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ADENOVIRUS VECTORS: PROMISE AND PITFALLS

ALSO:

HIV Persistence Workshop Report

Global Fund Woes

EDITOR'S LETTER

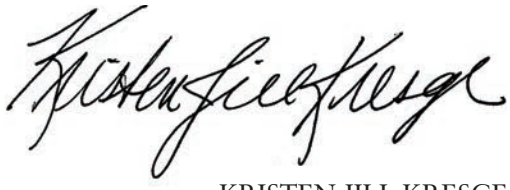
When I think back to when I first started writing for *IAVI Report*, I can recall being concerned that there wouldn't be enough happening in AIDS vaccine research to justify authoring article after article on the topic. Was I wrong! Seven years later, I am still surprised by just how much is happening in vaccine research and related fields, and how many new developments there are to report on.

In this issue, we tackle the most recent developments in HIV cure research and some of the parallels between this field and vaccine research (see page 12). Another feature article examines how the global recession has triggered a funding crisis at The Global Fund to Fight AIDS, Tuberculosis and Malaria, which provides a quarter of the international funding for HIV/AIDS programs throughout the world (see page 8).

On the vaccine front, we describe the details of two new trials that began recently (see page 17), and also review two recent studies that highlight both the promise and the pitfalls of using adenovirus (Ad) vectors as the delivery apparatus for HIV vaccine candidates (see page 4). The first Ad vector-based candidate (Ad serotype 5), tested in the STEP trial, failed to provide any effect. Researchers are still analyzing data from this trial, and another trial of the same vaccine candidate, and are learning more about how pre-existing immunity to Ad5 may hamper immune responses to HIV. Unfortunately, their analysis suggests that other Ad vectors based on different serotypes might also face these same problems. Yet, these alternate Ad vectors have also shown promise in pre-clinical studies. For now, researchers are pushing ahead with testing these alternate Ad vectors.

Finally, on a personal note, I want to introduce a few changes that will be taking place at *IAVI Report*. Starting in March, I will be taking maternity leave and handing over the reigns as Editor to Unmesh Kher, a gifted writer and editor whose work has graced the pages of everything from *Nature* to *TIME*. Because of my leave, we will also be publishing four print issues of *IAVI Report* instead of six this year. To fill the gap, we will be publishing more online-only features, so I hope you will visit www.iavireport.org often.

I'm sure that upon my return, there will be plenty of news and progress to report, baby-related and otherwise.



KRISTEN JILL KRESGE

The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. IAVI supports a comprehensive approach to addressing HIV and AIDS that balances the expansion and strengthening of existing HIV-prevention and treatment programs with targeted investments in the design and development of new tools to prevent HIV. IAVI is dedicated to ensuring that a future AIDS vaccine will be available and accessible to all who need it. IAVI relies on the generous donations from governments, private individuals, corporations and foundations to carry out its mission. For more information, see www.iavi.org.

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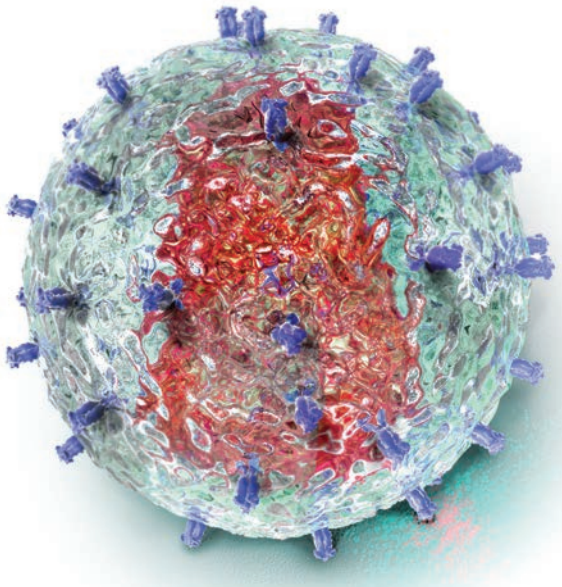

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[ON THE COVER]

The cover image shows a cloud of about 45,000 CD8⁺ T cells, represented as dots, from one healthy individual; this is about the number of CD8⁺ T cells that can be found in 0.1 ml of blood. The cells are arranged in a 3-dimensional space according to how similar they are in their expression of 16 surface markers and nine functional markers (such as cytokines). The more similar they are to each other, the closer they are together. Naive cells are green, central memory cells are yellow, effector memory cells are blue, and short-lived effector cells are red. The image is the result of a 25-dimensional data set—from simultaneous mass cytometry analysis of 25 different markers—projected into three dimensions that account for most of the variation in the data (see page 19).

