A recent study suggests a novel treatment might flush out latent copies of HIV hiding in the body—and re-ignites discussion over the challenges of eradicating HIV infection

By Philip Cohen

There’s a four-letter word that HIV researchers don’t use very often: Cure.

But that word did grace a recent report authored by David Margolis of University of North Carolina, Chapel Hill and a collection of other notable HIV researchers (Lancet 366, 549, 2005). After describing an unusual treatment for HIV-infected people that might be targeting the burden of latent virus, the researchers wrote that the “finding, though not definitive, suggests that new approaches will allow the cure of HIV in the future.”

The ensuing interest was not surprising. The story was widely reported in the mass media, thousands of websites picked up the story, and among the authors’ colleagues grew a buzz of criticism over whether it was worth raising hopes of a cure in those already infected given what the test-of-principle study of four patients actually accomplished. “I used the word, so I’ll take the heat,” says Margolis. “But the position being staked out by some on the
are tackled will have implications for when an effective AIDS vaccine is developed and this has many closely watching how the debut of this important vaccine plays out. “This is a sort of test case for HIV vaccines and there will be a lot of lessons on acceptability and delivery,” says Kahn. “Similar moral issues may also be raised.”

**Behind the virus**

HPV is a double-stranded DNA virus that most studies suggest infects at least 25% of sexually active adults—with one longitudinal study suggesting a cumulative prevalence close to 80% in a cohort of adolescent women in the US (Carr. Opin. Obstet. Gynecol. 17, 476, 2005; J. Infect. Dis. 191, 182, 2005). Variations in worldwide prevalence estimates are due to differences in the molecular sensitivity of DNA assays used to detect HPV infection around the world. More than 120 types of HPV infect humans and a third of these can cause genital infection (Vaccine 10.1016/j.vaccine.2005.09.054). These are further classified as high- and low-risk based on their oncogenic properties, with 13 to 18 types that are associated with cancer and two that predominantly cause genital warts and low-grade cervical lesions.

HPV types 6 and 11 are the main culprits for genital warts, causing 90% of benign cell proliferation, which is much less serious but can also serve as an important marker for infection with high-risk HPV types. Two of the high-risk HPV types, 16 and 18, are responsible for 70% of the cervical cancer cases worldwide, according to Kahn, but the predominant HPV types can vary geographically and these two types are not as common in sub-Saharan Africa or Asia as they are in North America and Europe.

There is limited research on the epidemiology of HPV infection in developing countries, but a study that pooled data from 11 case-controlled studies in 2506 women with cervical cancer in Morocco, Mali, Colombia, Brazil, Paraguay, Peru, Thailand, the Philippines, and Spain reported that HPV type 16 was the most common type with an overall prevalence of 59%, reaching 70% in some countries (N. Engl. J. Med. 348, 6, 2003). The second most common HPV type was 18, with an overall prevalence of 15%, followed by types 45, 31, and 35. The authors suggest that an effective vaccine against these five HPV types could prevent about 90% of cervical cancers worldwide, but suggest that regional variation in predominant HPV types should be considered in the creation of vaccines specific to a geographic region. Philippe Monteyne, vice president of worldwide operations for GSK’s cervical cancer vaccine program, acknowledges limited regional differences in HPV types but says the GSK vaccine “is really useful on a worldwide basis.”

Although the natural course of cervical cancer isn’t fully understood, screening protocols including Pap tests have been established to detect cervical cell abnormalities as early as possible. Many infections with HPV can be transient and cleared effectively by the immune system, but HPV becomes dangerous when persistent infection with an oncogenic type occurs. This can lead to high-grade precancerous lesions known as cervical intraepithelial neoplasia (CIN), and eventually to non-invasive and then advanced cervical cancer. Persistent HPV infection is also strongly implicated in other malignancies, including anal and oral cancer in men and women. Routine Pap smears are used to detect abnormalities in the cells of the cervix and HPV-DNA testing is also available to confirm infection and to identify the specific viral types. Catching cervical cancer in its earliest stages has substantially reduced the rate of mortality due to this disease in the US. “Most cervical cancer in this country is in women who don’t get screened,” says Kahn. Often these are women without medical insurance who are not receiving regular gynecological care.

But women in developing countries face the highest rates of cervical cancer, with India bearing almost a third of the world’s mortalities from this detectable disease. “The vaccine is the solution for these countries where mandating good screening programs is difficult,” says Monteyne.

There are also concerns that Pap smear results, or their interpretation, can be inaccurate, confirming a need for a vaccine even in countries with rigorous testing procedures. The value of the results from this qualitative test is highly dependent on analysis by a trained and conscientious professional. Laura Koutsky, an epidemiologist at the University of Washington, presented graphic images at the AIDS Vaccine 2005 meeting of women who had normal Pap smears but were found to have substantial cervical lesions when evaluated by a more invasive cervical colposcopy procedure.

**Self-assembly line**

Preventive HPV vaccines that are in development may help alleviate the reliance on screening methods in the future. Some HPV vaccine candidates might also be effective for therapy of...
moderate CIN (Curr. Opin. Obstet. Gynecol. 17, 476, 2005). A Phase I/II trial with one candidate, a modified vaccinia Ankara (MVA) virus vector carrying E2 HPV antigens, resulted in elimination of all cases of grades 1-3 CIN in 34 of 36 women, when delivered by direct inoculation into the uterus (Hum. Gene Ther. 15, 5, 2004).

This and other therapeutic candidates are mostly in the preliminary stages of development but both Merck’s and GSK’s preventive candidates could soon be on the market. Both consist of a recombinant form of the HPV L1 protein that encodes the nucleocapsid and can self assemble into a virus-like particle (VLP), a non-replicating shell that resembles an actual virus particle closely enough to fool the immune system into thinking it is encountering a natural HPV infection.

Initial data showed that these self-assembled VLPs from different HPV types are highly immunogenic, inducing both strong antibody and cellular immune responses. “Interestingly the vaccine induces even higher levels of antibody response than those induced in a naturally-occurring infection,” says Mark Feinberg, a vice president in Merck’s vaccine division. Studies have shown that the antibody response to the vaccine is up to 40 times greater with some HPV types.

Merck’s vaccine candidate, known as Gardasil, is now in Phase III testing in over 25,000 men and women and an application was recently submitted to the US Food and Drug Administration (FDA) for approval and licensure. Gardasil is a quadrivalent VLP vaccine consisting of recombinant L1 proteins from types 6, 11, 16, and 18, administered with an aluminum adjuvant. The company recently presented data from one of their Phase III trials involving 12,167 women aged 16-26 in Brazil, Colombia, Denmark, Finland, Iceland, Mexico, Norway, Peru, Poland, Singapore, Sweden, the UK, and the US.

In women that received 3 doses of the vaccine there were no cases of high-grade CIN or non-invasive cervical cancer associated with the types included in the vaccine as compared to 21 cases of either in the placebo group over an average of 17 months follow up. “It’s really hard to do better than that,” says Feinberg. Follow-up data so far shows that the immune response generated by the quadrivalent vaccine lasts at least 2.5-3 years after completion of the 3-dose regimen, but researchers at Merck are still monitoring the persistence of protection afforded by the vaccine.

A secondary analysis found that in women who received at least one injection the vaccine reduced the risk of developing high-grade precursor and non-invasive cancer associated with types 16 and 18 by 97%, a more real world example of the vaccine’s efficacy since many people are unlikely to return for all 3 inoculations. Only one participant had precancerous lesions or non-invasive cervical cancer in the group receiving Gardasil, while there were 36 in the placebo group.

Following closely behind Merck’s candidate is the HPV vaccine in development at GSK in Rixensart, Belgium, in collaboration with MedImmune. Researchers at the company expect to file for licensure with the European Medicines Agency (EMEA) in the first half of this year and are also involved in ongoing discussions with the FDA. The vaccine, known as Cervarix, is also a VLP vaccine but includes L1 proteins from types 16 and 18 only. “We have a different strategy,” explains Monteyne. “These are difficult decisions you have to make early on in the development of a vaccine and we wanted from the very beginning to focus on women and cervical cancer.”

Another difference between the Merck and GSK candidates are the adjuvants. Cervarix relies on a proprietary adjuvant developed at GSK, called AS04, which is a bacterial molecule co-formulated with aluminum. Vaccine administered with AS04 resulted in significantly higher antibody titers than with a standard aluminum salt adjuvant. Monteyne also credits the AS04 adjuvant with extending the duration of the immune response, now shown at up to four years after vaccination.

