B cells. One strategy for coaxing the B cells along the correct differentiation pathway is to use sequential immunizations with different antigens designed to focus the resulting antibody response on conserved neutralization targets on the HIV envelope. To help achieve this goal, researchers are also investigating how to best exploit mechanisms that provide crucial help to developing B-cell responses, particularly follicular dendritic cells and T-follicular helper cells (Tfh).

Switching to approaches designed to rapidly eliminate virus-infected cells, Alter cited the example of Louis Picker's replicating cytomegalovirus (CMV) vector-based SIV vaccine, which induces and maintains effector $CD8^+$ T-cell responses at mucosal sites where infection occurs. By contrast, most vaccines induce central memory T cells. These require activation by viral antigens to generate a swarm of effector T cells and this process takes days, too long to prevent systemic HIV infection. A possible alternative method for sustaining effector T cells in mucosal tissues was recently described in a mouse model of herpes simplex infection. This strategy, dubbed "prime and pull," employs vaccination to prime virus-specific T-cell responses, followed by local application of chemokines to pull the T cells to the mucosa where they establish a resident effector population (*Nature* **491**, 463, 2012).

Beyond T cells, non-neutralizing antibody responses can also mediate killing of infected cells by recruiting innate effector cells such as natural killer (NK) cells and monocytes. Immune correlates studies have implicated an antibody-mediated cellular cytotoxicity (ADCC) mechanism in the protection observed in the RV144 trial, the first vaccine trial to show any efficacy in blocking HIV infection, spurring efforts to gain a deeper understanding of ADCC and develop means to fine-tune the response with vaccination. The relatively new sciences of systems biology and transcriptomics (the study of the RNA transcripts produced by the genome in response to a particular circumstance or stimulus) are also uncovering previously unknown immunological pathways that appear to contribute to the efficacy of licensed vaccines (*Nat. Immunol.* **15**, 195, 2014), and these findings may also be applicable to HIV vaccine design.

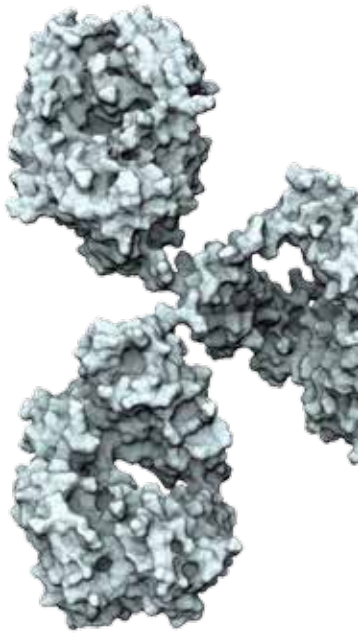
Given all this, Alter concluded that the stage is set for significant progress toward a successful vaccine in the coming years, and several subsequent presentations at the conference expanded on areas touched upon in her talk.

Margaret Ackerman, assistant professor of engineering at Dartmouth College, took up the theme of enhancing protective antibody activities with an approach described as "systems serology." Ackerman explained that antibodies possess domains that interact with Fc receptors on a variety of immune cell types including NK cells, monocytes, and dendritic cells. These interactions are critical for the induction of effector activities such as ADCC, and studies in both macaques and humanized mice have shown that even bNAbs depend on Fc receptor interactions to mediate optimal protection (*Nature* **449**, 101, 2007; *Cell* **158**, 1243, 2014).

The aim of systems serology is to capture data on all the key contributors to beneficial antibody activity to inform the design of vaccine strategies. One factor that contributes to better antibody activity is antibody subclasses. As an example of how vaccine regimens can modulate these responses, Ackerman compared the results of the RV144 trial—which tested the canarypox vector-based vaccine ALVAC in combination with an AIDSVAX B/E protein boost—and a previous trial known as VAX003 that tested AIDSVAX B/E alone. The RV144 vaccine regimen induced significantly greater amounts of immunoglobulin (IgG)3 antibodies, which are associated with potentiated effector function, while vaccination with only AIDSVAX in VAX003 skewed the response toward the IgG4 subclass of antibodies, which are linked to diminished Fc-mediated effector activity (*Sci. Transl. Med.* **6**, 228ra38, 2014; *Sci. Transl. Med.* **6**, 228ra39, 2014).

The glycosylation profiles of antibodies are also important. Ackerman found that in HIV-infected individuals, control of viral load in the absence of treatment is associated with the ability of virus-specific antibodies to recruit both NK cells and monocytes as effectors, which in turn is associated with more extensive antibody glycosylation (*J. Clin. Invest.* **23**, 2183, 2013).

Ackerman's lab has now developed a system that integrates over 1,000 salient measurements from a single sample to identify predictors of desired antibody activities. The approach has the potential to be combined with other techniques, including transcriptional profiling and assessments of cytokine responses, to better understand and modulate the response to vaccine candidates, with the ultimate goal of maximizing protective efficacy. Another possible application is to optimize the design of antibodies intended for delivery via passive immunization or gene transfer by improving their activity and bioavailability.



More shots, more protection?

Following the surprising results of RV144, researchers began preparations for a slew of studies designed to improve upon the modest 31% protection the vaccine regimen afforded. The RV305 trial, involving participants from RV144, was designed to investigate whether two additional booster shots with ALVAC, AIDSVAX B/E, or both (at baseline and 24 weeks later) would improve IgG3 antibody responses targeting the V1-V2 region of the HIV envelope and/or drive antibody gene maturation toward characteristics associated with bNAbs. Georgia Tomaras, associate director of research at the Duke University Human Vaccine Institute, presented results from this study at CROI.

An analysis of a blinded subset of participants in RV305 demonstrated significant enhancement of the IgG4 but not IgG3 antibody response compared to results obtained during RV144, in contrast to the desired outcome. There was evidence of a transient increase in ADCC activity and neutralization of tier-1 viruses (those that are considered the easiest to neutralize), but not broad neutralization, after the first but not second booster immunization. Detailed characterization of antibodies isolated from vaccinated volunteers showed an increase in somatic mutation frequency from a mean of 2.9% with the original regimen, to 6.7% after the additional boosters, and a greater prevalence of long HCDR3 sequences, which are a characteristic of bNAbs (2% versus 21% of antibodies with HCDR3 longer than 22 amino acids). Notably, there were a proportion of IgG3 antibodies identified among those with long HCDR3s. Ongoing studies are now exploring the specificities and functional attributes of the B-cell lineages responsible for producing these more matured antibodies. Tomaras, like Ackerman, highlighted the need to better understand the dynamics of antibody responses to vaccination and the implications for functionality.