Image courtesy of Evan Newell, Stanford University. A different version of this image appeared in *Immunity* **36**, 142, 2012.

Adenovirus Vectors: PROMISE AND Possible Pitfalls

New studies show the promise of alternate serotype adenovirus vector-based HIV vaccine candidates, but also raise questions about their ability to overcome pre-existing immunity issues

By Andreas von Bubnoff

More than four years ago, the HIV vaccine field was shocked and disappointed when a Phase IIb trial known as STEP was halted ahead of schedule because there was no evidence that the vaccine candidate protected against infection or controlled the virus in those who became infected. The candidate, developed by Merck and known as MRKAd5, used an adenovirus serotype 5 (Ad5) vector to deliver the HIV antigens Gag, Pol, and Nef, and was widely considered to be one of the most promising in clinical trials at the time.

Even worse, subsequent analyses of the STEP trial indicated that MRKAd5 actually led to an increased risk of HIV acquisition in a subgroup of vaccinees who were uncircumcised and had pre-existing Ad5 antibody immunity due to natural exposure to this serotype of the common cold virus. In addition, pre-existing Ad5 seropositivity was found to dampen HIV-specific cellular immune responses to MRKAd5.

To circumvent these issues of pre-existing immunity, a few research groups have been exploring vaccine regimens that use alternate serotype Ad vectors that are less common worldwide, including the serotypes Ad26 and Ad35. These vectors also elicit different immune responses than Ad5, according to Dan Barouch, professor of medicine at Harvard Medical School and chief of vaccine research at Beth Israel Deaconess Medical Center (BIDMC), who led a recent study that provided promising results with vaccine regimens containing these vectors.

The study found that among several prime-boost regimens that could partially protect rhesus macaques from challenge with simian immunodeficiency virus (SIV) mac251, considered one of the tougher challenge viruses in pre-clinical studies, the two that were best overall were Ad26 combined with modified vaccinia Ankara (MVA) or with Ad35 (*Nature* **482**, 89, 2012). Based on these results, researchers are planning to test an Ad26/MVA regimen in Phase I clinical trials.

However, there is some concern that alternate serotype Ad vectors may not entirely sidestep the issue of pre-existing immunity. Another recent study by Nicole Frahm, associate director for laboratory science at the HIV Vaccine Trials Network (HVTN), and her colleagues has shown that pre-existing T-cell responses to Ad5 can also dampen cellular immune responses to the HIV inserts of MRKAd5 (*J. Clin. Invest.* **122**, 359, 2012). These T-cell responses are cross-reactive, targeting conserved sites that are shared by multiple Ad serotypes. Such a dampening effect,

therefore, might also be an issue for vaccines that use rare serotype Ad viruses as vectors. What that would mean for vaccine efficacy is still unclear, but it suggests that Ad-specific T-cell responses should at least be monitored when using rare serotype vectors in human trials.

The promise of protection

Before Barouch's recent study, vaccinating rhesus macaques with live-attenuated SIV was the only approach capable of protecting them from the difficult to neutralize and highly pathogenic SIVmac251 challenge. However, this approach is thought to be too dangerous to test in humans because later studies found that the attenuated viruses used as a vaccine could regain their infectiousness and become pathogenic again.

Last year, Norman Letvin of Harvard Medical School and colleagues reported that a DNA/Ad5 prime-boost vaccine regimen that delivered the SIVmac239 Gag, Pol, and Env immunogens could partially protect rhesus macaques from repeat low-dose rectal challenge with SIVsmE660, but not from challenge with SIVmac251, which is more difficult to neutralize than SIVsmE660 (see *Research Briefs*, *IAVI Report*, May-June 2011).

