Bangkok
After RV144

Plus:
PrEP in the real world
A good deal has changed since US and Thai researchers showed the world in 2009 that HIV can be prevented by vaccination, fueling much excitement and follow-up study. The field has, for one thing, made considerable headway in unraveling the immunology of the modest protection observed in that trial. Meanwhile, other new preventive tools—such as pre-exposure prophylaxis (PrEP) and microbicides—have come into their own and irrevocably altered the landscape of AIDS vaccine development. Cure research has surged in parallel, bringing a new kind of hope to those who were certain they’d live out their lives with HIV.

We touch on many of these developments in this issue of IAVI Report. Our lead story profiles the HIV vaccine research unit of the Armed Forces Research Institute of Medical Sciences in Bangkok, which conducted the Thai trial. It covers what vaccinologists there are doing now to build on the nominal protection observed in the landmark study. The story also details how research in Thailand into acute infection and early treatment is advancing progress toward a functional cure for HIV.

Our other feature explores whether PrEP, now a proven method of HIV prevention, can be designed to work in the real world. Perhaps the biggest barrier to this goal is adherence—getting uninfected people at high risk for HIV infection to take pills that will mitigate that risk, and to take them every day for a long, long time. The feature describes how researchers hope to overcome this rather steep challenge in a number of PrEP demonstration projects that are planned or already underway.

Which brings us to Regina McEnery, the author of that report. After more than five years writing for IAVI Report and its sister publication, VAX, Regina has, for reasons we cannot fathom, up and left us to live in Massachusetts. We already miss her, but she has promised to stay in touch—and to keep reading us online.

We think this is an excellent idea. In fact, we think it’s so good that we’d like to encourage everyone to do the same. So, please, visit IAVIReport.org, and come back frequently. Like us on Facebook. Tweet our blogs. Tell your mom, dad, coffee guy, and anyone else you can think of to read us online. Seriously. We like putting out this report and would like to know that people know we’re here. So, please, follow Regina’s lead and do stay in touch.

– UNMESH KHER
Bangkok After RV144
The Thai capital already has one of the largest collections of samples from HIV vaccine trials. Now, it is also becoming one of the best places to study acute infection.

Preparing for PrEP
We know it works. But how do you get people to take it?

Research Briefs
Researchers solve high-resolution structure of HIV co-receptor CCR5.

[ ON THE COVER ]
This image captures the predominant movement of an HIV-1 gp120 protein and the attached sugar chains during 30 nanoseconds. It was calculated by a computer simulation of the interactions of all of the 7,500 atoms and roughly 320,000 water molecules that surround this gp120 protein. The gold ribbon surface illustrates the range of movement for the protein with the outer extremes in red. The gold dotted surface illustrates the range of movement for the sugars (red sticks). The image was generated as part of a project aimed at understanding the effect of sugars on the dynamics of the gp120 protein and in particular the V3 loop, which can be seen extending downwards at the bottom. The focus on the V3 loop is due to its important role in determining whether a specific virus uses the CXCR4 or CCR5 chemokine receptor for cell entry.

Image courtesy of Natasha Wood, South African National Bioinformatics Institute, University of the Western Cape.
The Thai capital already has one of the largest collections of samples from HIV vaccine trials. Now, it is also becoming one of the best places to study acute infection.

By Andreas von Bubnoff

A droning hum permeates the squeaky-clean sample archiving room on the ground floor of a brand-new laboratory of the Retrovirology Department of the Armed Forces Research Institute of Medical Sciences (AFRIMS) in central Bangkok. This white noise is a byproduct of dozens of freezers that contain one of largest and arguably most valuable collections of blood and tissue samples from HIV vaccine trials conducted in Thailand.

Many of the 1.2 million samples, stored since the early 1990s, were taken from participants in the RV144 trial, which was completed in Thailand in 2009 and demonstrated that HIV could be prevented by vaccination. The observed efficacy was, at 31.2%, nominal, but some of the samples it yielded may yet have a great deal to contribute to HIV vaccine design. “This is a national treasure,” says Nicos Karasavva, the assistant chief of the Department of Retrovirology at AFRIMS, which conducted the RV144 trial and is a joint collaboration between the US Army and the Royal Thai Army. “These samples are shared internationally by many, many investigators.”

The sample archiving room is part of the new “HIV Vaccine Research Center of Excellence” laboratories located about 300 meters from AFRIMS headquarters in Bangkok. They are built out of a former warehouse, where the Royal Thai Army Medical Department used to store medical supplies and equipment.

The new labs are emblematic of the prominence Thailand—and Bangkok in particular—has attained in the competitive world of HIV vaccine research. That prominence isn’t likely to be fleeting: In addition to analysis of RV144, AFRIMS researchers are busy designing and testing modified vaccine regimens that build on RV144 and, in the next few years, hope to begin a follow-up efficacy trial in a cohort of men who have sex with men (MSM) in Thailand. More recently, the city has also made a mark on HIV cure research: Thailand’s largest HIV testing facility in downtown Bangkok, just about two miles away, is becoming one of the best places in the world to study acutely infected people.