Other researchers are also mining the original RV144 results for clues. In a late-breaker talk by Shelly Krebs, scientist and principal investigator at the US Military HIV Research Program (MHRP), data was presented on how vaccination influenced antibody evolution in RV144 participants from a different perspective. The question Krebs sought to answer was whether receipt of the vaccines affected the development of antibodies in those subjects who became HIV-infected during the trial despite vaccination. The hypothesis behind the study was that these individuals might generate neutralizing antibodies more rapidly than is normally seen in natural infection as a result of the priming of memory B-cell responses by immunization. But, as frequently happens in science, the results turned this hypothesis on its head.

There was no overall difference in the breadth or potency of antibody responses during post-infection follow-up but, strikingly, only placebo recipients developed antibodies with greater than 70% neutralization breadth. The single exception was an individual in the vaccine arm who only received a single injection of the ALVAC prime. There was some evidence for increasing neutralization capabilities over time, but it was significantly less in the vaccine recipients compared to placebo, or even compared to historical data from unvaccinated HIV-infected individuals. Krebs and colleagues are now attempting to explain this observation and are considering two possibilities: either that vaccine-induced antibodies restricted viral diversity and thereby limited neutralization breadth, or that those RV144 participants capable of making good antibody responses did not become infected, leaving only those with poor antibody responses in the group of vaccinees that acquired HIV. A new round of studies evaluating viral diversity and host genetics will be conducted to assess which of these two explanations appears more plausible, but for now, Krebs favors the latter.

A little help from your friends

The topic of Tfh help for B cells that was alluded to by Alter was discussed further by Hendrik Streeck, chief of the Cellular Immunology Section at MHRP. The vast majority of this $CD4^+$ T-cell subset resides in lymph nodes, making routine analysis of Tfh responses challenging. Researchers have therefore been searching for markers that might reliably identify the small population of Tfh in the periphery. Streeck unveiled new evidence that production of the cytokine interleukin (IL)-21 is an ideal candidate for this marker.

In studies of HIV-infected individuals, HIV Gag-specific and Env-specific Tfh could be identified in blood based on IL-21 production, with both populations equally capable of providing help to $CD8^+$ T-cell responses. However, there was a bifurcation when it came to B-cell responses—Gag-specific Tfh-supported B-cell proliferation and maturation, whereas Env-specific Tfh were better at driving antibody class switching (the shift from IgM to IgG antibody production). Interestingly, a preliminary analysis of samples from past vaccine trials revealed that the RV144 vaccine regimen, which provided slight protection, induced significantly higher levels of IL-21-producing Tfh in the periphery than HVTN 505, which evaluated the efficacy of a

prime-boost regimen comprising a DNA prime and Ad5 vector boost encoding three different Env proteins from clades A, B, and C, along with clade B Gag, Pol, and Nef and failed to provide any protection against HIV infection (*N. Engl. J. Med.* **369**, 2083, 2013). Streeck's research suggests that more detailed investigations of Tfh will complement the work being conducted in the antibody arena.

Getting away without antibodies

Shortly before the start of CROI, a buzz-worthy paper in *Nature* captured headlines in publications from *The New York Times* to *Science*. The paper was on a potential alternative to traditional vaccination spearheaded by Michael Farzan, professor and vice chairman of the Department of Immunology and Microbial Science at The Scripps Research Institute in Florida (*Nature* **519**, 87, 2015). Farzan's approach involves a protein designed to bind to the sites on the HIV envelope that interact with the CD4 and CCR5 co-receptors, thereby blocking viral entry into target cells. Named eCD4-Ig, it has unprecedented breadth and potency, neutralizing 100% of a large panel of HIV isolates including tier-2 and 3 viruses, CXCR4-tropic viruses (those that gain entry using the alternative CXCR4 co-receptor), and those resistant to bNAbs targeting the CD4 binding site. Additionally, eCD4-Ig inhibits SIVmac239 and HIV-2 *in vitro*.

To deliver eCD4-Ig, Farzan adopted the adeno-associated virus (AAV) vector gene transfer approach developed by Phil Johnson, director of The Children's Hospital of Philadelphia Research Institute. At CROI, Farzan noted that the potency of neutralization achieved with eCD4-Ig should translate into activity against HIV at the levels achievable *in vivo* with AAV. Furthermore, studies in macaques suggest eCD4-Ig is less immunogenic than bNAbs; "it looks more like self," Farzan stated, meaning there is a reduced likelihood that it will provoke an anti-eCD4-Ig immune response that could block its antiviral effect. In an experiment conducted in macaques, an AAV encoding eCD4-Ig was mixed with a separate AAV encoding the gene for tyrosylprotein sulfotransferase 2 (TPST2). The TPST2 is needed to render the eCD4-Ig protein active via a process called sulfation. Four animals received the AAV mixture and four served as controls. Increasing doses of the challenge virus SHIV-AD8 were administered at weeks 8, 11, 16, 20, 26, and 34, with all controls eventually becoming infected. All eCD4-Ig immunized macaques remained uninfected and Farzan added that, since the publication of his paper, these animals have resisted two additional higher dose challenges of 1,600 and 3,200 picograms.

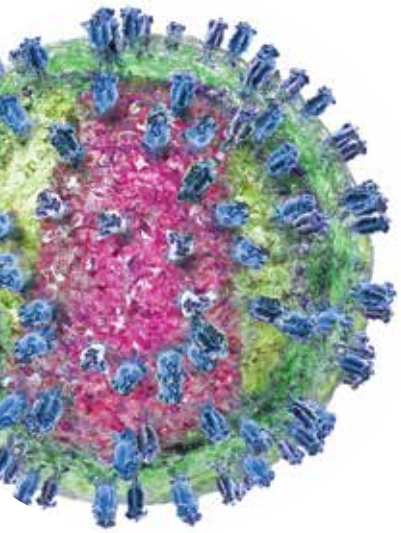
As was evidenced by quotes from other scientists in the media coverage of Farzan's paper, the results have generated considerable excitement. Farzan believes that AAV-delivered HIV inhibitors, whether eCD4-Ig or bNAbs, are "closer to realization than any conventional vaccine approach." Future plans include the development of an "off switch" that could curtail AAV production of encoded proteins in the event of safety issues, and therapeutic studies in SIV-infected macaques.

Antibodies and a cure

Interest in bNAbs and antibody effector functions is not only dominating the vaccine field these days. It has recently extended into cure research. A key rationale is that bNAbs may be able to promote destruction of HIV-infected cells, a task that cannot be accomplished by ART alone. Barouch reviewed the possible role of bNAbs in cure strategies. His laboratory bears credit for spurring interest in the topic with the publication of a paper in 2013 demonstrating that a cocktail of bNAbs could suppress viral replication and reduce proviral DNA in macaques infected with the hybrid SIV/HIV strain SHIV-SF162P3 (*Nature* **503**, 224, 2013).