GSK currently has 5 ongoing Phase III efficacy trials with Cervarix in 28,000 female volunteers. Last year they reported the results of one Phase II trial in more than 1100 women aged 15-25 in North America and Brazil who received 3 doses of the vaccine formulated with the AS04 adjuvant (Lancet 364, 1757, 2004). The vaccine was 100% effective at preventing persistent infection with the two strains of virus in the vaccine and 92% effective against transient viral infection. For women who received at least one injection the vaccine was 95% effective against persistent infection and 93% effective at preventing CIN, with only one woman in the vaccine group and six in the placebo group developing cervical lesions.

There may also be good news about the ability of the vaccines to prevent persistent infection with different HPV types because the L1 protein is one of the most highly conserved. At the annual HPV conference in Vancouver in
Our major rationale is to increase the percentage of individuals in a population that are immune

Mark Feinberg

Mark Feinberg, GSK, reported activity of its HPV vaccine against strains 31, 45, and 52, which can also cause cancer. Several previous studies have provided evidence for cross-immunity between genetically-related HPV types including 16, 31, 33, 35, 52, and 58. Monteyne calls this preliminary cross-protection data "extremely exciting". This phenomenon is not seen in natural infection and Monteyne is hopeful that it will mean the GSK vaccine can be given to women already infected with HPV types not in the vaccine and perhaps clear an established infection. Merck is also investigating potential cross-reactivity of Gardasil but is yet to report any data.

Implications for HIV

Since both HPV and HIV can be sexually-transmitted and enter the body through the same tissues, researchers have been studying the link between these two infections. The cervical lesions caused by persistent HPV infection can enhance women’s risk of acquiring HIV because of increased bleeding and the recruitment of CD4+ T and dendritic cells to the cervical mucosa, believed to be a target site for establishment of HIV infection in women.

This may also be true with the anal manifestations of HPV infection. A study presented at last summer’s International AIDS Society Conference in Brazil found that anal HPV infection was independently associated with HIV acquisition in a cohort of 1409 men who have sex with men (MSM; Abstract no. TuOa0403).

Several studies have also found that HIV-infected individuals are at greater risk for acquiring HPV and the two prove to be perilous partners. Co-infected individuals are more likely to develop severe lesions than those infected with HPV alone. It is estimated that HIV-infected women are three to five times more likely to develop cervical lesions due to HPV infection. HIV’s ability to hinder the immune system may be at the root of this problem, either directly or indirectly, because it allows HPV to persist longer, making cancer development more likely. Even people on highly active antiretroviral therapy (HAART) for HIV infection are more likely to develop serious anal and cervical lesions and there has been no decline in the incidence of cervical or anal cancer since the initiation of HAART.

Good for the gander?

With results showing that anal HPV infection can increase the risk of acquiring HIV, many researchers eagerly await data on the efficacy of HPV vaccines in men. This information won’t be coming from GSK. In keeping with their strategy of developing a cervical cancer vaccine the company has chosen not to test their vaccine in men and, with a name like Cervarix, there is little confusion about the vaccine’s intended target.

Merck, however, is studying their more androgynously-named vaccine in both male and female adolescents, as well as in cohorts of MSM. “HPV does cause significant disease burden in males,” says Feinberg, who sees clear benefits for giving Gardasil to men, including preventing anal and penile cancer and genital warts.

It is also likely that vaccinating both men and women will increase herd immunity and decrease overall the number of life-threatening infections in women. “Our major rationale is to increase the percentage of individuals in a population that are immune to an infection,” Feinberg adds. Models show that 90% of the population would need to be vaccinated to eliminate strains 16 and 18.

Merck has yet to report results on the efficacy of Gardasil in male volunteers and their submission to the FDA will only be based on safety and immunogenicity data in women, but Feinberg says the vaccine looks quite good, especially in young men. This is consistent with results from female volunteers where researchers saw significantly higher immune response in younger individuals.

Vaccinating adolescents

Ideally a preventive HPV vaccine would be administered prior to infection, which for such a common virus means vaccinating girls and boys before they become sexually active. This has researchers discussing whether parents will be willing to have their children vaccinated against an STI, perhaps even as early as age 9. Some conservative religious groups have vocally opposed the idea of vaccinating young adolescents (age 9-12) against HPV because they say it could promote sexual activity at an earlier age. As with HIV, these groups instead favor abstinence messages.

Vaccinating young adolescents also means that the vaccine would be administered by pediatricians or family practice physicians and not gynecologists who have more experience diagnosing and treating the manifestations of genital HPV infection. Some researchers like Kahn worry that this lack of
familiarity could affect the physician’s willingness to suggest HPV vaccination. “Cervical cancer is just not a disease of children and adolescents,” she says. “It’s a disease primarily of middle-aged women.”

Kahn and colleagues have conducted several studies to gauge the willingness of pediatricians to recommend HPV vaccines to their patients and their parents or guardians, who will be required to provide consent. Significantly more of the pediatricians and family physicians she surveyed would recommend an HPV vaccine to girls than boys and to older versus younger patients (J. Pediatr. Adolesc. Gynecol., 18, 6, 2005). Kahn concludes that the vast majority of parents and healthcare providers want this vaccine, but she admits that there will be some pediatricians who just aren’t comfortable giving a vaccine for an STI.

Another important factor for the acceptance of any new vaccine is education, and this is especially true for an HPV vaccine since public awareness of the disease and its possible implications is so low. A study that tested the knowledge of parents about HPV vaccines found that a single-page information sheet on the virus and its effects was able to sway 20% of parents who originally declined vaccination for their children.

Meanwhile both Merck and GSK are trying to steer clear of acceptance issues with the vaccine and stay focused on getting the vaccine approved. “There definitely will be some acceptance issues, but our role is not to enter it,” says Monteyne.

Acceptance of the vaccine will also be critical in some developing countries where discussion of sexual activity is particularly difficult with respect to young girls. India has one of the highest rates of cervical cancer and there is concern that societal restrictions may make it difficult to vaccinate only girls.

Another complication in some developing countries is getting adolescents into the clinics. Much of the progress with vaccination programs in developing countries has relied on infant immunizations and as Feinberg says, “There really isn’t any infrastructure in developing countries for administering vaccine to adolescents.”

So for HPV vaccines to be successful in these societies there will need to be public health campaigns and perhaps a new structure for vaccine delivery. These are significant challenges that some international organizations are now beginning to address. The Program for Appropriate Technology in Health (PATH), an advocacy group in Seattle, received a planning grant from the Bill & Melinda Gates Foundation to explore ways to make HPV vaccines available in developing countries. Their initial focus is on countries that already have active vaccination programs and also high levels of HPV disease burden, which currently includes India, Peru, Vietnam, and Uganda, but even in these countries there are substantial barriers to vaccination. “While there may be vaccine programs on the books aimed at adolescents, only a tiny proportion of girls and boys in those age groups are reached,” says Jacqueline Sherris of PATH. In some regions of India less than 10% of adolescent girls are receiving the recommended tetanus toxoid vaccine.

PATH is also working on a proposal to the Global Alliance for Vaccines and Immunization (GAVI) to explain why funding should be allocated for the purchase of HPV vaccines. The price for the Merck or GSK vaccines won’t be set until after they receive approval but according to a report from the American Academy of Microbiology (Vaccine Development: Current Status and Future Needs, www.asm.org/Academy/index.asp?bid=38323), the HPV vaccine could well be prohibitively expensive for use in developing countries. “We expect to look at a range of strategies to encourage an affordable supply,” says Sherris.

New research into the epidemiology of HPV infection by region may also be an important consideration in the implementation of these vaccine programs, but without a doubt introducing these vaccines in developing countries “could have a tremendous impact on mortality,” says Kahn.

Both companies are also looking at ways to make the vaccine widely available while ensuring a return on their investment, which according to the Wall Street Journal could be profits that exceed US$1 billion annually. Neither company is exploring advance market commitments (see If you build it, they will pay, IAVI Report 9, 3, 2005) as a mechanism for making the vaccine available throughout the world, but both are looking to form public-private partnerships with organizations like GAVI or the United Nations Children’s Fund (UNICEF) to get the vaccine to those in need. “It’s very exciting to have this vaccine that works so well, but there is still much work to be done,” says Feinberg.
other side is that curing HIV isn’t possible. And we don’t know that. If you tell everyone this is impossible, it becomes self-fulfilling, because no one will work on this and no one will fund it.”

Many researchers who study places that HIV might hide in the body, collectively known as the “reservoir,” agree with Margolis that the goal of HIV eradication is worth pursuing, even if they are more reluctant to talk in terms of the c-word. “We just need to be a little bit humble about what we can achieve in the face of a tough problem like this,” says Robert Siliciano of the Johns Hopkins School of Medicine, Baltimore.

But the sort of caveats researchers have raised about the Lancet paper reveal a lot about the scientific and technical difficulties in studying the reservoir and the large range of opinion on the feasibility of HIV eradication. Ask a dozen researchers about the prospects of eliminating HIV infection and you’ll get a dozen shades of opinion ranging from optimism to pessimism surrounding a central fact: no one actually knows how or exactly where the virus is hiding, or the best way to lure it out and destroy it.