Building on RV144

AFRIMS is perhaps best known for its contributions to HIV vaccine development, and its researchers continue to parse the immunology of RV144’s success. Beyond that, they are conducting trials—such as RV305 and RV306—to better understand the immune responses observed in
RV144 so that they can improve and prolong those responses (see VAX July 2013 Primer on Understanding the P5 Partnership).

They have completed vaccinating participants in RV305, in which 162 RV144 vaccine recipients got a boost with the same vaccine components used in the original trial—the canarypox-vector-based ALVAC-HIV and the AIDSVAX B/E gp120 protein)—either alone or in combination. The goal is to amplify existing immune responses to identify which ones correspond to each part of the vaccine regimen. Enrollment is about to begin in RV306, a 360-person trial that replicates RV144 in unvaccinated volunteers, who will also get an additional boost of ALVAC, AIDSVAX, or both six months after the last vaccination. In both RV305 and RV306, the researchers will also study immune responses in mucosal tissues and secretions, which wasn’t possible in RV144 because mucosal samples weren’t collected in that trial.

Karasavva says he doesn’t do too much hands-on research anymore. But he has been keeping his hands busy on one set of experiments, in which he uses a microscope to track the movement of fluorescently labeled HIV particles. He hopes to see if HIV moves slower in semen, vaginal or rectal secretions taken from vaccine recipients in trials including RV305, than in secretions taken from placebo recipients. If it does, then that could mean that vaccine-induced HIV-binding antibodies are dragging on the virus, slowing its passive movement toward target cells in the mucosa.

The work is part of a collaboration Karasavva has going with Tom Hope from Northwestern University. The goal is to see whether antibody-mediated trapping of viral particles in mucus might have played a role in the protection observed in RV144, by slowing down or immobilizing HIV. The hypothesis is interesting because the antibodies wouldn’t necessarily have to be neutralizing for this kind of protection to work (see Protection without neutralization?, IAVI Report Blog, Feb. 14, 2013).

Catching them early

While AFRIMS is perhaps best known in HIV research circles for its contributions to HIV vaccine development, its staff also participates in acute HIV infection studies. One example is RV217, a US Military HIV Research Program study in East Africa and Pattaya, Thailand, that follows acutely infected people from just a few days after infection.

AFRIMS also collaborates on a study of a cohort of acutely infected people in Bangkok called RV254. The members of this cohort are mostly identified at the “Anonymous Clinic,” perhaps one of the best places in the world to identify, and study, acutely infected people: Last year, close to one fifth of all new HIV infections recorded in Thailand were identified at the clinic. Some 1,500 of the 15,000 people who came there tested positive, making the Thai Red Cross AIDS Research Centre (TRCARC), which runs the clinic, the largest HIV testing center in Thailand—a country of over 65 million people.

The clinic’s central location in downtown Bangkok is partly what makes it such a hub for HIV testing. The clinic is called “Anonymous” because, when it was founded in 1991, testing places asked for the names of all those tested and were required to report anyone testing positive to the government. The “Anonymous Clinic” was the first clinic in Asia where people could get tested without giving their names, says Nittaya Phanuphak, one of the leading investigators at the clinic and the deputy director of SEARCH, an HIV/AIDS research partnership that includes TRCARC and AFRIMS.

The government no longer requires the reporting of positive cases. Still, Thai citizens are only eligible for two free HIV tests per year—paid for by the government—if they provide their national ID card and number. While their names aren’t reported to the government if they test positive, their national ID numbers will still be in a health insurance database to check that they aren’t getting more than their two free annual tests. To avoid that, Phanuphak says, some of the people who come to the “Anonymous Clinic” prefer paying out of pocket.

The high volume of visitors and the presence of researchers makes the clinic an ideal locale for the study of acute infection. “We are in a good position, in the sense that we are located in downtown Bangkok [and] have access to this Anonymous Clinic, [where] a lot of people come for routine testing,” says Jintanat Ananworanich, the director at SEARCH, who studies acute infection at the clinic. What’s more, the clinic is the only place in Thailand—other than blood banks—that routinely uses real time nucleic acid testing (NAT) to detect acute HIV infection immediately after people come in. NAT measures HIV RNA in the blood and can detect infection just a few days after transmission, before any HIV-specific antibodies appear in the blood.
Ananworanich and her colleagues were the first to use NAT in Thailand in 2008 to identify 11 cases of acutely HIV infected people who were antibody negative (J. Acquir. Immune Defic. Syndr. 49, 151, 2008). That study was retrospective, which means that it involved analysis of blood samples that had been collected before.

Encouraged by these results, Ananworanich and her colleagues launched what is perhaps the largest effort to date to find acutely infected people to enroll participants in the RV254 study: Since mid-2009, they have screened every person who comes to the Anonymous Clinic for HIV testing using NAT. Speed is essential to catch as many people as possible in the earliest, antibody-negative phase, Ananworanich says, adding that clinic staff can obtain test results and enroll eligible candidates in studies in the first two days after someone walks in for testing. “We can turn things around pretty fast,” she says.