Barouch has since embarked on follow-up studies combining bNAbs and ART. He described results of an experiment intended to model chronic HIV infection in which a total of nine macaques were infected with SHIV-SF162P3 for seven months, then given either ART (comprising tenofovir, emtricitabine, and the integrase inhibitor dolutegravir) plus four monthly infusions of the bNAb PGT121, or ART alone. Five animals received the combination ART/antibody, while four received only ART. In both groups ART was maintained for 20 weeks. There was rapid and complete suppression of viral load in all macaques but significant declines in the viral reservoir in peripheral blood and gut—as measured by proviral DNA—were only seen in the bNAb recipients. The difference in proviral DNA levels between the groups was driven by three of the five macaques given bNAbs, with little change evident in the other two.

When ART was stopped, the two animals in the bNAb group showing no reduction in the reservoir experienced a typical viral load rebound, as did those on ART alone. But in the three cases where proviral DNA was diminished, there was an extended period of virological control, with one rebounding after nearly a year of sustained virus control. Barouch concluded that while bNAbs were not completely curative, they did appear to



contribute to reservoir clearance. He suggested combinations of antibodies and agents capable of activating the latent reservoir may be needed to achieve greater effects, and larger macaque studies exploring this concept are now in progress.

Barouch also offered a glimpse at preliminary data from a macaque study modeling very early infection. Thirty-two animals were infected intra-rectally with SHIV-SF162P3 and divided into four groups of eight. The groups received no treatment, ART alone, ART plus the bNAb PGT121, or PGT121 alone. The interventions were initiated three days after challenge, with ART lasting for four weeks and PGT121 administered as a single infusion. The groups receiving ART or PGT121 briefly displayed detectable viral load prior to suppression, while recipients of the combination showed no viremia during treatment. After ART suspension, viral load rebounded in the majority of animals in all groups, but there was a significant delay in those given ART plus PGT121. Detailed virological and immunological analyses are ongoing.

Next steps include the development of bNAb cocktails, with Barouch noting that the combination of PGT121 with a recently discovered potent bNAb PGDM1400 (*Proc. Natl. Acad. Sci. USA* **111**, 17624, 2014) was able to neutralize 98%-99% of a panel of circulating HIV isolates *in vitro*, suggesting a dual antibody approach could be highly effective. Human trials of PGT121 infusions are planned, first evaluating safety and then looking at its activity in different populations of HIV-infected individuals. The studies will include evaluations of antiviral activity in participants with detectable viral loads and effects on the reservoir in those on ART. Questioned about the mechanism by which bNAbs might impact the HIV reservoir, Barouch explained that some infected cells likely express the viral envelope on their surface, making them susceptible to ADCC or other antibody-mediated effector activities.

Passive immunization with bNAbs was also addressed by Diane Bolton, chief of Animal Models and Pathogenesis at MHRP, who outlined plans to conduct a clinical trial of the bNAb VRC01 in acutely HIV-infected individuals in Thailand. The trial, RV 398, is due to start later this year. As part of the planning process, Bolton performed a macaque study comparing ART (tenofovir, emtricitabine, and the integrase inhibitor raltegravir), ART plus VRCO1, and ART plus two other bNAbs selected due to their greater potency against the SHIV-SF162P3 challenge virus used in the experiment: VRC07-523 and PGT121. The design of Bolton's monkey study differed from Barouch's early infection experiment: animals were infected with SHIV-SF162P3 at baseline and on day 10, daily ART was begun in the first group, and a single infusion of the bNAbs was administered to the latter two groups. The bNAb recipients began receiving ART at day 21 following infection. This allowed Bolton to document that ART and the dual bNAb combination were equally potent at reducing acute viremia. VRC01 lowered viral load by about a log prior to ART, which Bolton pointed out was consistent with its lower *in vitro* potency against the challenge virus. All animals ultimately achieved undetectable viral loads on ART. Analysis of proviral DNA levels in lymph nodes on day 17 showed comparable declines across the groups, with one exception: those given dual bNAbs showed significantly less proviral DNA in naïve $CD4^+$ T cells. There is a caveat, however. Bolton pointed out that the bNAbs used in the study were human so their Fc effector functions may not have been fully intact in macaques, which could lead to an underestimation of their impact. The RV 398 trial should reveal if superior activity is achievable in HIV-infected individuals.

James Whitney, assistant professor of medicine at Beth Israel Deaconess Medical Center and Harvard Medical School, delivered the cure-related presentation that garnered the most attention at the conference. Whitney debuted results from a macaque study designed to evaluate whether a toll-like receptor (TLR)7 agonist could reverse viral latency. TLRs are innate immune receptors involved in the recognition of pathogens and stimulation of several TLRs has been shown to activate latent HIV *in vitro*.

Ten macaques were infected intra-rectally with SIVmac251 and, 65 days later, started on a daily ART regimen of tenofovir, emtricitabine, and dolutegravir. At day 320 post-infection, four animals were administered escalating doses of the TLR7 agonist every other week (0.1mg/kg, 0.2mg/kg, and 0.3mg/kg) with the highest dose repeated four additional times. The other six animals received placebo. Receipt of the TLR7 agonist was associated with transient activation of both NK and $CD8^+$ T cells, and there was some evidence of low-level $CD4^+$ T-cell activation. Viral loads remained undetectable until after the second 0.3mg/kg dose, at which time two of the four macaques displayed transient viral load blips. The three subsequent TLR7 agonist doses prompted consistent blips in the two-log to three-log range in all four animals. Measurements of proviral DNA showed modest but significant reductions, including in the lymph nodes and colons in three out of the four macaques. No significant changes in any of the measurements occurred in the

continued on page 19

The Trimer TRANSFORMED

A sculptor fixes her gaze on HIV vaccine research for an exhibit called *The Art of Saving a Life*, one of many examples of how contemporary art is engaging with science

By Michael Dumiak

Robin Shattock had never been asked quite this way about his work until last summer, when the Imperial College molecular biologist, HIV vaccine researcher, and mucosal infection and immunity expert found himself on the phone with Katharine Dowson, a sculptor based in south London. Shattock was explaining to her the intricacies of the structure of HIV's shadowy envelope protein.