The SIVmac251 challenge virus used in Barouch's study also set a high bar for protection because it contained different viral sequences than those in the vaccine candidates, a so-called heterologous challenge. The vaccine carried the *gag*, *pol,* and *env* genes from SIVsmE543, an SIV strain closely related to E660. The Env protein in the vaccine candidates was 18% different in its amino acids from the Env in the SIVmac251 challenge virus—a difference that is greater than the difference between HIV strains in an infected person, but smaller than the difference between HIV clades. Barouch says the choice of a tough challenge was intentional. "We wanted to see whether vaccines made from a heterologous strain could offer an acquisition effect as well as a virologic control effect against a particularly hot virus challenge."

To make the challenge even more vicious, the researchers used a dose of challenge virus that was about 100 times more infectious than HIV is in humans, so that one exposure to the challenge virus infected at least half the control animals. They compared how well four different prime-boost vaccination regimens (DNA/MVA, MVA/MVA, Ad26/MVA, and MVA/Ad26) carrying the 543 Gag, Pol, and Env immunogens protected groups of eight animals from the 251 challenge. They vaccinated the animals with a prime, followed by a boost six months later, and then gave them six weekly rectal challenges starting at month 12. All primes were done once except the DNA prime in the DNA/MVA regimen, which consisted of three monthly vaccinations.

It took just one challenge to infect at least half, and three challenges to infect all of the control and the MVA/MVA vaccinated animals. By contrast, it took two challenges to infect half the animals vaccinated with DNA/MVA, and three challenges to infect half the animals vaccinated with the Ad26/MVA and MVA/Ad26 regimens. Seven of the eight animals in all three heterologous prime-boost regimen groups became infected after all six challenges. This means that all three heterologous prime-boost regimens reduced the per-exposure risk of infection by about 80%.

In a separate experiment, researchers vaccinated 16 macaques with an Ad35/Ad26 vaccine regimen and challenged them with SIVmac251 in a similar way. This too resulted in about 80% protection per exposure. However, macaques that received the same vaccine regimen without the *env* gene were not protected, suggesting Env was required for protection. "It continues to add credence to why Envelope is going to be important to include [in vaccines]," says Nelson Michael, director of the US Military HIV Research Program and one of the senior authors of the recent study led by Barouch.

The researchers also found that the Ad26/MVA and Ad35/Ad26 heterologous prime-boost regimens not only provided about 80% protection per exposure, but also resulted in at least a two log or 100-fold lower set-point viral load 84 days after the last challenge. The Ad26/MVA regimen reduced set-point viral load by 2.32 logs, while Ad35/Ad26 lowered it by 2.18 logs. "For the combined endpoint of acquisition and virologic control, the two optimal regimens are the Ad26/MVA regimen and the Ad35/Ad26 regimen," Barouch says. "[This] shows that optimal vaccine regimens can indeed offer at least a partial acquisition effect as well as a virological control effect against a heterologous challenge with a difficult to neutralize virus."

For Michael, the "clear winner" of the two was the Ad26/MVA regimen, because the set-point viral load was undetectable in three of the seven Ad26/MVA vaccinated animals that got infected, but in only one of the 13 Ad35/Ad26 vaccinated animals that got infected.

Because some, but not all, animals vaccinated with the different vaccine regimens were protected or showed viral load control, researchers were also able to determine immunological correlates of protection and of viral load control. For protection, antibodies binding to Env were most important, although neutralizing antibodies to easy-to-neutralize viruses were also relevant, according to Barouch. For viral load control, nine different cellular and humoral immune responses were important, including Gag-specific cellular immune responses. This is consistent with previous observations that viral load control is associated with Gag-specific cellular responses, Michael says. "The view from 35,000 feet is antibodies for infection and cells for virologic control," he says. In addition, the best viral load control, which was seen in the Ad26/MVA vaccinated animals, correlated with an unusually balanced immune response between central and effector memory $CD4^+$ and $CD8^+$ T cells compared with the DNA/MVA vaccinated animals, which showed no viral load control, Michael adds.

The observation that binding antibodies, as well as neutralizing antibodies, were the most important correlates for protection from SIVmac251 suggests, Barouch says, that antibody effector functions other than those measured in traditional neutralization assays may also be relevant for protection. "There is more research that's needed to understand the full spectrum of antibody effector function," he adds.