The numbers needed to find acutely infected people are huge: Out of 60,000 samples tested between mid-2009 until the end of last year, 5,500 were positive, and only 110 were in the acute infection stage. Of those, 60 were only positive in the NAT test. Exactly how long after infection patients remain antibody free is unclear; but judging from what these patients told clinic staff about when they were most likely infected, Ananworanich suspects that they had been infected for two weeks or less. The other 50 acute infection samples contained HIV-specific IgM antibodies but were still negative for IgG antibodies, which appear a little later, suggesting that they had been infected for about three to four weeks.

Ananworanich and colleagues enrolled 104 of these 110 acute cases in RV254. Almost all of the volunteers also agreed to immediate start of antiretroviral therapy (ART), which has enabled the researchers to conduct a long-term study of the effects of early treatment initiation.

**Aiming at the reservoir**

Initial results from 75 of the RV254 volunteers show that early treatment can drastically reduce the size of the HIV DNA reservoir in the blood. In the patients who started ART two weeks or less after infection, the reservoir was...
undetectable initially and remained undetectable for at least one year of treatment. Of the people who started treatment between three and four weeks after infection, half initially had a detectable reservoir, which became undetectable one year into treatment. What’s more, most of the acute treatment starters had fewer latently infected long-lived central memory CD4+ T cells than people who started treatment during chronic infection, suggesting that their reservoir was shorter lived and might be easier to eradicate.

To see if a more intense treatment regimen can reduce the reservoir even further, Ananworanich and her colleagues randomly assigned half of the RV254 volunteers to a more intensive five-drug highly active ART (HAART) regimen, as opposed to the normal three-drug HAART. So far, Ananworanich says, she hasn’t seen much of a difference in reservoir size between the two groups.

Early treatment starters in RV254 would appear to have a better chance of getting functionally cured. “The more I treat these patients and see the reservoir data, the more I’m excited that maybe this is doing something good for them,” Ananworanich says.

This is consistent with findings from the French VISCONTI cohort of so-called post-treatment controllers. These are 14 people who started treatment on average 39 days after infection and controlled the virus after treatment was stopped (see Is it Ever Too Early?, IAVI Report, Sep.-Oct. 2012).

To test if post-treatment control is also possible in the RV254 volunteers, Ananworanich and colleagues want to combine early ART with other treatments that boost the immune system or that target the reservoir to see if this results in a functional cure. Their plan is to interrupt treatment to check if some can control viral load either without treatment, or after treatment with therapeutic HIV vaccines or drugs such as SAHA that activate the HIV reservoir.

**Cutting transmission**

A smaller reservoir isn’t the only advantage of early treatment. Data from RV254 also show that treatment during acute infection makes people less infectious. At the IAS conference in Kuala Lumpur earlier this year, Eugene Kroon, a clinical trial physician at the TRCARC and SEARCH, reported data from 74 of the early treatment starters in RV254 that showed that viral loads in semen and anal washes, and in some cases also in blood, were undetectable one year after treatment started. Mathematical modeling suggested this would reduce infectiousness by 80%. What’s more, volunteers reported increased use of condoms, fewer sexual partners and less unprotected intercourse, suggesting that concerns that early treatment could lead to increased risk behavior may be overblown.

The RV254 volunteers could only be offered immediate treatment because they were part of a clinical study; otherwise, the current Thai guidelines recommend starting treatment only below a CD4+ T-cell count of 350, a number that will likely change to 500 next year, in response to the new WHO guidelines, Phanuphak says. Meanwhile, the Thai government is considering an expansion of HIV testing coupled with the immediate treatment of key affected populations such as MSM who test positive (a strategy known as “test and treat”), if further studies show this is feasible and reduces infectivity without promoting risky behavior.

To see if this is the case, the Anonymous Clinic and two government-run hospitals in Thai provinces outside Bangkok have launched a test and treat demonstration project. It involves testing about 800 MSM and transgender women every six months, and offering immediate ART to anyone who tests positive, Phanuphak says. Researchers will then check their viral load in semen, and in anal and neo-vaginal secretions, and test participants for sexually transmitted diseases as a marker for risk behavior.

Phanuphak says, suggests that almost one quarter of HIV transmissions already happen during the first few weeks of infection, which could be prevented if people were diagnosed and started treatment early enough.

What’s more, Phanuphak says she has done modeling studies with a colleague at the Thai Ministry of Public Health that shows that just treating everyone in the general population won’t suffice to attain the national goal of reducing new infections from 8,000 to 3,000 per year by 2016. Rather, test and treat efforts will have to focus primarily on key affected populations, including MSM, to reach that goal.

That’s because HIV incidence is climbing fastest in MSM in Thailand. Mathematical models, Phanuphak says, predict that from 2012 to 2016, 41% of new infections will be among MSM, and that if nothing changes, half of all new infections will soon be of MSM. Consistent with that,
around 90% of the RV254 volunteers are MSM, she says, even though MSM only accounted for roughly one third of the 60,000 samples that were initially screened (another third were females, and the rest were heterosexual males.)