"It was challenging to think about how to communicate the complexity of the science in a visually appealing way," Shattock recalls. "We now have these exquisite, finely detailed structural models of the [HIV] envelope protein. It's a beautiful looking structure," he says. "We were trying to give some sort of artistic representation to the complexity but also the beauty of the problem we are trying to solve." The problem Shattock and scores of other researchers are working on is developing vaccine candidates that can induce antibodies which can attack and eliminate HIV.

"We know where these broadly neutralizing antibodies interact with this [HIV Envelope] structure. What we don't know is how to present that to the immune system in order to reliably induce a broadly neutralizing response," Shattock says.

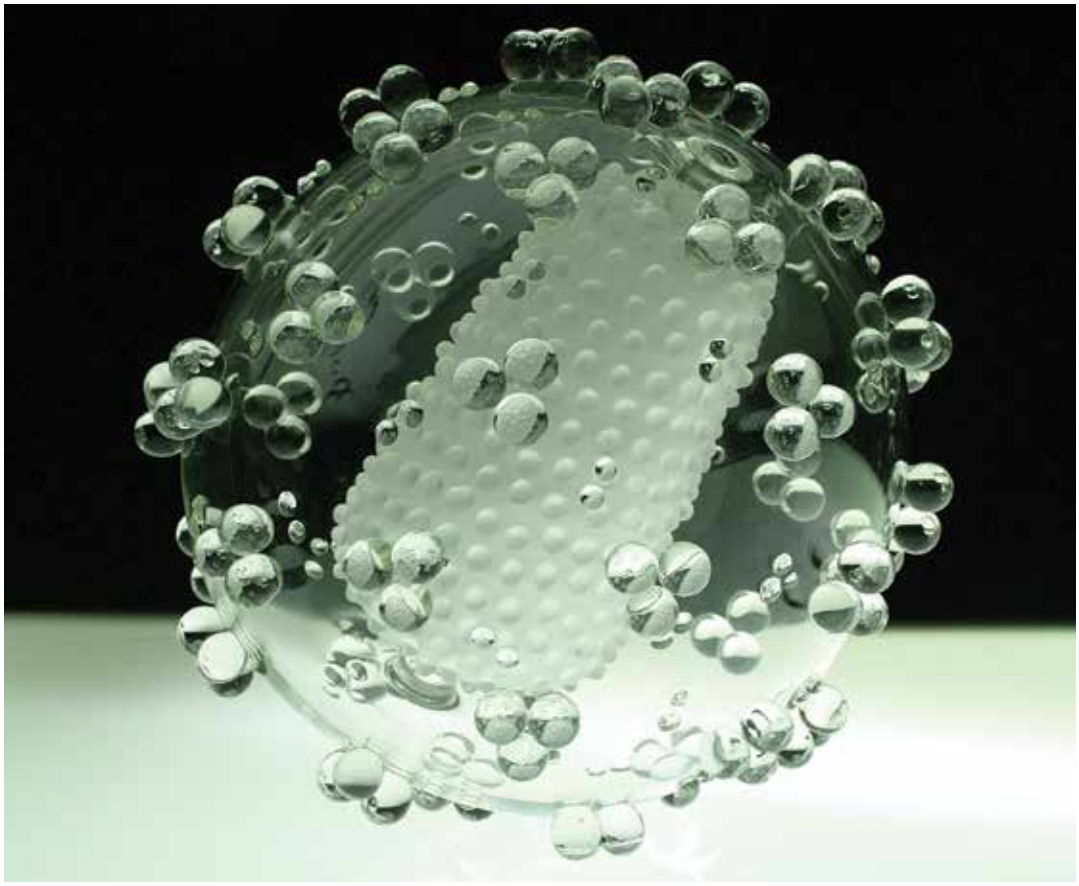
Dowson, who first started blowing glass sculptures of stomach linings in a rented Paris studio some 25 years ago, uses glass and light as her primary media in creating works that often draw on science for inspiration and increasingly rely on collaboration with researchers. Her works include a laser etching inside a glass brick of a cousin's cerebral venous malformation and a 3D model of Dowson's own brain.

Together, she and Shattock plotted the steps that led Dowson to casting *A Window to the Future of an HIV Vaccine*, a work consisting of eight separate scored, polished crystal blocks that when placed against one another in a certain way reveal the ephemeral shape of the trimeric HIV envelope protein, which was etched by laser inside. The lethal virus seems to be a floating cotton wisp. The sculpture is not displayed in order. One block placed to the side symbolizes the as-yet-unsolved puzzle of developing a vaccine against HIV.

Dowson's work became one of the standout pieces of a Bill & Melinda Gates Foundation-sponsored campaign called *The Art of Saving a Life* (http://artofsavingalife.com), which invited

Luke Jerram
Pathogens in glass

"With a bit of color, you can make a virus look incredibly dangerous, or incredibly toxic, or incredibly beautiful," says the Bristol, UK, multimedia artist. "You find out very quickly that viruses don't actually have any color." Jerram produces his millions-of-times oversized viral sculptures using translucent glass.
Photos by Luke Jerram.



HIV, lipid membrane, glycoprotein, and capsid

Katharine Dowson
Window to the future of an HIV vaccine

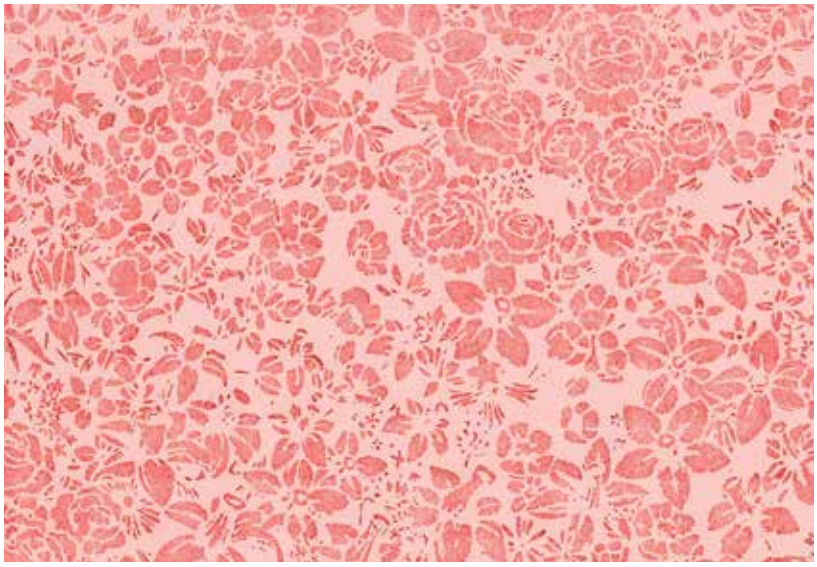
"They look a lot like flints," Dowson says about the HIV trimer models she studied for producing her crystal-block sculpture in the exhibit *The Art of Saving a Life*. "They are very hard." She continues to work with the trimer as a subject for future work. "I'm sketching them. I'm drawing them. It's in my head."