Today most researchers seem to lie on the glass-is-half-empty side on the question of HIV eradication. But that wasn’t always the case. Talk about wiping out HIV infection was more popular in the late 1990s when powerful combinations of antiretroviral (ARV) drugs—highly active antiretroviral therapy (HAART)—first became available and were bringing some people with AIDS back from the brink. Where drugs were available, the death toll of the disease plummeted and the level of virus in the blood of infected people dropped until no such cells could be detected by conventional assays of the time, about 500 HIV copies/ml of blood.

Even so, it was clear that drugs could only attack actively replicating virus. They could not touch another potential source of HIV—copies integrated into the chromosomes of resting CD4+ memory T cells. By their nature, these cells could be long-lived and the fear was that if the drugs were eventually withdrawn, some antigen would reactivate these cells and release the latent virus. But David Ho at the Aaron Diamond AIDS Research Center in New York and his colleagues calculated that if viral replication was completely stopped, then existing latent cells harboring HIV could die off in less than three years (Nature 387, 188, 1997).

But researchers were to discover that while HIV can’t run away from HAART, it can hide. This became clear when researchers experimented with removing drugs from HIV-infected people who had been receiving HAART for more than three years: the virus rebounded to pretreatment levels within weeks. “That means you are dealing with exponential growth from very small numbers of virus,” says Siliciano. “It means partial reduction in the size of the reservoir is essentially useless. You are stuck with the virus unless you get every last latently-infected cell.”

And Siliciano’s measurements suggested that this reservoir of cells was extremely stable, displaying a half life of 44 months (Nat. Med. 5, 512, 1999). If that’s true it means that even if HAART completely suppresses HIV replication and no new virions are produced, the ticking time bomb of latent cells would not be eliminated from the body for at least 60 years. When that finding was published, HIV eradication strategies switched their focus to directly targeting this source of HIV. The tactic adopted by several labs was to stimulate the CD4+ memory T cells to reactivate the virus, making it susceptible to drugs, an immune response, or some other therapy. These wake-up strategies involved treating patients with T-cell stimulating factors like the cytokines IL-2 and IL-7.

But while some researchers were able to see impressive reductions in the size or composition of the pools of latent HIV in CD4+ memory T cells, the effect wasn’t strong enough to purge the reservoir. One striking report came from Tae-Wook Chun and Anthony Fauci at the National Institute of Allergy and Infectious Diseases and colleagues in which they treated two HAART-recipients intermittently with IL-2. At first this treatment appeared successful. The number of latent HIV cells dropped until no such cells could be recovered from the patients. However, when the drugs were removed viral levels rebounded in just a few weeks (Nature 401, 874, 1999).

There was an obvious complication with these approaches, however, that could explain their failure. Attempts to stimulate resting CD4+ memory T cells to release virus also creates more active CD4+ T cells, and it has since become clear that these cells are HIV’s preferential replication ground. That’s
why researchers began looking for a more subtle approach which could stimulate the slumbering HIV genome without prompting widespread activation of T cells.

Switching on the HIV genome requires the work of proteins called transcription factors that bind near the viral promoter and recruit the RNA polymerase machinery that transcribes the viral genome into RNA, which is then used to make protein. Margolis’ team determined that in some latent cells the HIV genome is silent because of the action of an enzyme called histone deacetylase (HDAC) whose job in the cell is to shut off regions of the chromosomal DNA that contain unused genes. HDAC makes these regions inaccessible to transcription factors by chemically modifying histones, proteins that package chromosomal DNA, creating a large protein and DNA complex known as chromatin. Margolis found that sometimes HDAC ends up remodeling the region of chromosomal DNA around the HIV genome so that it is packed with histones and inhibitory proteins that cover the viral promoter, thereby blocking transcription factors from entering.

So researchers in Peter Dervan’s laboratory at the California Institute of Technology developed hairpin-shaped molecules called polyamides that were designed to bind near the promoter of an integrated copy of HIV, freeing it from histones and allowing transcription to proceed. Margolis and his team used these polyamides to show in vitro that specifically opening chromatin around the integrated HIV genome induced HIV production from latent cells taken from patients. But these chemicals had never been used as approved drugs and so wouldn’t be available to use in people for many years, if ever. But in 2001 Margolis’ work unexpectedly took a giant step forward. “It was exactly what I had been looking for,” he says. The answer, however, didn’t come from his experiments. It arrived in his email inbox.

He was sitting in his office, eyes glazing over as he quickly skimmed through electronic tables of contents from the dozens of journals he regularly checked. One suddenly caught his eye. Sandwiched between reports of a novel protein from mouse testes and another on the biochemical minutiae of adrenaline pumping chromaffin cells was a seven page article titled “Histone Deacetylase Is A Direct Target of Valproic Acid, A Potent Anticonvulsant Mood Stabilizer, and Teratogen.” (J. Biol. Chem. 276, 36734, 2001). For Margolis, it was an exciting moment.

Not only did the paper show that valproic acid inhibited the enzyme Margolis believed suppressed HIV transcription, but it is an FDA-approved drug used to treat many conditions including epileptic seizures and bipolar disorder. “Here was something I could give to patients right now,” he says. His team first confirmed the drug could awaken HIV when applied in vitro to CD4+ memory T cells purified from infected volunteers. They also found valproic acid did so without activating the T cells or making them susceptible to new infection (AIDS 18, 1101, 2004). Armed with that data, Margolis and his colleagues felt they were ready for the clinic.

They recruited four HIV-infected volunteers who were receiving HAART and had viral loads below the threshold of current standard assays, about 50 HIV copies/ml. In a leukopheresis process that takes a couple of hours per patient, their blood was pumped through a centrifuge and white blood cells spun out. Cells expressing surface proteins characteristic of resting memory T cells were purified and then stimulated to reactivate any HIV, enabling the researchers to determine the size of the latent HIV reservoir at the start of the study.

The volunteers then intensified their ARV therapy and added valproic acid to their drug regimen and three months later the level of latently infected cells was measured again. Based on reports on the stability of the latent HIV reservoir reported in the literature, the researchers set a level of 50% decline as significant. In three of the patients, they measured declines of 68, 72, and 84%. While this level of reduction is unlikely to have any clinical benefit, the downward trend was encouraging.

This pilot study has its weaknesses, which Margolis readily acknowledges. The study involved only four volunteers and had no control group. And even an 84% reduction is on the border of confidence of the assay, say researchers who regularly conduct measurements of latent HIV in memory T cells. Also, since the patients received two additional drugs as well as HAART—they were also given the fusion inhibitor T20 (or enfuvirtide) in order to make sure any reactivated released virus did not infect neigh-
boring cells—in theory either drug or their combination may have been responsible for any depletion of latently-infected cells, making the mechanism of depletion unclear. Finally, one patient receiving the ARV zidovudine as part of HAART developed anemia during the study, possibly because valproic acid increases the bioavailability of this drug. This side effect sets limits on how widely the treatment could be used. But as the first test of an HDAC inhibitor in the clinic, many experts agree the results are intriguing and deserve to be followed up with a larger study.

Blanekteed, bulldozed, or barely replicating?

But some of the questions raised about the paper reflect wider disagreement in the field. Some experts are wondering, for instance, whether the mechanism of latency that Margolis’ team is targeting is the most important one. Siliciano believes that latently-infected cells are a major contributor to the HIV reservoir responsible for rebound, but he thinks valproic acid is targeting the wrong process to awaken the virus in most of these cells.

Consider that by inhibiting HDAC valproic acid helps remove a blanket of inhibitory proteins that obscure the promoter of the integrated HIV genome, preventing it from expressing its genes and spooling off RNA copies of itself to form new viruses. This would suggest that latent HIV would be located in transcriptionally inactive regions of chromosome. But Siliciano says that recent studies have revealed that HIV actually prefers to integrate into active genes, which suggests a completely different mechanism by which its genome could be shut down: transcriptional interference (Trends Mol. Med. 10, 525, 2004). In this scenario the HIV genome is silent because the cellular gene in which it has integrated is so active that transcription factors can’t gain access to the viral promoter—they are bulldozed out of the way by active cellular transcription. “RNA polymerase complexes would be going right through these HIV genomes all the time,” explains Siliciano.

And there could be many other reasons why the virus may lay fallow. For example, some factors that HIV needs to transcribe its genes may not be fully active in some cells. Indeed, Dean Hamer at the US National Cancer Institute and his colleagues have shown in vitro that some latent HIV genomes can be revived by synthetic molecules called dacylglycerol lactones. These molecules stimulate protein kinase C, an enzyme known to boost HIV transcription by driving the activation of the cellular transcription factors NF-kB, c-Jun, and TAR-binding factors, as well as the virally encoded Tat transcription factor (J. Virol. 77, 10227, 2003).