That’s a big change from the 1990s, when the HIV epidemic in Thailand mainly affected heterosexual people. (Indeed, RV144 was conducted in a heterosexual population.) Since then, safe sex campaigns, such as the Thai government’s 100% condom campaign, have been very successful in reducing HIV incidence in heterosexual populations. Among the most colorful of such efforts is perhaps a restaurant called “Cabbages & Condoms” in Bangkok. Founded by activist and former politician Mechai Viravaidya to raise awareness of condom use, it features lamps and life-size dolls covered with condoms. The bill comes with a condom instead of mints.

Enticing people to the test
But just getting people to get tested and treated, let alone early, is a challenge. For one thing, many health workers in Thailand still believe that one has to wait months after infection for any HIV tests to come back positive, says Phanuphak. Further, she says, many doctors are skeptical about the benefits of early treatment. Many doctors also know little about the symptoms of acute retroviral syndrome, such as fever, muscle pain, sore throat, diarrhea and, in some cases, oral ulcers or skin rash. Some of those who do know are uncomfortable about asking patients about their sexual history, Ananworanich says. “If you are a doctor practicing in Bangkok and have a young man who has these symptoms and you don’t ask about whether he is an MSM, had unprotected sex and all that, that’s a lost opportunity,” she says.

To address these issues, Phanuphak and her colleagues from the Thai Ministry of Public Health have been participating in government-funded training sessions for thousands of nurses and doctors in several Thai provinces since 2012.

Patients are also hesitant to get tested: Even though every Thai citizen can get free testing twice a year, about two thirds of the people who test positive at the clinic already have a CD4+ T-cell count of 350 or less, Phanuphak says. “I don’t think that we are really very successful at getting people at the very early stage,” she says.

Many don’t get tested because they are afraid of the social stigma of HIV infection, or for fear that they might not get a job if the results become public, Ananworanich says. Many companies in Thailand require HIV tests, and nursing students were recently expelled from nursing school after they tested positive, and some of them are now suing the school, Ananworanich says. “I think [stigmatization] prohibits people from coming for testing, because they don’t want to know,” she says.

Continued on page 14
Clinics in Southern California will, in the next few months, begin enrolling 400 volunteers for a study examining a particularly troublesome aspect of pre-exposure prophylaxis (PrEP) against HIV. PrEP trials have, of course, been done before. But the focus of the study this time around is somewhat different—reminiscent of nothing quite so much as mom begging you to, please, eat your peas. In this case, however, it won’t be moms doing the cajoling but HIV prevention researchers. The folks getting cajoled, meanwhile, will be men who have sex with men (MSM) in a pattern and manner that puts them at great risk of HIV infection.

Turns out the Southern Californian researchers won’t be the only ones doing this routine. Nearly two dozen similar studies examining adherence are either already up and running or about to start. Their ultimate aim is to figure out how to translate PrEP into a practical public health intervention. Large-scale trials have now established that PrEP using the antiretroviral drugs tenofovir and a tenofovir/emtricitabine combo called Truvada can dramatically lower the risk of transmission in high-risk men and women. But to do so, they have to be taken faithfully, and they often are not. And most studies have not been designed to vet possible solutions to that fundamental problem.

To be sure, the problem is not, well, easy-peasy. Researchers would like to make PrEP as attractive to adults as a gummy-bear multivitamin is to toddlers (and some adults). But even in the context of a clinical trial, where volunteers are monitored regularly by researchers, getting people to take pills faithfully has been an uphill battle.

The results were mixed. The international iPrEX study found, for instance, that daily Truvada lowered HIV risk among MSM by 54%. Similarly, Partners PrEP (see Treatment is Prevention, IAVI Report, July-Aug. 2011) established that a single dose of tenofovir (TDF) reduced the risk of infection by 62% among a cohort of 4,758 serodiscordant couples (SDCs), while daily Truvada reduced HIV infection risk by 73%.

Most recently, a daily dose of oral tenofovir lowered HIV incidence by as much as 50% in a study of 2,413 injection drug users in Thailand (see IDU study rounds out PrEP picture, IAVI Report Blog, June 26, 2013). Yet other studies, such as VOICE and FemPrEP (see Oral PrEP trial in women stopped early, IAVI Report Blog, April 18, 2011), which both happened to focus on high-risk heterosexual women in Africa, didn’t...
show any efficacy. (VOICE evaluated topical and oral tenofovir as well as Truvada, while FemPrEP only focused on Truvada.)

In all of these studies, adherence was found to be the primary—perhaps sole—determinant of PrEP efficacy. “We must never forget that PrEP is not just a pill,” says Mitchell Warren, executive director of AVAC, the HIV prevention advocacy group based in New York. “It is a program, a new intervention that includes an antiretroviral pill. We know the pill can work. But we have not systematically designed or assessed the programs that might best translate that ability to work into public health and prevention.”

Hence, this new phase of PrEP research—a collection of demonstration projects, off-label studies and pilot studies that are all, to one degree or another, searching for ways to make PrEP practical.

Demonstration projects are designed to focus more on program design for new interventions than on assessing efficacy. As such, they can be helpful to the rollout of novel interventions in the real world. Open label studies describe a type of clinical trial where both the investigator and the study participant know the treatment being administered. In the case of PrEP, the approach is being used to examine and improve compliance.