Image courtesy of the Bill & Melinda Gates Foundation.

Methicillin-resistant Staphylococcus aureus

Detail from Papilloma virus

Two viral spikes on a crystal Adenovirus



Vik Muniz

Flowers—The Beauty of Vaccines

With *Flowers — The Beauty of Vaccines*, the Brazilian artist collaborated with MIT synthetic biologist Tal Danino to make a large-scale, digitally-colored print of the microscopic image of liver cells infected with vaccinia virus. "You look at something that you are completely familiar with, that you think you've seen before," he says. "Halfway through the process you realize you're looking at something completely different."
©Vik Muniz / Image courtesy of the Bill & Melinda Gates Foundation.

With *Vaccines as Love Serum*, sculptor Mauro Peruchetti uses gummi-like pigmented resin to bring a smile to children (and reduce fear of needles).

Image courtesy of the Bill & Melinda Gates Foundation.

artists to tell stories about vaccination. *Saving a Life* includes conceptual and decorative work, text, film, music, and illustrative and abstract art; 38 pieces in all. A brief exhibition of these pieces accompanied a donor's conference held in Berlin this January for Gavi, The Vaccine Alliance, an organization that aims to distribute vaccines to children in the poorest countries of the world.

To create *A Window to the Future of an HIV Vaccine*, Dowson met Shattock's team at the red-bricked St. Mary's Hospital campus of Imperial College in London. The arches and columns and Victorian lobby give way to a modern research lab where Dowson consulted with Shattock and his team, pored over the recent scientific literature, and above all observed the HIV protein structures that team members were showing her on screens around the lab. Shattock's team contacted Andrew Ward and Ian Wilson at The Scripps Research Institute in La Jolla. The two had published the molecular crystal structure of an HIV trimer structure, dubbed BG 505 SOSIP.664, in complex with a neutralizing antibody. For researchers, solving this structure allows detailed modeling of the trimer and mapping of the sites on this structure that remain constant — and are therefore most vulnerable to neutralizing antibodies. It's all done in the hope that this information will aid in the design of new vaccine candidates able to present these sites in a manner that can preferentially elicit broadly neutralizing antibodies. It was a dazzling array of visual information and complexity for Dowson to absorb. "You've got to edit it down and pare it down until you get the absolute essence. You have to grapple to make it simple," she recalls.

HIV is anything but simple. Dowson wanted to capture the many facets of the virus in her work. "It's a fragile virus in many ways, still so robust and so difficult. It's very evil, yet it's a beautiful-looking thing as well." Artists engaging with science-related material, or with scientists themselves, often wrestle with how illustrative or abstract their work should be. For Dowson, and her *Window to the Future of an HIV Vaccine*, the trimer was the perfect vessel for creating an inspired sculpture representing broader themes. By dividing her sculpture into eight pieces and using highly polished material that creates a play of reflection and transparency, she's made what could be a straightforward illustration into a shimmering, elusive, and sometimes sinister object.

Other pieces from *The Art of Saving a Life* directly reference scientists and health workers, such as Fatoumata Diabaté's *Ebola Trials* photographic essay, Mauro Peruchetti's gummi-like *Vaccine as a Love Serum* resin figures, and Vik Muniz's *Flowers—The Beauty of Vaccines*. Muniz's work is a digitally colored, wall-sized print of liver cells infected with vaccinia virus (the virus used to inoculate against smallpox). It was done in collaboration with Tal Danino, a synthetic biologist at the Massachusetts Institute of Technology (MIT).

Exploration of the relationship between science and art goes back to Da Vinci and Darwin. But Arthur I. Miller argues that a new kind of art is now emerging. His recent book *Colliding Worlds* illustrates the evolving relationship between science and art over the course of 80 interviews—including one with Dowson. "The artists I considered for my book are artists whose work can reflect back on science. These are artists who collaborate with scientists; artists who know a lot of science, who are willing to read the conceptual part of science."

Miller, a Bronx native with a physics doctorate from MIT, founded the University College of London's department of science and technology studies. His interest in art and science was first sparked by attending *9 Evenings: Theater and Engineering* in 1966, which featured Billy Klüver of Bell Labs and Robert Rauschenberg. He himself collaborated with artist Fiorella Lavado in the 2010 Berlin exhibit *Weaving the Universe: from Atoms to Stars*.

In his book, Miller draws connections between the emergence of Cubism and the physics of Albert Einstein and Niels Bohr. Cubism is a highly influential break in 20th-century art, strongly associated with Pablo Picasso, which prized a radical change in technique and the use of geometry to present multiple simultaneous perspectives of a single form. Miller argues that the insights leading to some of these scientists' most groundbreaking work can be attributed to their ability to use imagery, thought experiments, and abstractions to refine and reimagine their experimental equations.

Einstein himself said the greatest scientists are artists as well. Miller points out that Neils Bohr read an influential book on cubist theory, *Du Cubisme*, which inspired the physicist to imagine an electron as both a particle and a wave. The resolved form depends on when and from what perspective it is viewed.

The relationship works the other way too. Scientific research is providing a well of inspiration for artists. London gallerist Robert Devcic cultivates this at his hub for art and science, GV Art. The gallery features several artists who collaborate with science as part of their everyday practice, including David Marron, who's also a paramedic, and Helen Pynor, who was once an aspiring molecular biologist. Dowson's brain scan and heart sculpture is now displayed in the GV Art window.

Devcic's love of science leads back to his boyhood love of fossils; his idea of a good collaboration is when researchers and artists go on a creative journey, getting to know one another without a pre-determined outcome. In his view, this is preferable to work that places the artist in position of being a transcriber. Miller and Devcic both touch on what is a sometimes sore nerve: Miller, who says sometimes all the attention goes to the art and not to the science that helped bring it into being, and Devcic, who says art should not be merely illustrating science. "Our artists investigate and pursue knowledge through the sciences with meaningful collaborations," Devcic says, "What we do not do is encourage our artists to be used by science organizations as a tool for communicating."

The UK-based Wellcome Trust is familiar with this tension, given its nearly 20 years of formal promotion of arts and science collaboration. In 1996 the Wellcome Trust launched SciArt, a decade-long grants program that jointly supported scientists and artists on projects such as Helen Storey's *Primitive Streak*, a fashion and design collection illustrating the first 1,000 hours of life, and the painter Mark Quinn's *Molecular Gaze*, a portrait of geneticist John Sulston using DNA extracted from Sulston's semen and grown in agar.