The finding that binding antibody to Env was an important correlate of protection in the most recent study suggests that SIVmac251 challenge might be a good animal model for what happened in RV144, the first vaccine trial to show modest protection from HIV infection, says Louis Picker, a professor of pathology at the Oregon Health & Science University who was not involved in the Barouch study (see *A Bangkok Surprise, IAVI Report*, Sep.-Oct. 2011). In RV144, the protective effect seemed to be due to Env binding and not neutralizing antibodies. "[SIVmac251] is actually a very good model to ferret out the correlates of this kind of protection," Picker says. "[Barouch and colleagues] were able in the monkey model to recapitulate that weak acquisition protection with a non-neutralizing antibody correlate. This model will help dissect the results of the Thai trial and provide a path forward to improving that kind of efficacy."

While there was 80% protection per challenge, Picker says, almost all vaccinated animals were infected after all six challenges, and it took just two additional challenges to infect half the vaccinated animals than it took to infect half the unvaccinated control animals. "This is significant but it's still weak," he says, adding that, "I think there should be more work in the monkey model to isolate and improve the effect before you bring it into humans."

Still, Barouch, Michael, and others are now planning to test the Ad26/MVA regimen in Phase I clinical trials in humans. The vaccine will carry mosaic antigens that were developed by Barouch and Bette Korber of the Los Alamos National Laboratory and are computationally designed to achieve optimal coverage of the many different versions of HIV circulating globally.

Alternative adenovirus vectors, including Ad26, have already been tested in the clinic and have been shown to be safe and immunogenic, Barouch says. Other HIV candidate vaccines with such vectors are also being tested. For example, Barouch is collaborating with the HVTN, IAVI, and the Ragon Institute to conduct IAVI B003/IPCAVD-004, a Phase I trial of different prime-boost combinations of Ad35 and Ad26 vector-based vaccine candidates (see *Vaccine Briefs*, *IAVI Report*, Sep.-Oct. 2010).

Reassessing pre-existing immunity

Despite the promising results from Barouch's study, there is concern that alternate Ad vectors may not be able to overcome some of the issues of pre-existing immunity. Until now, researchers have measured Ad5-specific antibodies in blood to characterize pre-existing immunity to Ad5 in STEP trial participants. Now, Frahm and colleagues looked for the first time at cellular immune responses to Ad5 in placebo recipients from the STEP trial a month after the time of the final vaccination, and found that about three quarters of the people with Ad5-specific antibodies also had Ad5-specific $CD4^+$ T-cell responses. To their surprise, more than half of the placebo recipients without Ad5-specific antibodies also had Ad5-specific $CD4^+$ T-cell responses. They used placebo recipients for the analysis because samples were not collected from STEP trial volunteers before vaccinations started.

This was unexpected, Frahm says, because it argues against the hypothesis that Ad5 seropositive STEP trial vaccinees were more susceptible to HIV infection because they had more Ad5-specific $CD4^+$ T cells that were activated by the vaccination, resulting in an increased pool of

HIV target cells. Frahm says that if this hypothesis were true, few, if any, Ad5 seronegative people should have Ad5-specific CD4+ T-cell responses compared with seropositive people, which is not what she and her colleagues found. In fact, they found that 93% of the seronegative vaccinees, but only 78% of the seropositive vaccinees, had such responses. "That's exactly opposite of what we were expecting," Frahm says.

However, her measurements were in blood and therefore don't shed much light on what happened in mucosal tissues, which is where the infection most likely occurred. Previous studies did not find any hints that mucosal CD4+ T cells in STEP trial vaccinees were easier to infect by HIV than in placebo recipients in terms of CCR5 expression, activation markers, or homing markers, Frahm says. However, these CD4+ T cells might have been taken too late after vaccination and from too few vaccinees to see any effects, she adds. To better address this question, the HVTN is conducting HVTN 076, a trial in Ad5 seronegative people from whom mucosal samples are taken right after the vaccinations with a DNA/Ad5 vaccine regimen to see if mucosal Ad5-specific CD4+ T-cell responses are higher in vaccinees than in placebo recipients.