Warren says these studies and others will be crucial to the future of PrEP. “We need to determine who really wants it, who needs it, who gets it, how do we help them get it, who pays, and who decides,” says Warren. “None of these questions got answered in the efficacy studies.”

The solution could lie, in part, in the ubiquitous cellphone. The Southern California demonstration project will try and boost adherence to Truvada by texting MSM volunteers a daily reminder. The drug was initially developed by Gilead Sciences to treat HIV, but a year ago the US Food and Drug Administration granted the biotech approval to market once-daily Truvada for reducing the risk of acquiring HIV in high-risk adults on the strength of the efficacy data (see FDA approves Truvada for use in PrEP, IAVI Report Blog, July 16, 2012).

The study will enroll men at clinical sites in San Diego, Long Beach and Los Angeles and is to be conducted jointly by the California Collaborative Treatment Group (CCTG) consortium, which includes the Harbor-University of California-Los Angeles, University of Southern California and University of San Diego (UCSD). It will not include any HIV-uninfected MSM who took Truvada as part of an earlier trial, says Richard Haubrich, professor of medicine in the Division of Infectious Diseases at UCSD, who heads the CCTG demonstration projects, known as ALERT.

Haubrich says half of the MSM will be randomized to a group that will receive safe sex counseling along with their Truvada. The other half will, in addition, get daily texts. The study was designed to test the benefit of using a text message intervention called iTAB (Individualized Texting for Adherence Building), which was developed by David Moore, an associate professor of psychiatry at UCSD.

The phrasing of the text messages can be deliberately generic—something like “Take your blue pill and stay healthy”—or more specific, such as, ‘Did you take your Truvada today?’ To keep them motivated, the men will also receive an accompanying “fun fact” from a pre-selected list of topics that are tailored to their specific interests and tastes. These might be fun facts about music, movies, sports, nutrition, and other topics. (Here’s one example: “In ‘Terminator 2: Judgment Day,’ Arnold Schwarzenegger received a salary of US$15 million; the 700 words he spoke translate to $21,429 per word”.)

“We all understand that the biggest challenge [in PrEP] is adherence to therapy,” says Haubrich. “So we chose to design a randomized study to use text messaging to augment [adherence]. The beauty of this system is that the men can control the wording of the message and they can respond that they took their medicine with a single key stroke.”

The hope, of course, is that the men will answer every day in the affirmative. If they do, they’ll get a reply, such as “Good job.” But the study participants also have the option of ignoring the texts, or ‘fessing up and saying, “No, I haven’t.”

“There is a lot of interest in cellular and mobile technologies for health uses,” says Haubrich. “It’s really a burgeoning field.” Haubrich says one of his colleagues is using a sensor that records ingestion of medicine and then wirelessly transmits the data to a clinician of the patient’s choosing, so that the clinician can keep track of the patient’s adherence.
Location, location, location

Another demonstration project involving MSM is looking at the acceptability, feasibility and safety of administering PrEP in different real-world clinical settings. The study, known as the Demo Project, is offering daily oral Truvada to 600 HIV-uninfected MSM and transgender women in San Francisco, Miami, and Washington, D.C. The cities were chosen because they are heavily impacted by HIV and have large MSM epidemics. In San Francisco and Miami, men are being recruited from sexually transmitted disease (STD) clinics, while men from Washington, D.C. are being recruited from a community health center. As adherence is critical to the effectiveness of PrEP, the primary goals of the Demo Project are to evaluate PrEP adherence among MSM and transgender women in real-world clinical settings and to determine factors related to adherence.

“We initially started with STD clinics, given the substantial proportion of MSM patients at risk of acquiring HIV in these settings, and subsequently added a community health center site as well,” says Albert Liu, director of HIV Prevention Intervention Studies at the San Francisco Department of Public Health and the lead investigator in the Demo Project. This diversity of sites will provide important information on staffing and space for effective delivery of PrEP in different care delivery systems, he says. “Typically, STD clinics aren’t set up to see people in an ongoing fashion in terms of longitudinal care, whereas community health centers are.”

Participants in the Demo Project will visit the clinic one month after initiating PrEP, and then every three months for clinical monitoring. While follow-up visits were monthly in PrEP clinical trials, PrEP demonstration projects will help determine whether less frequent clinical monitoring can provide adequate support and safety monitoring to PrEP users. This question is important, he says, because otherwise healthy individuals may not be inclined to visit the doctor all that often. Frequent clinic visits may also burden the healthcare system, so finding strategies to streamline PrEP delivery will be important in the scale-up of PrEP, he added.

“We want to see what the interest level in PrEP is and who will want to take it,” says Liu. “We will also be looking closely at patterns of adherence over time and, importantly, whether and how their sexual behaviors and practices change while using PrEP.”

Revisiting a success

While most of the demonstration projects focus to one degree or another on the MSM community, a project launched last year in Kenya and Uganda is following up on results of the Partners PrEP study of SDCs. This demonstration project will be enrolling 1,000 SDCs in which HIV-infected partners are not yet on antiretroviral therapy (ART) because they either do not meet the treatment guidelines, only recently discovered they were HIV-infected or treatment-eligible, or because they have chosen to opt out of therapy. The project will explore how couples use ART and PrEP to reduce HIV risk: uninfected partners will be offered PrEP as a bridge to ART use in the infected partner, with PrEP discontinued once the infected partner initiates and sustains adherence to ART.