The SciArt initiative began with the idea that the collaboration would spur new data as part of the project, but this proved hard to maintain. The SciArt program therefore wound down in favor of art awards with a broader remit, says David Cahill Roots, a former theater producer and now senior arts adviser at Wellcome.

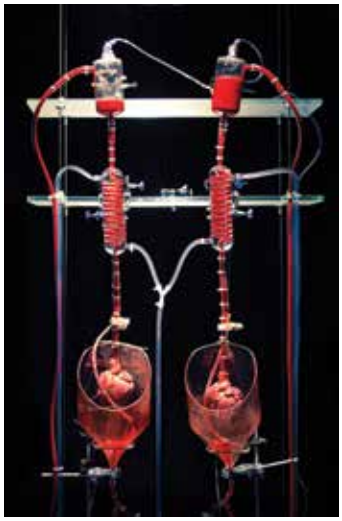
Wellcome has also supported many artists by sponsoring shows and, as with Bristol-based artist Luke Jerram, purchasing work for the trust's growing collection. Jerram brings a unique perspective as a colorblind artist who does not formally collaborate with scientists, though he does closely consult with them. He works with scientific glassblowers to produce large-scale glass replicas of pathogens, including several of HIV and of the H5N1 strain of the influenza virus. The sculptures are close representations of viruses, made millions of times larger. Given Jerram's (colorblind) view, however, these sculptures are created of clear glass without the tint or dye often given to scientific illustrations.

Wellcome is not the only science grant funder with an artistic bent, and is far from alone as a scientific institution promoting collaboration with the art world—not just for raising public awareness of science, but because many researchers say they see a benefit in working with artists. Wellcome's peers include Champalimaud Neuroscience in Lisbon, the University of Western Australia's SymboticA lab (from where Devcic first heard of Helen Pynor), and the Max Planck Society in Germany. Devcic is currently working on developing a project with the Max Planck Institute of Evolutionary Anthropology studying rest in human behavior, and one of the most active Max Planck organizations in the arts is in Dresden's Institute for Molecular Cell Biology.

Pynor, the former biologist, is collaborating with molecular biologist Jochen Rink, whose group works with planarian flatworms and pluripotent stem cells to study tissue formation and regeneration. Rink and Pynor's work represents a deeper than normal collaboration—Pynor is spending many lab hours culturing cells to be used in the project. Together, the artist and scientists are extracting living fibroblast cells from chicken meat from the supermarket—and if that is successful, to see whether they can convert those into lines of living stem cells. Pynor will later use the project as part of a conceptual artwork about the permeable lines between life and death.

It's Rink's first time working with an artist. He's getting the kind of experience Dresden's Institute of Molecular and Cell Biology finds valuable. "It's great to have discussions comparing the scientific method to the artistic method," he says. "I am doing this for my own personal curiosity. I like working with Helen simply because of the different frame of thought. It is stimulating, and I am curious to see how Helen will turn a scientific result into a piece of art." ■

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



In *The Body is a Big Place*, Helen Pynor collaborated with Peta Clancy in using perfusion and pig hearts to illustrate the permeability of life and death.

***The Body is a Big Place*; 2011 Helen Pynor and Peta Clancy; photo by Geordie Cargill; image courtesy of Helen Pynor.**

Opening the *ENVELOPE*

The premier gathering of HIV vaccine researchers showcased a healthy dose of progress in understanding HIV's structure and how it can be utilized to engineer better vaccine candidates

By Yegor Voronin and Noah Sather

***Yegor Voronin** is senior science officer at the Global HIV Vaccine Enterprise.*

***Noah Sather** is an assistant professor at the Seattle Biomedical Research Institute.*

"Three atomic-level structures of HIV envelope were released over the past two years," said Peter Kwong of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID). "At this meeting, I've already seen six new trimer structures, and the meeting is barely half over."

The meeting was the Keystone Symposium on HIV Vaccines, which took place March 22-27 in Banff, Canada, and, for the record, Peter counted a total of 10 new HIV envelope (Env) structures that were unveiled there, reflecting the rapidly accelerating pace of progress in defining the structure of HIV's outer surface protein. This sentiment extended to the whole meeting, as several directions of HIV vaccine research, none of which are completely new, showed spectacular progress in the recent months.

Epitope mapping reveals surprises

New structures of Env illuminate how the genetic diversity of HIV results in the diversity of protein structures, which allows the virus to efficiently evade the immune system. They also reveal the conserved motifs that are targeted by antibodies capable of neutralizing a broad swath of HIV isolates (so-called broadly neutralizing antibodies or bNAbs). At Keystone, Pamela Bjorkman from the California Institute of Technology presented data showing that an antibody referred to as 8ANC195 binds to HIV Env in a manner different from all previously identified bNAbs. Although initial analysis showed that 8ANC195 competes with antibodies targeting the CD4 binding site (CD4bs) on Env, crystal structures revealed that it actually binds to a distinct region nearby and that its epitope spans the gp120 and gp41 subunits of Env.

How bNAbs bind is also important. The antibody N6, which was discovered in Mark Connors' laboratory at NIAID and presented on at the meeting by Jinghe Huang, binds to the well-known CD4bs epitope; however, the way this antibody binds is unique. The heavy chain of N6 interacts with the epitope in a very flexible manner that tolerates viral variability and the light chain moves aside, allowing the heavy chain to bind in a unobstructed way that increases the antibody's neutralization breadth and potency.

As multiple research groups continue to isolate bNAbs and decipher how they bind to HIV, the view of the Env protein is radically transforming. Previously researchers believed that there were just three or four specific sites on the surface of Env targeted by bNAbs in a very specific manner. But it turns out the vast majority of the Env surface is targeted by one bNAb or another (see Figure, p. 18). This is great news for researchers who are attempting to elicit bNAbs via vaccination because it provides multiple options for designing immunogens. The challenge now is focusing the immune response on these conserved regions and not the variable parts of the virus.

From structure to immunogen

A better understanding of Env's structure dovetails with efforts to create better recombinant immunogens that mimic the trimeric shape in which Env

exists on the surface of viral particles. Traditional approaches to create soluble Env trimers resulted in immunogens that had little resemblance to the native structure. Over 15 years ago, John Moore and colleagues began working on an approach to create an immunogen that more faithfully reproduces the native trimer. They preserved the natural cleavage site between the gp120 and gp41 subunits of HIV Env, but introduced mutations that generally stabilized the trimeric structure and linked the monomers with di-sulfide bonds. The resulting protein is referred to as BG505 SOSIP.664 (see *CROI: Progress on Prevention and Cure*, *IAVI Report*, Vol. 18, Issue 1, 2014). Because this method of trimerization creates Env trimers that adopt native-like conformation, it enabled elucidation of the many trimer structures seen at this year's meeting and is leading to unprecedented insight into Env's structure.