Some researchers also question what role latent cells play in sustaining the reservoir given the numerous other possible places that HIV might conceal itself. “Reservoir is a garbage bag term, I don’t think it is one location or type of cell,” says Roger Pomerantz of the pharmaceutical research company Tibotec. To him the rapid recovery of virus after HAART cessation suggests the primary source for this rebound is not latent virus at all. “I think you have to have ongoing replication for it to happen so fast,” he says.

In 1999 Pomerantz’s team found evidence for this “cryptic” replication when they analyzed blood from 22 HIV-infected people on HAART with ultra-sensitive detection methods. They found that virus was present in the blood, but at levels below the 50 copies/ml level of detection of conventional tests (JAMA 282, 1627, 1999).

In retrospect, it isn’t surprising to find some viral replication. No drug combination is perfect. Cocktails that are 99.9999% effective would, by definition, allow for replication at one millionth of pretreatment level of about 10 billion virions per day. And that would be the case even if the drugs could reach every nook and cranny in the body. In fact, the availability of drugs in different tissue compartments can vary considerably. And other factors can limit how well the drugs operate. Many ARVs depend on cellular enzymes to convert them from precursors to an active form and not all cells carry the same levels of these enzymes. It also turns out that some cells are equipped with membrane-spanning molecular pumps called P-glycoproteins (PGPs) whose job it is to spit toxins out of cells. But unfortunately PGPs do just as good a job removing some drugs, particularly protease inhibitors.

The relative importance of low levels of viral replication versus latent virus for sustaining the reservoir, and the relationship between these two viral sources, is...
unclear. Siliciano favors the idea that leaks of latent virus from sporadically activated resting cells are the major source of rebound virus, suggesting that activation of the latent virus and its destruction is crucial to eradication. This viewpoint is partly based on the evidence of the longevity of this source of virus.

But some evidence suggests that latently-infected memory T cells may be less stable than Siliciano’s data suggests. A recent paper from Chun and Fauci argues that the latent reservoir is frequently refilled by virus replicating in sporadically-activated CD4+ T cells, extending the apparent half life of latently-infected memory T cells (J. Clin. Invest. 115, 3250, 2005). This leads the authors to propose a very different recipe for eradication: intensification of therapy to further cripple replication, plus drugs to dampen activation of latent cells.

Intriguingly, the Margolis paper could be taken as evidence for either model. Remember that besides valproic acid, the researchers intensified therapy aimed at stopping replication of the virus with a fusion inhibitor. So any actual reduction in the level of the latent reservoir could have been caused either by direct depletion of the reservoir, or slowing its refilling by active replication or a combination of both effects. Margolis suspects that viral latency and low-level replication may contribute to viral persistence to a greater or lesser extent in different patients on different regimens, and that both obstacles will need to be breached before eradication can be achieved.

A set of new studies may resolve some of these issues. One trial headed by Joseph Eron at the University of North Carolina at Chapel Hill is studying whether just the addition of T20 to HAART accelerates the decay of the latent HIV in CD4+ memory T cells (www.clinicaltrials.gov/ct/show/NCT00051831). Margolis and his team have begun a new small study in which his patients receive only HAART plus valproic acid. And Siliciano is looking for signs of rapid reservoir depletion in the “natural” experiment of HIV-infected people who were prescribed valproic acid because they are bipolar, or have seizures or headaches. “We’ve found there are lots of people who have been on valproic acid plus HAART for a long time,” he says.

Ultimately, developing the perfect cocktail of drugs to purge HIV may require understanding all the ways the virus manages to evade destruction by HAART for so long. Researchers say it will be important to develop better animal models of viral latency, such as SIV in the macaque, to take the hunt for hidden virus to a whole new level. “We can give the animals drugs and if the virus disappears from one compartment we can see if it’s gone somewhere else or just disappears,” says Margolis. “We can look through all the blood and every tissue, if that’s necessary. We can’t do that in people.”

Even when all of HIV’s hiding places are mapped, the prospects or timeline for turning that knowledge into a cure is uncertain. Hamer, for example, has argued that multiple steps may be required to allow HIV-infected people to live drug-free: developing HAART regimens that completely suppress viral replication throughout the body, developing drugs that activate latent HIV copies, targeting those activated cells for destruction, and some ongoing form of non-drug therapy (he suggests genetically-engineering cytotoxic T cells as one possibility) to stop any residual HIV copies from rekindling the infection (Curr. HIV Res. 2, 99, 2004).

But Hamer also retains a guarded optimism shared by many reservoir researchers. “There’s no evidence that HIV is genetically programmed to persist in the body,” he says. Instead, he argues, the virus simply benefits from the limits of current therapy. “So eradication might actually turn out to be quite simple. Nobody knows. Adding one right drug to HAART may push the virus down to a level where it doesn’t rebound. There’s no evidence that’s not the case.”
What are the key challenges you still face as a treatment activist in your country?

Unfortunately there are still many challenges in South Africa regarding treatment. In our country about 800,000 people currently need treatment and fewer than 110,000 are receiving it. Of these, fewer than 70,000 are in the public sector. That’s quite sad. There is also the need to establish second- and third-line regimens for people that fail their initial treatments and provide access to ARV treatment for children.

All of these problems are worsened by some serious mixed messages from our government, including denial by some of the science of HIV infection. This political and scientific denial really reinforces very deep, personal denial for many South Africans. The government isn’t utilizing the strong and open HIV-positive movement, which doesn’t exist in many other countries, to create further progress and that makes all of our tasks as activists much bigger. It’s particularly difficult for the individual who discovers they have HIV and then doesn’t have access to a doctor or nurse who understands what the issues are.

What is the situation with HIV prevention efforts in South Africa?

We don’t simply have a crisis of treatment; we also have a critical crisis of prevention. Our country had 500,000 new HIV infections last year and it’s critical that we act on that. It’s critical that we look at why the ABC [abstinence, be faithful, use a condom] message has failed. You cannot reduce prevention of HIV to a simple slogan. It is a caricature of what needs to be a comprehensive prevention program that is linked to serious treatment and care issues.

I think all of us know that prevention is the key to ending the epidemic and that means we have to find new tools, like vaccines and microbicides. But there isn’t a magic bullet and there’s not going to be one for...
a long time so we have to use the array of tools that we have at the moment, whether it is barrier methods like male and female condoms or programs to prevent the mother-to-child transmission of HIV. We have some decent programs on prevention, but currently we’re not doing enough to scale them up or to encourage openness about their use.

Why haven’t activists done more prevention advocacy?

For many activists their inhibition is discussing basic science. Unfortunately all of us that have worked in prevention haven’t developed the scientific literacy that needs to go along with a serious understanding of the social problems and inequality that inhibit behavior change. There is now some understanding of how gender and economic inequality hamper prevention efforts and put people at risk, but there isn’t a scientific understanding of prevention tools and how they can be used.

I remember when we were first starting to do HIV work and all we worried about was giving out condoms. We never said how the condom prevented transmission of the virus and it’s a tragedy that it took politicians and the Catholic Church to make us explain exactly how these tools work and get us to think about the science of prevention in a way we didn’t before.

There are numerous prevention service organizations with people who talk about condoms or voluntary counseling and testing, but I am yet to come across someone in those programs who actually understands the science. It’s just a simplistic ABC message, which is why these messages are so counterproductive because they actually stop people from thinking. Our first job as activists in South Africa was actually on the prevention of mother-to-child transmission and many of us who started TAC actually began in HIV prevention and human rights work. Now it’s sort of coming full circle as we are trying to make sure that what we learned in treatment goes back into prevention.

According to the latest report from UNAIDS and WHO on the status of the global epidemic, the HIV prevalence rates among pregnant women in Kwazu-Natal, one of the hardest hit provinces in South Africa, is around 40%. Is there still debate about the use of single-dose nevirapine as a way to prevent mother-to-child transmission?

I have never believed in having one standard for the north and another for the south, but you have a situation in many countries where there are no antenatal services for poor women and so single-dose nevirapine is a first step. It provides an entry point for building the antenatal and treatment services that are needed. To automatically say that this regimen is third class and you either have to have the best or nothing at all is not practical. Even single-dose nevirapine is reaching less than 10% of people who need it. That frustrates me. We’re still delaying both prevention and treatment significantly.

Presumably it’s even more difficult to explain the basic science involved in the research and development of vaccines and microbicides. How can this be accomplished?

