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.

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.

Entrance of the separate counseling area for MSM at the Anonymous Clinic in Bangkok with posters that promote getting tested and the adamslove.org web site. Photo by Andreas von Bubnoff.

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.

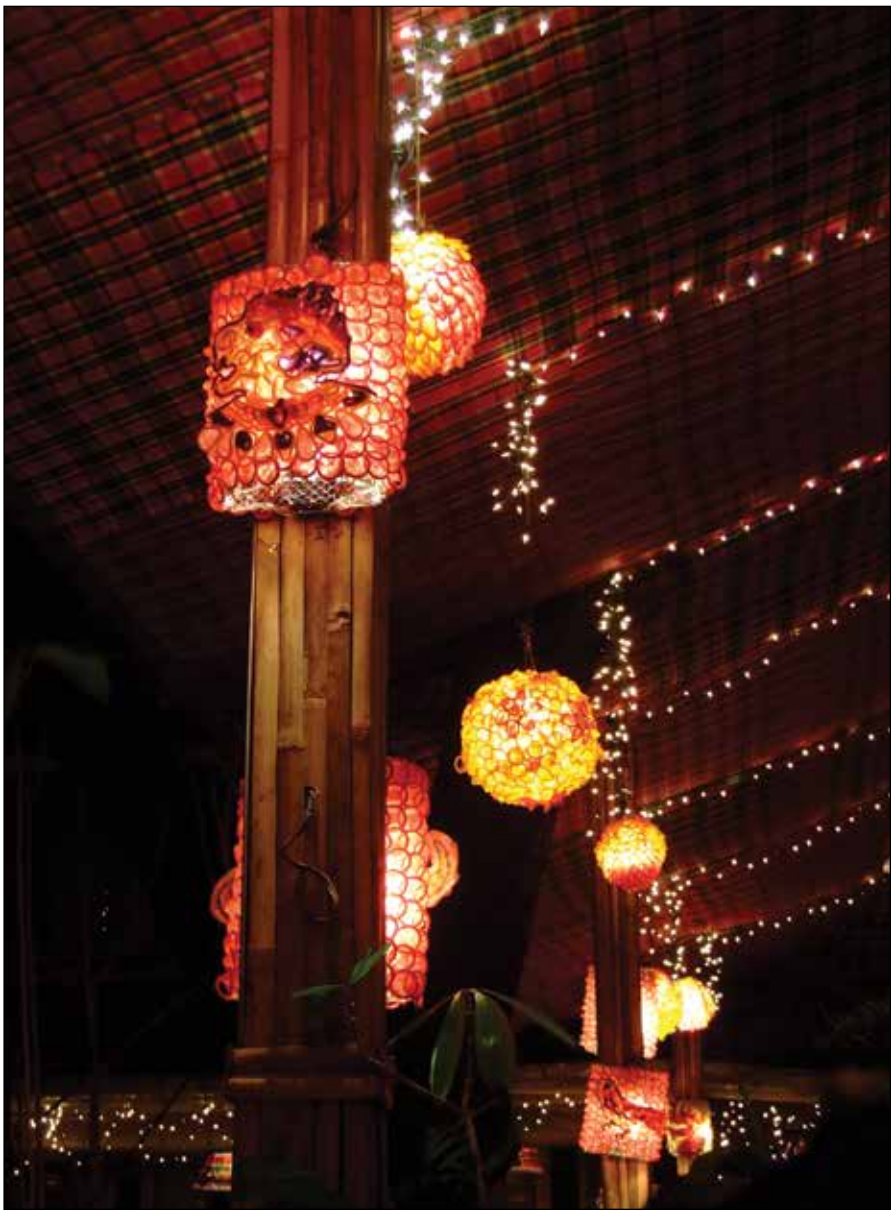
The RV254 volunteers could only be offered immediate treatment because they were part of a clinical study; otherwise, the current Thai guidelines recommend starting treatment only below a $CD4^+$ T-cell count of 350, a number that will likely change to 500 next year, in response to the new WHO guidelines, 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.

Mathematical modeling, 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,



Condom lamps at the Cabbages & Condoms restaurant in Bangkok. Photo by Andreas von Bubnoff.

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.

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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.

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." ■