Until recently, only a very small number of Env proteins were sufficiently stabilized using the SOSIP approach, but as reported by Gordon Joyce of NIAID, this is no longer the case. Joyce and colleagues applied a design approach based on the SOSIP trimerization method to approximately 160 different Envs from various clades and identified approximately 40 that formed stable native-like trimers. Most of the new trimers are clade C, but clades A and B were also represented in the panel. These new SOSIP trimers give a more broad representation of the global Env diversity and have the potential to provide valuable insights into the structural differences among the major clades of the virus and how these differences are overcome by bNAbs.

Somewhat ironically, and indicative of how rapidly the field is progressing, just when the SOSIP approach begins to gain momentum and bear fruit, some researchers are already working on various ways to improve the SOSIP technology. As explained by Rich Wyatt of IAVI's Neutralizing Antibody Center at The Scripps Research Institute (TSRI), one promising approach is to create native flexibly-linked (NFL) trimers, in which the cleavage site between gp120 and gp41 subunits is replaced by a flexible amino acid linker and the trimer structure is stabilized by modification of a small number of critical amino acid residues. Javier Guenaga, a member of Wyatt's laboratory, reported on recent progress in optimization of the amino acid sequence of Env to promote formation of stable NFL trimers. The resulting trimer was shown by negative stain electron microscopy (EM) studies to adopt a well-ordered, native-like structure and was found to bind only bNAbs and not the undesirable narrowly neutralizing antibodies. Although they are still in the early developmental phase, NFL trimers are thought to have several desirable attributes—this approach has been successfully applied to Envs from both clades A and B, simplifies production methods, and increases yields during manufacturing.

Regardless of whether they were created by SOSIP or NFL technology, the native-like trimers have a number of potential uses. They can be complexed with bNAbs to study the fine details of Env-antibody interactions using crystallography or EM, or be used as reagents to fish out antibodies that specifically recognize the native Env conformation. Some researchers also expect that they will be vastly improved immunogens, presenting to the immune system a biologically relevant target that is even more stable than the protein on the surface of the virion. So far immunogenicity data have only been presented for the BG505 SOSIP. Joyce Hu from the laboratory of Shane Crotty at the La Jolla Institute for Allergy and Immunology, reported that BG505 was poorly immunogenic in mice, generating antibodies able to neutralize tier-1 viruses (the easiest viruses to neutralize), but not the harder to neutralize tier-2 viruses. This disappointing result may be specific to mice, which have a B-cell receptor (BCR) repertoire that appears to have difficulty targeting highly-glycosylated epitopes.

Data presented by Rogier Sanders of the Weill Medical College of Cornell University showed that immunization of rabbits and macaques with BG505 SOSIP trimers resulted in potent neutralization of tier-2 autologous virus (virus that bears the un-modified BG505 Env that was used to create the SOSIP trimer). Sanders says that previous vaccine approaches have not induced meaningful neutralization against tier-2 viruses, what he refers to as "a level of neutralization that would protect against a robust virus challenge." He also stressed that the appearance of homologous neutralization is a promising first step, which needs to be followed by additional immunizations designed to increase the breadth of the response.

Engaged ancestors

One of the major findings from the research on the appearance of bNAbs in HIV-infected individuals is that most bNAbs go through an unusually large number of rounds of mutation and selection in germinal centers. As a result of this process, the antibodies become quite different from the so-called unmutated common ancestor (UCA) or germline antibody. Recently several groups showed that previous Env immunogens bound the germline forms of bNAbs very poorly, possibly explaining why vaccine strate-

HIV Envelope

The model of HIV Envelope, showing in various colors footprints of broadly neutralizing antibodies (bNAbs) on one of the monomers in the trimer (top image, side view; bottom image, top view). As researchers discover more bNAbs, new sites of vulnerability are being discovered.

Figure courtesy of Gabriel Ozorowski of The Scripps Research Institute.

gies to date haven't induced neutralizing antibodies with broad specificity. Some Env proteins will bind the germline versions of bNAbs if certain N-linked glycosylation sites are altered (*J. Exp. Med.* **210** (**4**), 655, 2013), but this was limited to mostly gp140 fused Env trimers and engineered outer domain (eOD) Env proteins, and was only observed for germline versions of VRC01-class bNAbs.

At Keystone, J.M. Medina-Ramirez, a member of Sanders' other laboratory at the University of Amsterdam, presented data on the design and characterization of a native-like trimer that is able to engage germline antibodies. Using knowledge gained from antibody function studies and crystal structure analyses, Medina-Ramirez and colleagues selected 18 amino acid changes that were predicted to allow the well-characterized BG505 SOSIP.664 trimer to bind germline antibodies. This modified trimer engaged multiple bNAb germline antibodies, including precursors of four different bNAbs that target the V1/V2 loops and three that target the CD4bs region of Env. Additionally, they confirmed that this germline-optimized BG505 SOSIP.664 was able to stimulate the germline version of the anti-CD4bs bNAb VRC01 in its membrane-bound form (as a BCR) similar to what would be displayed on the surface of a B cell. Stimulating the germline BCR is a critical first step for antibody development. Medina-Ramirez says this is therefore a suitable protein immunogen for trying to initiate the first steps in eliciting bNAbs by vaccination.

Leonidas Stamatatos, one of the meeting's organizers, also reported progress in the design of germline-optimized immunogens from his work at the Fred Hutchinson Cancer Research Center. Previously his group created a more classical Env construct (a fused trimeric gp140), which despite not being a native-like trimer, was capable of binding the germline version of VRC01. Now, he's developed highly modified versions of this construct in which a portion of the variable loops (V1-V3) was removed and targeted mutations were introduced to expand its binding to almost all known germline VRC01 antibodies. Stamatatos highlighted that interaction between a BCR created from one such germline precursor and the germline-targeting immunogen that they developed was shown in a transgenic mouse, a more relevant model than *in vitro* studies.