South Africa is one of the few countries where there is a relatively good understanding of microbicides among activists and increasingly within civil society because there are some really good researchers in the country. And all of us that are activists, whether in prevention or treatment, now have a much clearer understanding of what we need to do to ensure that there is access to information about microbicide and vaccine development. It’s difficult to explain the science of microbicides and vaccines, but no more difficult than treatment. HIV treatment has allowed us to become engaged in science and it’s time that we became a lot more scientifically literate about HIV prevention.

We need to find a way to reach out to a broader community and find people who love to talk about basic science and then bring them into the HIV movement so that we get to the point where the conversation about HIV vaccines, microbicides, and new medicines is an informed scientific conversation. There has to be a certain level of scientific literacy within communities because otherwise they can be exploited by either quacks or people who wish to misuse science for commercial or political ends.

I also believe it’s important that we as activists don’t try to undermine the outcomes of science. Whether it’s favorable to what we believe or not, we have to support the integrity of the scientific process.

Recently there has also been a great deal of discussion about male circumcision to prevent HIV infection in men based on the results
of a study in South Africa. How do you think the international community should react to this?

As soon as there's a scientific consensus we need to move with rapidity. But first we have to be aware of and prepared for every single pitfall. You have to consider situations where young men will go and get circumcised in a bush with unclean implements, without having been tested for HIV.

It’s really critical that there be a global and urgent summit to discuss an appropriate way to respond to this. If the reduction is valid, then it will be an important intervention and it should be offered to every man who wishes to do it, along with condoms and other means of protection.

Many African countries face problems with infrastructure and lack of medical centers or trained physicians. Is this a problem in South Africa?

It’s not South Africa’s major problem but there is a problem with human resources. I was just looking at some research that said 12-16,000 of our nurses and doctors work outside South Africa. There are also 55,000 trained nurses inside the country that are working outside the healthcare system. So there’s a huge potential pool of people that just need better pay, improved conditions, and minor retraining to be brought back into the system.

You were in New York City recently to attend a Global Health Summit sponsored by TIME magazine. Do you think it is important for the international media to keep global health issues in the news?

I think it is a major step forward that the US media in particular is talking about global health problems and raising it as an issue to inform Americans. Now this needs to be matched with the mobilization of civil society in the US on health, both locally and globally. It’s very important to raise the issue of global public health and not just in terms of economic consequences or cost-effective strategies, but on what Helene Gayle [director of AIDS programs at the Bill & Melinda Gates Foundation] referred to as the policy of being a good neighbor and if my neighbor is sick then I should do something about it.

In that sense we still have a long way to go. We have to create a consensus that everyone has the right to life and everyone has the right to health care. And that includes understanding that the right to life is about a life with dignity.

What role has the South African media had in covering the country’s epidemic?

The media in South Africa has played a critical role in discussing HIV. They raised awareness on the government’s delay on providing treatment and on a range of other issues. There’s still a lot more the media can do, but it’s much better than almost anywhere else that I’ve seen. They’ve been dealing with the issues in a non-sensationalist and non-judgmental way and clearly laying out what still needs to be done.

South Africa is now hosting a Phase II vaccine trial and a Phase III microbicide trial. Do vaccine and microbicide trials in general receive much attention in the South African media?

Microbicides and vaccines get coverage, but the problem with the publicity has been with talking about them as magic bullets. This causes a degree of skepticism, both in the public and the activist community, about the potential for microbicides and vaccines. Skepticism is good for most things, but I think we need to eliminate this type of skepticism because it can paralyze us from taking action or wanting more information about these important strategies. There’s no way we can proceed with an infection of this nature that continues to infect millions of people across the globe, and at least half a million people a year in our country alone, without educating ourselves.

We need to ensure that we understand the range of measures that need to be taken to end the AIDS epidemic. We can end the epidemic but there are at least two things we have to do: find a vaccine for tuberculosis (TB) and HIV. So I would like to see organizations like IAVI work closely with AIDS and TB activists. We have to end the solo approach to treatment and prevention and look at the broader impact and use of HIV as a way to promote really good medical care for everyone’s benefit.

As the AIDS vaccine community begins discussing the possibility of testing vaccine candidates in adolescent volunteers there will undoubtedly be discussion about South Africa since there is such a high prevalence rate among 15-24 year olds. Is there any momentum building for this type of trial?
There's no momentum for it and there's not enough talk about the young people. I think that's certainly an area where we need to do some work. There's obviously a range of consent and possible infection issues involved, but the fact is clear that if you stand at least a 1 in 10 chance of getting infected then there's a duty to prevent that. And just as we try to advocate for condoms in school, we should advocate for very good trial practices for adolescent volunteers.

What advice would you give to the activist community?

TAC is regarded as one of the strongest movements in the country and as one of the strongest movements of people living with HIV in the world, yet I don't believe we reach 1 in 100 people in our country, maybe a little more or a little less. But there are 46 million people in our country. And in any other country the burden of dealing with such a public health crisis would not fall on organizations like ours, it would fall on the state, so we have to reach more people. We have the capacity. In our organization more than half of our activists are under 25. I'm really one of the oldest and I think these young people are essential. But we don't have the resources to reach as many people as we want.

Still we all must continue to educate ourselves, spread the message, and ensure that there's money available. But then also start looking three, five, even ten years ahead. What happens when a vaccine or microbicide becomes available? Do we have the systems ready for it? How do we make sure that access is once again not going to be limited? Discussion about vaccines allows us to talk about issues with intellectual property that rewards research and development and allows companies who want to make a profit to do so, while at the same time ensuring the widest possible access everywhere. Every person has the right to decent health care whether it's in the US, China, India, or South Africa. ☛
Inventions innovate thyself

To accelerate the discovery of an affordable AIDS vaccine and other crucial health resources, experts say they will need to invent new ways to use intellectual property

By Philip Cohen

Last summer, AIDS vaccine aficionados turned their attention to Brazil partly for the science and partly for the spectacle. The science emanated from the 3rd International AIDS Society Conference on HIV Pathogenesis and Treatment where the presentations had a strong focus on a wide array of prevention strategies: from circumcision and diaphragms to vaccines. The spectacle was provided by the continuing war of words between Brazil’s government and the American pharmaceutical company Abbott over its patent for the antiretroviral (ARV) Kaletra.

Brazil had announced plans to manufacture a cheaper generic form of the drug (which is also known as lopinavir/ritonavir), arguing that Kaletra’s high price was making it impossible to reach their treatment goals. The country’s health minister forged an agreement with the company to respect the patent in return for a cut-rate price—but then he promptly resigned. The new health minister announced he had to rethink the agreement. Local AIDS activists and at least one other government official at the IAS conference openly criticized the deal as too costly.

After many months of heated negotiations, a new deal between Brazil and Abbott was finally reached in October. Shortly after, another battle between a government and Big Pharma grabbed the headlines. This time Taiwanese officials—driven by fears of a potential bird flu outbreak—threatened to produce a generic version of Roche’s influenza drug Tamiflu if the company did not grant a license by the end of the year. Roche began discussion over a licensing deal, but decided it could supply the drug more cheaply than a Taiwanese company under a voluntary license. According to a November press release on the company’s website, Roche will provide Taiwan with an additional 1.3 million treatments, making a compulsory license “unnecessary.”

These public struggles illustrate an issue that has long been on the minds of AIDS vaccine developers—the need for new ways to strike a balance between preserving the innovation incentives of intellectual property (IP) without sacrificing the affordability of new medicines.

Intellectual property describes a range of knowledge-based assets that give their owner exclusive ability to sell some product or service. The best known form of IP is a patent, a published description of an invention which grants its holder a legal monopoly for use or sale of that invention for a number of years. But some of the IP relevant to vaccine production can also be in the form of carefully guarded data or know-how, such as secrets about processes that allow vaccine production to be scaled up.

Most of the time you pay for some new technology, whether it’s an iPod or antiviral, somewhere an inventor responsible for ideas or IP inherent in the device gets a bit of money. While consumers traditionally pay the price for IP, the rationale for its existence is that society at large benefits from financially rewarding creativity and innovation. The argument goes that without exclusive marketing rights secured by IP, companies would never embark on risky research towards innovative products because another company that didn’t make the same investment could simply copy the final product and sell it at a lower price.

But the incentive of IP and other aspects of the market-driven system have failed to deliver some crucial health technologies. For one, the uncertainty of an adequate market for treatments or preventives for diseases that mostly afflict people in developing countries has failed to entice many companies to invest in research and development in these areas. In other cases medications are developed but, partly because of the IP involved, they are not available at prices within the budgets of countries where these health technologies are desperately needed.

Navigating the set of laws and contracts that define IP is infamously difficult. Even some experienced lawyers, for example, refer to the legal framework of patents as “the thicket.” And if the legal status quo wasn’t complicated enough already, IP laws are currently undergoing a wave of reform in the US (see box) and internationally.