Jared Baeten, a University of Washington associate professor of global health who was a

The pharmacokinetic factor

Adherence appears to be the primary determinant of PrEP efficacy, but could differences in drug levels in tissues also play a role?

The answer: Sort of.

According to Craig Hendrix, director of the Drug Development Unit at Johns Hopkins University, concentrations of tenofovir disoproxil fumarate (TDF) and tenofovir diphosphate—the active form of TDF—are much higher in homogenized colon tissue than in homogenized vaginal tissue 24 hours after dosing. The differences are not so striking, however, in CD4+ T cells extracted from tissue. Hendrix, who has extensively studied chemoprevention of HIV, made this observation with colleagues in a recently published study of six healthy women who were administered a single oral dose of TDF and then tested for drug concentrations in blood and colon and vaginal tissue (AIDS Res. Hum. Retroviruses 2013, doi: 10.1089/aid.2013.0044). Samples were taken up to 15 days after the dose was administered.

The study sought to answer questions about dosing frequency in PrEP regimens. But the higher drug concentrations in colon tissue could also, perhaps, explain why less than perfect PrEP adherence is less detrimental to men who have sex with men, who primarily transmit and acquire HIV through anal intercourse. High-risk heterosexual women, on the other hand, are primarily exposed to HIV through vaginal intercourse. “The risk of receptive anal intercourse is roughly 20 times higher than receptive vaginal intercourse,” notes Hendrix. “So if the concentration of [TDF] is 100 times higher in colon tissue, there may be a net benefit.”

But Hendrix says this initial benefit subsides in the first week after dosing because the half-life of the drug is shorter in rectal tissue than in vaginal tissue. “The critical issue going forward is how much drug has to be present for PrEP to be effective,” says Hendrix. “In other words, if one did take the drug reliably on a less than daily schedule, how well would that work? If one could take a smaller amount of drug a day, might that reduce cost and side effects?” —RM
A tough group to crack

While the data from SDCs will almost certainly be useful, the work being done among younger, high-risk heterosexual women from Africa is likely to be even more informative. Not only are HIV incidence rates dramatically higher among this demographic, but engaging them in PrEP studies has proved difficult as well.

“How do you figure out how to make PrEP feasible in this group? It’s a million dollar question,” says Jeanne Marrazzo, a professor of medicine at the University of Washington and the principal investigator in the VOICE trial, which ended on a disappointing note. “Why didn’t the women use it? And among the women who did it, what were they like and what motivated them? That is the conundrum—where we are right now.”

The VOICE study—which was conducted in South Africa, Zimbabwe and Uganda—showed adherence differed geographically. “We are still trying to figure out who took the product,” says Marrazzo. “What we do know is that, in general, women who were older, married, and who had more children were more likely to have taken the product. That played out in terms of countries. In Zimbabwe and Uganda, the women were more likely to be married. In South Africa, a minority were married. So the question becomes: what was it about those [married] women that facilitated taking the product? Is it because they had control, that they were in more stable relationships?”

Because VOICE did not find PrEP to be effective in the evaluated population, a demonstration project would be fruitless. But Marrazzo says researchers are planning to follow up on the results to try and get a better handle on why adherence in this group proved so difficult. They will be meeting with women in the study, their male partners, and community leaders to determine whether particular perceptions about PrEP might have persuaded women to stop taking the study drug.

“Some of the stuff will be pretty important,” says Marrazzo. “We are learning, I think, that we may not have appreciated how much the concept of using ARVs as prevention was really new and not necessarily well understood among the women.”

Marrazzo says discussions with the women revealed distrust about the use of ARVs to prevent HIV. “When we talked to them about why women didn’t use the product, they said, ‘Why would I want to take that drug that people take when they are sick?’ Maybe we were not attentive enough to the concerns these women may have had about taking products that are clearly associated with HIV infection.”

Marrazzo says members of the VOICE team also plan to hold focus groups with women from the VOICE study. Women will be placed in groups according to how adherent they were with the study drug, says Marrazzo. “Am I sure we will get answers? No. The barriers to disclosure can be huge. I just don’t think we really appreciated how challenging that was.”

PROUD of PrEP

As researchers begin to sort out why PrEP failed to work in high-risk women in Africa, another pilot study in the UK known as PROUD is trying to determine if it might be feasible to study the impact of PrEP effectiveness—in this case Truvada—and its impact on risk behaviors and STDs among MSM in a real-world setting.
Sheena McCormack, a professor of clinical epidemiology at UK’s Medical Research Council Clinical Trials Unit (MRC CTU) and a principal investigator of the study, says it is still unclear whether taking a pill that they now know reduces their risk of acquiring HIV will prompt gay men to use condoms less frequently with a larger number of sexual partners, and thereby increase their risk of STDs. “To make a measurable difference to the HIV epidemic in the UK, we would probably need a large number of gay men to take PrEP,” McCormack says. “This is why it is unclear whether offering PrEP for free to the entire MSM community would be cost-effective overall in the UK.”