Bart Haynes of the Duke University Medical Center discussed another germline targeting approach—the idea of mimicking the natural process of bNAb development. In contrast to rational engineering of Env immunogens with the goal of optimizing binding to germline bNAb precursors, this approach relies on identifying the naturally occurring HIV Envs to stimulate development of bNAbs. This approach is feasible because of the detailed investigation of co-evolution of antibodies and viruses in HIV-infected individuals that develop bNAbs. Haynes and colleagues identified the antibody germline precursor stimulated during natural infection and also identified the stepwise mutational changes that the initial germline version underwent during the affinity maturation process. They also tracked the corresponding changes that occurred in Env sequences as the virus escaped the antibody responses. This detailed examination allows identification of the variants of Env that drive antibody maturation from the initial ancestor, through various intermediates, to the final bNAb. By creating immunogens that correspond to these key Env variants, Haynes and colleagues aim to recapitulate the natural development of bNAbs via vaccination.

Efforts to test these immunization strategies in animal models are already underway and some of the first results in rhesus macaques were presented at the conference. Haynes and Larry Liao from the Duke University Medical Center presented immunization studies with Env variants from a volunteer that eventually developed antibody CH103, which targets the CD4bs. The virus that established infection in this volunteer carried the Env that was able to bind the germline version of the CH103 antibody. In addition to that Env, researchers also created immunogens based on Envs found at weeks 52, 78, and 100, because their analysis suggested that those Envs played an important role in driving the development of the CH103 bNAb. They tested the Env from the infecting virus on its own and in combination with the later versions of Env, which were either combined as a mixture or administered sequentially one after the other. All three approaches resulted in appearance of antibody intermediates at initial stages of CD4bs bNAb lineages, which was an encouraging finding. In macaques immunized sequentially with these Envs, neutralization assays showed appearance of more potent and broad antibodies than in the other two groups, validating the overall approach of attempting to mimic the natural evolution of antibody maturation. However, neutralization titers in plasma were not very high and, therefore, more work is needed to improve upon this initial success.

Joe Jardine, a member of William Schief's laboratory at TSRI, presented another exciting study involving germline targeting immunization strategies. In an effort to target the germline precursors of VRC01-class antibodies, they have developed the eOD-GT8 60mer—a self-assembling nanoparticle

carrying an engineered form of the outer domain of gp120. This protein particle is able to bind germline antibodies that express VH1-2*02, the heavy chain gene that is predominantly used by the human immune system to generate VRC01-like antibodies. To directly explore whether this immunogen could drive the evolution of a VRC01-like antibody by vaccination, their collaborator David Nemazee at TSRI created a transgenic mouse carrying just the heavy chain of the germline version of VRC01. The light chain of the germline antibody is not provided, so the antibodies produced in these animals consist of the germline VRC01 heavy chain and various light chains coming from the natural mouse repertoire. After immunization with the eOD-GT8 60mer, two important observations were made. First, a large number of antibodies appeared with light chains that have just five amino acids in the CDR-L3 loop, despite the fact that such short light chains are extremely rare in mice. VRC01-like antibodies require a light chain that has such a short CDR-L3 loop, so this result bodes well for plans to elicit such antibodies in humans. Second, in response to vaccination the heavy chain of the germline antibody was undergoing somatic hypermutation and many of the mutations that appeared were identical to mutations found in VRC01, indicating that antibody maturation followed the same pattern that led to the appearance of VRC01 in the HIV-infected individuals from which it was isolated. While the transgenic mouse system is quite artificial, these results indicate that immunization with the eOD-GT8 60mer can lead to selection of the necessary heavy and light chains and drive antibody evolution. Together with Liao's findings, these studies strongly suggest that engaging germline versions of antibodies and guiding them towards a desired specificity is a feasible approach.

Next frontiers

As immunization studies with native-like trimers and germline-optimized immunogens get underway, investigators are focusing on closely monitoring humoral immune responses to vaccines using recent advances in high-throughput technologies, such as next generation sequencing and B-cell cloning. These approaches allow almost real-time evaluation of vaccines at the sequence population level. The goal of such studies is to gather information that may guide development of better immunogens or immunization strategies.

Gunilla Karlsson-Hedestam of the Karolinska Institutet reported on her laboratory's progress in defining anti-Env vaccine-specific immune responses in nonhuman primate studies. The researchers begin by using cell sorting techniques to separate B cells expressing anti-Env antibodies. The heavy and the light chain genes from single B cells are cloned and used to produce antibodies that are tested for binding to and neutralization of HIV. Epitope mapping techniques provide information on the regions of Env trimers that were targeted by these antibodies, thus giving the researchers a clear picture of the immunogenic properties of the tested Env. In parallel, they leverage high throughput Illumina next generation sequencing to study heavy chain genes of the entire population of anti-Env B cells. This approach provides minute details on the types of B-cell receptors being stimulated by the vaccine being tested and on the levels of antibody maturation that they are able to achieve, allowing researchers to monitor and evaluate vaccine-induced antibody responses in unprecedented detail. ■

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placebo group. When ART was interrupted, there was no difference in the kinetics of viral rebound between the groups, but set point viral loads were significantly lower in TLR7 agonist recipients.

Whitney explained that the TLR7 agonist used in the study is an analog GS-9620, a Gilead Sciences compound already in clinical development for hepatitis B and C infection. Early phase human trials have found GS-9620 to be safe and a study in people with HIV on ART has now been launched. GS-9620 also successfully activated latent HIV in $CD4^+$ T cells isolated from HIV-infected individuals (abstract #417). Results from the clinical trial are eagerly anticipated.

The increasing enthusiasm for cure research remained evident at CROI, but John Mellors, director of the HIV/AIDS Program at the University of Pittsburgh, sought to quell any misconceptions about immediate prospects. "The reality is that progress will be slow, and will grind out over years to decades until we have a functional cure for a significant fraction of HIV-infected individuals—there are many pieces to the puzzle that need be put together to solve the problem." ■

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Upcoming HIV-Related Meetings

MAY 2015

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

JUNE 2015

3rd International Conference on HIV Prevention & Infection Control
June 16-19; Geneva, Switzerland
More information: icpic.com/index.php/conferences/icpic-2015

JULY 2015

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

SEPTEMBER 2015

US Conference on AIDS
September 10-13; Washington, DC
More information: nmac.org/2015usca

World STI & HIV Congress
September 13-16; Brisbane, Australia
More information: www.worldsti2015.com/ehome/index.php?eventid=91027&

OCTOBER 2015

15th European AIDS Conference
October 21-24; Barcelona, Spain
More information: www.eacsociety.org/conferences/eacs-conference/conference.html

NOVEMBER 2015

International Conference on AIDS & STI in Africa (ICASA)
November 22-27; Tunisia
More information: icasa2015tunisia.org

3rd International Conference on HIV/AIDS, STDs & STIs
November 30-December 2; Atlanta, GA
More information: hiv-aids-std.conferenceseries.com

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