Brazil and Taiwan claim their rights to manufacture, respectively, generic Kaletra and Tamiflu under a World Trade Organization agreement known as the Trade Related Intellectual Property Systems (TRIPS) agreement. This agreement obligates developing countries to adopt minimum standards to protect IP
While the TRIPS treaty has been shaking up the global patent landscape, independently the United States has been busy revamping its own IP laws which could indirectly affect AIDS vaccine development.

The Cooperative Research and Technology Enhancement Act, also known as the Create Act, was signed into law in December 2004. It allows companies and academic institutions working with research partners to share confidential information without jeopardizing future patent applications. Until now such disclosures could be considered “prior art” raising questions on the novelty of the technology—a reason patent applications can be denied. Protecting IP arising from collaborations is important since AIDS vaccine research increasingly involves different partners working together.

In June, a bill called the Patent Reform Act of 2005 was introduced in the US Congress to modernize American patent law. The reforms, which are being hotly debated, are intended to streamline the resolution of patent disputes and bring the US system in line with international norms such as giving priority to the first person to file a patent, rather than the first person to invent. Some experts worry that if these reforms are enacted, companies will gain an upper hand over academic institutions in pursuing AIDS vaccine research.

Using IP as incentive is a controversial component of proposed US anti-terror legislation known as Bioshield II. The idea is that in return for developing new antiterror tools a company would be eligible for a range of incentives, including “transferable” patent extensions which will not apply to the antiterror technology but, say, one of their best-selling drugs. The legislation specifically lists HIV as one agent that may be used as a weapon of mass destruction and HIV/AIDS vaccines as a terror countermeasure under the provisions of the act.

rights in exchange for other trade benefits of WTO membership. A few years ago the possible impact of TRIPS on the affordability of medicine to developing nations became the focus of a major debate in the WTO. This resulted in the Doha declaration, which asserted that during a public health crisis developing countries have the right to grant “compulsory licenses,” allowing local companies to produce medicines without permission from the patent holder.

But overall TRIPS is leading to a strengthening of intellectual property rights, which experts feel could have global implications for development of new therapies. For instance, Brazil’s current ability to provide low cost ARVs to its citizens is the result of a network of pharmaceutical firms from developing countries that grew up with no tradition of patent protection and, for that reason, can supply drugs at bargain basement price. ARV treatment programs in Kenya, South Africa, Malawi, and Thailand similarly rely on a discounted global supply chain including Indian, Chinese, and Korean companies. But as countries adapt to the provisions of TRIPS, this situation is rapidly changing. Just this year India changed its laws to recognize patents on pharmaceutical compounds issued after January 1, 2005 and all TRIPS signatories need to revise their laws by 2016. One concern that now looms is that in the post-TRIPS world where unlicensed generics on new medications are illegal, drug costs could soar out of the reach of many poor countries.

TRIPS could hold similar ramifications for an AIDS vaccine. Vaccine designers often must incorporate existing IP into new vaccines and the holders of those patents may be entitled to royalties from the sale. For instance, the world’s first genetically-engineered vaccine—against hepatitis B virus—required 14 patented components and royalties amounting to 13% of total sales. But vaccine production also depends on undisclosed know-how, which would make it harder for developing countries to produce the vaccine since these processes would need to be reverse engineered even if a local company resorted to a compulsory license to get around existing patents. Even so, companies worry that they could have the same sort of showdown over prices that Abbott now faces with Brazil, says Phillip Gomez, director of vaccine production at the Vaccine Research Center of the US National Institutes of Health. “I think one of the biggest fears some large pharmaceutical companies have is that they will own a successful HIV vaccine,” he says. “They will then be under tremendous pressure to produce billions of doses to sell at next to nothing.”

An AIDS vaccine might also represent special challenges for IP management, says Helen Kettler of the Bill and Melinda Gates Foundation. “Given the number and difficulty of the unanswered [scientific] questions, there is a critical need for greater collaboration and information-sharing across the HIV vaccine field, especially on issues related to early-stage vaccine discovery,” she says. Also, she says that since developing countries are making significant contributions to the research and testing of vaccine candidates, ethics demand that they be rewarded for their efforts. She points out that these issues are why the plan for the Global HIV/AIDS Vaccine Enterprise pinpointed creating an “enabling environment” for IP as an important step towards accelerating progress.

So if IP represents a potential obstacle to the AIDS vaccine field, what’s the best way to overcome it? James Love, director of the Washington-based Consumer Project on Technology, thinks that the best solution is to do away with the current system of IP entirely. Love has argued that public health would be better served by systems where essential public health research is publicly funded, monopoly protections of IP are suspended, and information is freely exchanged as in the open source software movement (PLoS Biol. 2, 147, 2004). “Historically it was a big, profound mistake to give inventors of new medicines an exclusive right to sell that medicine. It’s not wrong to give them money, but it’s wrong to give them a monopoly.” As an alternative, he points to legislation recently proposed in the US.
Congress called the Medical Innovation Prize Fund which would set aside part of the US government’s budget to pay enormous cash rewards to researchers who create certain innovative drugs or vaccines, but removes the monopoly protections of IP. “This way you divorce the market for the product from the market for innovation,” he says. He admits that such a radical rewrite of health policy will take years to implement, and be fiercely opposed by companies, but thinks consumers will support the legislation.

But less drastic measures can overcome barriers that IP may represent to affordability, says Richard Mahoney who just completed nine months as the interim CEO of the Centre for the Management of IP in Health Research and Development (MIHR) in Oxford, UK. MIHR promotes innovative IP practices to address social and economics inequalities. He believes that some of the uneasy relationship that now exists between large companies eager to protect IP and governments, non-governmental organizations (NGOs) and other nonprofits such as public-private partnerships (PPPs), stems from the relative inexperience these public sector agencies have with IP. “If you work for the private sector, there are units of lawyers and business managers around you who worry about IP,” says Mahoney. “But the discipline of IP in the public sector is very, very embryonic. It’s mostly a large, dark space.”

To help PPPs shed some light on IP, MIHR and the Initiative on Public-Private Partnerships for Health organized a workshop dubbed “Dealmaking and Intellectual Property Management for Public Interest” in November 2004. There, PPPs met to compare experiences brokering IP deals and learn from each other. “What was clear from all the case studies presented is that you can’t have a general contract. Each one needs to be tailored for the needs of each partner,” says Jerry Sadoff, president of the Aeras Global TB Vaccine Foundation, which hosted the event.

The fine details of such deals are rarely publicly disclosed, but based on case studies presented at the meeting Sadoff says almost every arrangement imaginable is being made. Even within Aeras Sadoff says the deals range from agreements with pharmaceutical companies who insist on maintaining all IP rights to vaccine candidates on which they collaborate, to other candidates for which Aeras owns all the IP and may license with companies for production. For TB vaccines for which his foundation doesn’t own the IP, Aeras gets assurances for reasonable prices for developing countries or makes deals where vaccines will be purchased at an agreed price above the manufacturing cost, or the company agrees to license their technology for others to scale up production for developing countries. If there is some general lesson, says Sadoff, it’s that with enough effort and enough lawyers on the payroll there is usually some way to add the IP concerns of both parties. “And if there isn’t, then you walk away from the deal,” he says. “And we’ve also done that.”

Mahoney says that reaching agreements on IP between a company and a nonprofit may sometimes be easier than between two profit-making companies. “What the public sector wants is complimentary and non-competitive to what the private sector wants,” says Mahoney. Companies by their nature are interested in the potentially lucrative markets for vaccines in developed countries, while nonprofits are concerned with supplying the vaccine to very poor countries where it may need to be sold at a much lower cost.

Gomez points out another way in which governments or PPPs and companies have complementary IP interests. “If we approach a company about working on HIV vaccines, we are only focused on HIV,” he says. “So our agreements try to leave them free to use any innovation in another more profitable part of their portfolio, for example cancer, while still allowing our HIV product to move forward.”

When it comes to IP for an AIDS vaccine, it’s important to remain optimistic and be prepared for hard work, says John Barton, an emeritus law professor at Stanford University who recently spent a year at the NIH studying vaccine technology transfer issues. “I’m sure there will be problems assembling the IP package for an AIDS vaccine. And I’m sure they are solvable, because the imperative is so great.”
Viral defense: Use it or lose it

Human proteins with the innate ability to fight off retroviruses are a hot topic since they could form the basis of novel therapeutic approaches. A recent report by Harmit Malik and Michael Emerman at the Fred Hutchinson Cancer Research Center and their colleagues suggests that at least one of these virus fighters is less powerful now than it may have been in the past (Curr. Biol. 16, 95, 2006).