Focusing on high incidence groups makes sense, but these populations might be the least likely to take the regimen, as was seen in the clinical trials with younger participants. These uncertainties, and lack of UK-specific data, explain why the UK has a position statement recommending that PrEP be used only in the context of a clinical research study, says McCormack.

To try and answer these questions, the MRC CTU and Public Health England (PHE) proposed a study of 5,000 MSM—half randomized to the immediate offer of daily oral Truvada in the combination prevention package and the other half to a deferred offer of Truvada after 12 months follow-up—to measure the effectiveness of PrEP and impact on risk behaviors and STDs. To determine whether this would be feasible, a pilot study called PROUD has been initiated. If it is possible to enroll 500 gay men to the study design in a timely manner and retain both groups, then the collaborators will reapply for funding—an initial request by collaborators was declined—for a trial that is large enough to address the question of effectiveness.

McCormack was optimistic that volunteers could be quickly recruited from participating publicly funded sexual health clinics, as this is where the majority of gay men are tested for HIV and STDs, and where HIV treatment is provided. “Having HIV prevention and treatment under one roof ensures excellent linkage to care, and we are lucky to have had this network since the start of the epidemic,” says McCormack. “In spite of this, we still have rising numbers of new infections in gay men.”

In reality, the PROUD study has recruited more slowly than anticipated. First, it took longer than expected to obtain the local approvals for each clinic, and so the study has only been at full capacity since July. Second, there is very little awareness of PrEP in the community and it has been difficult for community organizations and clinics to promote it, as PrEP is not currently available in the UK.

“I worry that we will end up concluding PrEP is a tool of no interest when we haven’t given it a fair shot,” McCormack told Baseline, a community magazine for people living with or affected by HIV and hepatitis. “I firmly believe we can promote condoms and PrEP together in a responsible way, and if we don’t rise to this challenge, we are failing the breadth of the sexually active community in the long term, but especially gay men right now.”

**Working with sex workers in Africa**

Another real-world PrEP experiment will include commercial sex workers (CSWs) from Johannesburg and from a rural community six hours from the South African city. The study, which researchers hope to continue over three years, will evaluate the uptake, acceptability and adherence to a proven PrEP regimen.

Professor Helen Rees, executive director of the Wits Reproductive Health and HIV Institute of the University of the Witwatersrand in Johannesburg says her research group proposed the study to address questions about PrEP acceptability. “We have learned a lot from the efficacy trials but more needs to be understood about the feasibility and acceptability of introducing PrEP,” says Rees. “And it will be difficult to roll out PrEP to a general population, so we are looking instead at targeted populations. Here in South Africa, modeling suggests that 20% of new infections are associated with CSWs and their clients.”

Rees says CSWs present particular challenges, their mobility being one, and their long-term commitment to daily PrEP another. Fortunately, says Rees, her group has established longstanding relationships with many CSW cohorts throughout the region.

Countries will have to figure out how to put research into practice, she says, or risk delaying the rollout of a proven intervention that could save thousands of lives.

**The PrEP forecast**

Warren of AVAC agrees that the public health community is still grappling with PrEP delivery. Even in the US, he says, where PrEP is recom-
mended for high-risk populations, medical providers lack awareness about current protocols. “There was a young man at a leading university [in the US] who went into the student health services and said he is at risk for HIV, that he had had unprotected sex, and that he would like to get on PrEP,” says Warren. “He was told, ‘Oh, you have to go to an infectious disease doctor. And the infectious disease doctor says, ‘No. That is only for sero-discordant couples.’ This guy was a perfect candidate for PrEP.” Warren says this anecdote is Exhibit A for why well-designed demonstration projects are needed to make the PrEP program work. The good news, he says, is that there is no shortage of such projects. In fact, the field is littered with them. What is missing, he says, is leadership and an overarching strategy. “There need to be some common elements, answering a core set of questions in a systematic fashion. We need some sense of shared vision, a common play book. If not, at the end of the day, we run the risk of having random acts of demonstration-project goodness that answer a bunch of questions.” Except, that is, the one that matters most: how to make PrEP work in the real world.

Ananworanich and her colleagues are therefore trying to address stigma as well, by reminding people that anyone can be infected with HIV, and by trying to make HIV testing seem about as run-of-the-mill as a liver function test. In September 2011, the TRCARC started an “edutainment” web site for MSM called adamslove.org, and a similar site for Indonesians late last year called temanteman.org, which doesn’t just focus on MSM. The sites discuss acute retroviral syndrome, emphasize that anyone is at risk, should get tested and doesn’t have to wait to do so, and that the widespread belief that people with HIV can’t stay healthy and live a normal life is wrong. “We are trying to send a new message: you can come [two weeks after infection]; with the NAT testing perhaps you can even come after one week,” says Ananworanich. They are also working on TV and radio ads with the Thai Health Promotion Foundation to spread these messages, Phanuphak adds.

To get more people to come to the clinic, they are also offering other services, such as cervical or anal cancer screens using Pap smears. People who test positive are offered further tests, such as checks inside the anal canal and of the cervix. There are even dental services for patients who receive ART as part of a clinical trial.

Because almost half of all new HIV cases are currently identified in MSM, the staff also tries to make such people feel more comfortable by offering them counseling on a separate floor and allowing them to leave the building through a separate exit. This, Phanuphak says, is to try to avoid any double stigmatization they might experience for being both gay and HIV positive.

Continued from page 8

The efforts seem to be paying off: 85% of MSM who come to the Anonymous Clinic for anal cancer screening also get an HIV test, Phanuphak says, and the number of people coming to the Anonymous Clinic for testing has almost doubled, from 8,000 in 2008 to 15,000 last year, Phanuphak says. MSM numbers have even quadrupled from 1,000 in 2008 to 4,000 last year.

Still, all these efforts to get people tested are useless if people don’t learn their test results, Phanuphak says. Data from the Thai Ministry of Public Health show that about one in three don’t return to get results if they have to come back another day, Phanuphak says. At the Anonymous Clinic, that’s not an issue, because all patients who come there learn about their test results on the same day. However, that’s not the case in many other places in Thailand, Phanuphak says, which is why the Thai Ministry of Public Health has a goal to achieve same-day testing in all Thai hospitals by the end of this year. That, she says, “could be a game changer.”

Over at AFRIMS, meanwhile, researchers continue studying the immune responses induced in RV144 and other vaccine trials, and sending samples from RV144 and other trials to researchers all over the world. Asked how much he thinks the sample collection is worth, Karasavva doesn’t hesitate: “I don’t think you can put a value to this,” he says. “Scientifically, the contribution of this site you cannot measure.”

As for the one place you should visit in Bangkok—he suggests the “Cabbages & Condoms” restaurant, and not only to check out the condoms. “The food,” he says, “is very, very good.”
Researchers solve high-resolution structure of HIV co-receptor CCR5

The fact that a drug is approved means—in most cases—that it works. But its mere approval doesn’t guarantee that anyone knows exactly how it works.

Take, for example, the antiretroviral (ARV) drug Maraviroc. It belongs to a class of ARVs called “entry inhibitors” because it keeps HIV from entering its target cells by binding to CCR5, one of the co-receptors HIV uses to slip into CD4+ cells.

Researchers know that Maraviroc binds to a part of CCR5 distinct from the part bound by HIV’s gp120 protein, so it isn’t merely outcompeting gp120 for access to CCR5. But exactly where Maraviroc binds to CCR5, and how Maraviroc binding renders CCR5 incapable of binding to HIV, has so far remained a mystery.

Now, a team of researchers led by Beili Wu, a professor at the Shanghai Institute of Materia Medica, has determined the structure of both co-receptors are available, it should be possible to develop drugs that inhibit HIV binding to both CCR5 and CXCR4. “We are working on a structure-based drug design to get an idea of how to make new drugs to inhibit both [co-]receptors,” she says.

A comparison of the new CCR5 structure to CXCR4, Wu says, shows small differences in the charge and position of atoms in the so-called “ligand binding pocket” of the two proteins. This pocket binds to a stretch of gp120 called V3 (see cover image), which determines whether HIV prefers to bind to CCR5 or CXCR4 to enter cells. Those differences may be what determines what kind of HIV variant can bind the two co-receptors, Wu says, adding that she was surprised that the differences were so small. This insight may now enable researchers to better understand just how the virus switches from preferring CCR5 early in infection to CXCR4 later in infection.

Even though it’s been known since 1996 that HIV needs to also bind to CXCR4 or CCR5 to enter cells, it took until 2010 to determine the structure of CXCR4, and the structure of CCR5 has been determined only now. One reason it took so long, Wu says, is that both proteins are G-protein coupled receptors (GPCRs), a class of proteins that is notoriously unstable and therefore difficult to crystallize.

To make CCR5 more stable, Wu and colleagues replaced the most flexible part of CCR5 with a more rigid protein, a common trick in structural biology. CCR5 was especially challenging, Wu says, because the rigid replacement proteins that had previously worked for other GPCRs such as CXCR4 didn’t work for CCR5. So Wu and colleagues had to find a new one. Another challenge: getting sufficient amounts of the CCR5 protein to make crystals.

Now that they know how to produce large amounts of high quality CCR5, Wu says, they can use it as an immunogen in a vaccine to induce antibodies that might keep HIV from binding to CCR5 and invading cells.

P. J. Klasse, a virologist at Weill Cornell Medical College in New York City who was not connected to the study, says he was impressed by the quality of the study. “They managed to solve the structure to a very high resolution,” says Klasse, who wrote a commentary on the finding in the same issue of Science. He says the finding now enables researchers to design better CCR5 inhibitors that bind the protein more strongly than Maraviroc and perhaps perturb its structure in a different way, making it harder for the virus to escape.

Still, Klasse adds, to better understand just how HIV gp120 interacts with CCR5 to enter cells in the absence of Maraviroc, it would be valuable one day to know the structures of the CCR5 co-receptor both when it is free and when it is bound to CD4-bound gp120 (in the absence of Maraviroc).

That, Wu says, is one of the things she plans to do next. —Andreas von Bubnoff
The Next Step
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