These researchers were looking at naturally-occurring variation in the human gene for TRIM5α, a protein recently implicated in the species-specificity of retroviral resistance in primates (see Making a monkey out of HIV, IAVI Report 9, 3, 2005). Previously, this group analyzed the primate lineage and found that TRIM5α had undergone sporadic episodes of rapid evolution for at least 33 million years, consistent with selection imposed by the emergence of new retroviruses.

The new work focused on whether naturally-occurring variants of human TRIM5α contribute to a range of susceptibility to different retroviruses. The researchers analyzed genes from people representing 37 different regions around the world.

The analysis yielded 20 single nucleotide polymorphisms (SNPs) in the human population. Based on sequence conservation and structural prediction, three of these were predicted to possibly affect the function of TRIM5α. These candidates were then expressed in cell lines to test their ability to confer resistance to retroviruses. Only one, which changed a histidine to tyrosine at position 43 (H43Y), detectably altered protein function—for the worse.

In one assay, the researchers looked directly at human B-lymphocytes taken from four individuals with two, one, or no copies of H43Y TRIM5α. Cells from the person with no copies of H43Y were able to potently restrict N-MLV, a mouse retrovirus susceptible to the human protein. The two people with two copies of this variant restricted the virus about 100 times less potently. And a single copy of H43Y rendered B cells from the last person about 10-fold less potent.

This defective version of TRIM5α is not rare. The researchers found H43Y in 20% of chromosomes. They suggest that H43Y may have been able to flourish because it hasn’t been necessary in recent human history to fight retroviruses, either exogenous ones or those endogenous in our chromosomes. They point out that the human genome sequence contains thousands of examples of endogenous retroviruses, all of which appear to be defective, and that humans only appear to be currently infected by two retroviruses, HTLV and HIV. It’s also clear that humans have at least one other layer of innate protection against retroviruses, a protein called APOBEC3G (see Guardian of the genome, IAVI Report 9, 2, 2005), perhaps rendering TRIM5α redundant.

The authors also suggest that diminished pressure from retroviruses could make TRIM5α as much a risk as a benefit. The H43Y SNP lies in the protein’s RING domain, which bestows on some proteins the ability to tag target proteins with ubiquitin that are then shuttled to the cell’s proteasomal disposal pathway. So, occasionally, TRIM5α may inappropriately destroy the cell’s own proteins, perhaps giving people who have eliminated this function a slight biochemical edge. The work suggests that any attempt to redirect innate antimicrobial activity against HIV will need to account for the fact that these systems may not be optimized in a significant portion of the human population.

How cells get hooked on HIV

HIV’s gp120 core protein participates in many important stages of the viral replication cycle and host response including binding coreceptor, viral fusion with target cells and sensitivity to antibody neutralization. Now, for the first time, researchers have been able to capture a snapshot of one domain of gp120 that is important in all those roles: the third variable region, or V3 (Science 310, 1025, 2005).

The HIV envelope spike is composed of six protein molecules, three of gp120 and three of gp41. Through gp120 the virus attaches to the CD4 protein on a target cell’s surface, shifts its structure to grab a coreceptor (either CCR5 or CXCR4), the binding of which then initiates a series of structural transformations in gp41 ending with the fusion of the virus and cell. Biochemical and genetic evidence suggests that V3 plays an important role in coreceptor binding. But presumably due to the flexibility of this region, V3-containing gp120 has proven difficult to crystallize, which has left its detailed structure unknown.

Peter Kwong at the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases and his colleagues began their hunt for V3 crystals by using robots to screen many different combinations of proteins, chemistry, and time points. They screened gp120 from 3 different clade B HIV-1 isolates mixed with a shortened version of CD4 and 1 of 6 anti-gp120 antibodies to create 13 different complexes. Each of those complexes was dissolved in 576 different solutions and left to form crystals. Pictures of each crystallization reaction were taken at time points out to 21 days and visually inspected. Promising candidates for crystal formation were then optimized for growth of larger crystals and good X-ray diffraction characteristics.

The structure derived from those crystals shows the V3 protein chain forming a highly extended “hook” 50 Å long, 15 Å deep and only 5 Å wide. In free virus, the authors suggest, this hook could reach across to associate with the other proteins of the viral spikes, potentially playing a role in driving their interaction or structure. But it also appears to be flexible enough for every surface of it to be exposed to antibodies, explaining why V3 is a major focus of antibody response to HIV.

In gp120’s CD4-bound conformation, V3 extends towards the target cell 30 Å from the gp120 core, ready to grasp the appropriate coreceptor molecules. Once bound, the authors also suggest V3 could act as a “rip cord,” linking coreceptor binding to deployment of the viral fusion program of gp41.
Microbicide interferes in viral affairs

Vaginal microbicides could be powerful new HIV prevention tools—monoclonal antibodies, CCR5 inhibitors, and other less specific antiviral agents have been studied and clinical trials of some compounds are ongoing. Now researchers at Harvard Medical School led by Judy Lieberman and David Knipe have extended the microbicide concept to harness a new technology: RNA interference (Nature 439, 89, 2006).

RNA interference is mediated by small interfering RNA (siRNA) molecules, short (21-23 bp) double stranded RNA molecules that complex with proteins to form the RNA-induced silencing complex (RISC). When the siRNA within the RISC pairs precisely with its target RNA strand, cleavage occurs and the target RNA is destroyed. The acute specificity of siRNA sequences has encouraged researchers to evaluate their efficacy in silencing viral gene expression and thereby mitigating infection and disease. The limiting factor, as with most gene therapy approaches, has been the delivery of the siRNAs to their intended site of action.

To investigate delivery and uptake of siRNAs, the researchers used the transgenic green fluorescent protein (GFP) mouse that constitutively expresses this protein in all cells. SiRNAs were complexed with a transfection lipid to facilitate crossing cell membranes and instilled into the GFP mouse vagina. The siRNAs were efficiently taken up by the vaginal and ectocervical epithelium, as well as the underlying lamina propria and stroma, and genetic silencing of GFP persisted for at least nine days.

They then looked to see if topical siRNA application could protect against herpes simplex virus 2 (HSV-2), a sexually-transmitted infection that is lethal in the mouse. SiRNAs complementary to the UL27 and UL29 genes—which encode an envelope glycoprotein and a DNA-binding protein, respectively—were instilled intravaginally into mice 2 hours before and 4 hours after vaginal challenge with 2LD50 of HSV-2. Only 25% of mice given an irrelevant siRNA survived to day 15, whereas 75% of those given UL29-specific siRNA survived.

This protection was not due to inflammation or induction of interferon-responsive genes. Also, the researchers did not find any indication of escape from the siRNA sequences, but acknowledge that this could be a bigger concern for RNA viruses like HIV that have a higher mutation rate than with DNA viruses like HSV-2.

One of the most striking features of the study was the efficient uptake of and lasting silencing by siRNAs in the vaginal mucosal layer, an important consideration for any practical microbicide. Whether a similarly efficient uptake and longevity of effect will be seen in primates, and more specifically humans, remains to be determined. Another crucial question is whether siRNAs can be effective against HIV in infection models; previous HIV/siRNA research has only been done in cell culture.

The research team now intends to evaluate the concept on HIV infection in primates. They plan to target highly conserved viral genes and HIV’s cellular coreceptor, CCR5, to see if down regulation of its expression augments any benefit of targeting HIV’s own genes.

What drives HIV envelope evolution?

One of the most frustrating problems that HIV researchers are confronting is the virus’ ability to escape from neutralizing antibodies (NAbs). HIV has an intrinsically high mutation rate and the env gene encoding the envelope glycoprotein, gp160, that is the primary target for NAbs evolves at an especially high rate. Immediately following infection, env genetic diversity is low and then narrows further, increasing to a peak after several years. As a result env is extremely genetically diverse and poses one of the trickiest challenges to vaccine development.

A number of factors contribute to this genetic diversity but the relative importance of each and the precise mechanism remain to be determined. Selection for CXCR4 coreceptor usage can generate env diversity, but this doesn’t happen until late in infection and involves only a limited number of amino acid residues. Escape from cellular immune responses also drives diversity in env, but cellular responses are usually stronger towards other HIV genes. Selection by NAbs, however, results in rapid, continuous evolution of viral escape at the phenotypic level, and so may be the most significant driving force.

Three mechanisms can contribute to this escape from neutralizing antibodies: point mutations, changes in glycosylation patterns, and insertions and deletions in the envelope. To explore the relative contribution of each, Douglas Richman at University of California at San Diego and his colleagues compared env genetic variation in a cohort of 13 recently HIV-infected men with different rates of escape from NAbs responses (Proc. Natl. Acad. Sci. USA 102, 18514, 2005). They measured NAb responses in a virus assay that used recombinant virus particles containing patient virus envelope proteins plus an HIV genomic vector with a firefly luciferase indicator gene insert.

As in previous studies, HIV-specific NAb responses varied greatly within the cohort. The researchers used the rate at which patients’ NAb responses decreased against successive autologous virus isolates as a measure of their rate of viral escape. After fitting data to a statistical model, they classified individuals as having high and low rates of viral escape. They then compared the patterns of env genetic variation in the virus from the two groups. There was no correlation between the rate of escape from NAbs and the rate of evolution of glycosylation sites, nor insertions and deletions. However there was good correlation between the rate of NAb escape and the rate of evolution of amino acid substitutions, consistent with previous observations in SIV-infected macaques.

This is contrary to previous in vitro work suggesting that a variable “glycan shield” due to mutations in glycosylation sites provides protection from NAb in recent infection (Nature 422, 307, 2003). The authors note that such NAb escape may require several substitutions at glycosylation sites and, since this will be reliant on the accumulation of single point mutations, this mechanism may be secondary.
First Phase II AIDS vaccine trial begins in South Africa

A clinical trial evaluating the safety and immunogenicity of tgAAC09, a recombinant adeno-associated virus (AAV) vector-based vaccine containing clade C HIV antigens, recently began at three sites in South Africa, including clinics in Soweto, Cape Town, and Medunsia. The randomized, placebo-controlled, double-blind trial will evaluate two inoculations with the candidate at three different doses and two dosing intervals. This is the country’s first Phase II AIDS vaccine trial and investigators will enroll and follow 78 volunteers over a period of 18 months.

Preliminary safety data on the candidate administered at lower doses was established in a joint, multi-country Phase I trial conducted in Belgium, Germany, and India. The candidate was designed by Philip Johnson at the Columbus Children’s Research Institute in Ohio and Children’s Hospital of Philadelphia and developed and manufactured by Targeted Genetics Corporation in Seattle.

The South African trial is a collaboration between Targeted Genetics and IAVI and is an important advancement in a country where 25 million people are currently estimated to be HIV infected. Other arms of this Phase II trial will occur in Zambia and Uganda, after receiving final regulatory approval in these countries.

South Africa is now also hosting another important HIV prevention trial involving the microbicide candidate PRO 2000, a vaginal gel consisting of a synthetic polymer that binds to HIV and acts as a fusion inhibitor, preventing the virus from infecting target cells. This placebo-controlled Phase III trial will enroll over 10,000 women volunteers in South Africa, Uganda, Tanzania, and Zambia, making it the largest microbicide trial to date. The trial began enrolling volunteers recently in Johannesburg and is being coordinated by the UK Medical Research Council.

Rwanda starts first AIDS vaccine trial

A Phase I AIDS vaccine trial to evaluate the safety and immunogenicity of a prime-boost vaccine regimen recently began enrolling volunteers at a site in Kigali, Rwanda. The two candidates, a DNA plasmid vaccine followed by an adenoviral vector vaccine, were developed by the Vaccine Research Center (VRC) of the US National Institutes of Health (NIH). This is the first AIDS vaccine trial to take place in Rwanda and is being conducted by the NIH, IAVI, and Project San Francisco, a research organization that has been working in Kigali for almost 20 years.

Volunteers in this placebo-controlled, double-blind trial (IAVI V001) will be randomized to receive either the multi-clade adenovirus serotype 5 (Ad5) candidate alone or the DNA/Ad5 candidates in a prime-boost sequence. The naked DNA vaccine is comprised of a fused gag/pol/nef construct from subtype B, the primary viral clade found in Europe and North America, and HIV env genes from subtypes B as well as A and C, which are the most common subtypes in Africa and parts of Asia. The boost vaccination is an Ad5 recombinant vector containing gag, pol, nef, and env genes. The adenovirus vector was developed by the VRC in collaboration with GenVec, who also manufactured the vaccine. The DNA components were manufactured by the California biotechnology company Vical.

This Phase I trial will also soon begin enrolling volunteers in Kenya and the prime-boost approach will be evaluated in HVTN 204, an ongoing Phase II trial at HVTN sites in North and South America, Haiti, Jamaica, Botswana, and South Africa. The DNA/Ad5 candidates will also be tested in other Phase I/II trials that are expected to begin soon at other clinical trials sites in Uganda, Kenya, and Tanzania in partnership with the US Military HIV Research Program.
New public private partnerships for AIDS vaccine development

Two new partnerships between private sector companies and non-profit organizations were recently established to focus on the development of novel AIDS vaccine candidates. The first between IAVI and Transgene, a French biopharmaceutical company, will focus on the development and manufacturing of an AIDS vaccine candidate using an adenovirus serotype 35 (Ad35) vector.

Two currently ongoing trials are using Ad5 as a vector, including Merck’s Phase Ib “test of concept” trial and the DNA/Ad5 prime-boost strategy being evaluated by the VRC (see Rwanda starts first AIDS vaccine trial, this issue). However, the immune responses induced by Ad5-based vaccines could be limited by pre-existing immunity to the viral vector in some populations. Researchers have therefore focused their efforts on the development of novel candidates based on other adenovirus serotypes, including Ad35, which naturally infect fewer people worldwide.

The vaccine candidate will be manufactured at Transgene’s facility near Strasbourg, France for eventual evaluation in clinical trials. IAVI has worked with Transgene in the past on the manufacturing process of modified vaccinia Ankara (MVA) constructs and on other characterization studies.

A second partnership between GlaxoSmithKline (GSK) Biologics in Rixensart, Belgium and the Institut Pasteur in Paris will concentrate on developing an AIDS vaccine candidate using the measles vaccine as a vector to carry HIV antigens. Researchers hope that this vaccine concept will induce similarly strong and persistent immune response to HIV as it does for measles. The measles vector technology will be licensed from Institut Pasteur to GSK and the initial project, which includes manufacturing the vaccine candidate and evaluating its safety and immunogenicity in two clinical studies, is supported by a grant from the European Union.

GSK is also working with IAVI on other AIDS vaccine vectors based on non-human primate adenoviruses. Several other public private partnerships have also recently been established to research and develop other vaccines, including collaborations between GSK and Aeras Global TB Vaccine Foundation for tuberculosis vaccines and GSK and the Malaria Vaccine Initiative to complete development of an advanced malaria vaccine candidate.

WHO and UNAIDS release update that focuses on HIV prevention

The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) recently released their annual report on the global AIDS epidemic. It highlights the progress made by some countries in lowering HIV infection rates despite a continued increase in the total number of people infected with HIV throughout the world, which now is estimated at 40.3 million. The report, AIDS Epidemic Update 2005 (www.unaids.org/Epi2005/doc/report.html), was released in advance of World AIDS Day on December 1 and focused on the importance of HIV prevention efforts and the need to increase and improve efforts that focus on behavior change throughout the world.

Kenya and Zimbabwe are two African countries where behavioral changes, such as an increase in the uptake of voluntary counseling and testing (VCT), a delay in the initiation of sexual contact, and a reduction in the number of sexual partners, are linked with a decline in HIV prevalence over the past few years. National prevalence rates among adults in Kenya dropped to 7%, while Burkina Faso also witnessed a decline in prevalence among young pregnant women in urban areas.

But there were still nearly 5 million new infections in 2005 and more than 3 million AIDS-related deaths. Sub-Saharan Africa was the hardest hit region globally, accounting for 64% of all new infections or more than 3 million newly HIV-infected people. Infection rates continued to rise in Mozambique, Swaziland, and South Africa.

Eastern Europe and Central Asia saw the sharpest rise in infection rates where a 25% increase in total infections now accounts for 1.6 million infected individuals. In these areas as well as in East Asia and Latin America—where there were 200,000 new infections—the epidemic is now being fueled by both injection drug use and heterosexual transmission. Pakistan and Indonesia are also now facing explosive epidemics among both injection drug users (IDUs) and sex workers. The only region that didn’t have an increase in the number of new HIV infections was the Caribbean, where there has been an increase in VCT services and condom use among sex workers.

“We really are failing to prevent this epidemic in most parts of the world,” says Jim Kim, director of WHO’s HIV/AIDS program. “And we have real opportunities to scale up prevention.” He said one of those opportunities is ensuring that some of the momentum created around access to HIV treatment programs in developing countries, including more available funding, is extended to HIV prevention efforts. This can help bolster existing programs, allowing countries to scale up VCT programs and focus on preventing mother-to-child transmission of HIV.

Efforts must also be made to improve the number and reach of harm reduction programs such as methadone maintenance therapy for IDUs and Kim says progress is also being made on this front. “China is committed to scaling up harm reduction in every province by 2008,” he says.

The greatest obstacles to expanding treatment programs are the need for more funding to increase the number of prevention workers and overcoming the stigma that is associated with accessing VCT and harm reduction programs in many countries.