Frahm and colleagues also analyzed T-cell responses in volunteers from a trial called HVTN 071, in which volunteers received the same MRKAd5 vaccine as was used in the STEP trial, but which was stopped after two of three vaccinations because the results of the STEP trial had been announced. In these volunteers, the researchers found for the first time that pre-existing Ad5-specific CD4+ T-cell responses before vaccination were associated with dampened CD4+ T-cell responses to the HIV inserts and with lower breadth of the HIV-specific CD8+ T-cell responses to the vaccine. In addition, the Ad5-specific CD4+ T cells recognized epitopes shared by many different Ad vectors, in addition to Ad5. "We are the first to really show what epitopes are targeted by these T cells and that these epitopes are really identical across the different adenoviruses," Frahm says. This cross reactivity suggests that the dampening effect on cellular immune responses to the HIV vaccine inserts might even affect vaccines that use rare serotype Ad viruses such as Ad26 or Ad35 as a vector.

Just how much this dampening effect would affect the efficacy of vaccines that use Ad viruses as a vector is unclear, Frahm says, because the magnitude of insert-specific responses needed for an HIV vaccine to be protective is unknown. Michael, who was not involved in Frahm's study but wrote a commentary on it in the issue of the *Journal of Clinical Investigation* where the study appeared, agrees. "What that means in terms of predicting efficacy no one knows," he says.

Also, the immune responses induced by Ad vectors are so high that the dampening effect may be negligible, Frahm says. "Just because [Ad vectors] are so good at inducing immune responses, they can overcome the pre-existing immunity and are still going to give a relatively decent immune response that may still be better than an immune response by a weak vector that may not have any pre-existing [immunity]." For now, she thinks these findings don't necessarily mean that alternative Ad vectors shouldn't be used in clinical trials or that people with Ad-specific T-cell responses need to be excluded from trials that use alternative Ad-vectored vaccine candidates, at least not until further studies suggest any deleterious effects on vaccine efficacy. But once a trial that uses alternative Ad-vectors shows efficacy, these effects are something to keep in mind when interpreting the results, she says. "We will definitely have to look if these immune responses at baseline have anything to do with efficacy."

"While Frahm raises concerns for cross reactivity between pre-existing T-cell responses to Ad5 and rarer serotype Ads, the impact of this observation needs to be directly tested in humans vaccinated with vectors such as Ad26," Michael says. "We are moving forward with testing Ad26 with MVA."

Barouch says he has data that show that most people have Ad26 and Ad35 T-cell responses (*Nat. Med.* **15**, 873, 2009), consistent with Frahm's observation that Ad-specific T-cell responses are cross-reactive, and is currently investigating whether these Ad-specific cellular responses affect the immunogenicity of alternative Ad vectors in humans.

Meanwhile, Gary Nabel at the National Institute of Allergy and Infectious Diseases' Vaccine Research Center is collaborating with the biopharmaceutical company Okairos to explore the use of chimpanzee adenoviruses as vaccine vectors. Nabel says that in humans, seropositivity against chimp adenoviruses is much lower than against human Ad5 and Ad26, and generally also lower than seropositivity against human Ad35. In addition, chimp Ads seem to have a similar ability to Ad5 to stimulate immunity, he says. ■

The Global Fund's UNCERTAIN FUTURE

A funding shortfall has raised concerns about the organization's ability to continue providing life-saving treatments and interventions

By Regina McEnery

A decade ago, the founding executive director of The Global Fund to Fight AIDS, Tuberculosis, and Malaria, Sir Richard Feachem, adopted the slogan "Raise It, Spend It, Prove It," to underscore the non-profit organization's role in dispersing life-saving drugs and interventions against these three major killers. But after the global economic downturn blew holes in the budgets of many of its major donors, and an internal review found evidence of misuse of donor dollars, the Fund, which provides about a quarter of all the money spent on international HIV/AIDS efforts and most of the malaria and tuberculosis (TB) spending in developing countries, is facing some major battles in upholding this slogan.

The Global Fund was created in 2001 after appeals by the G8 nations and the United Nations. A permanent Secretariat was established in Geneva in 2002. The idea behind the Fund was that the world was losing the battle against AIDS, TB, and malaria, and that to reverse course there was a need to dramatically increase resources and direct those resources to the areas of greatest need. The Global Fund worked hard at remaining true to that goal. Feachem, who presided over the non-profit public-private partnership from 2002 to 2007, says the agency resisted pressure to adopt other global health priorities and stuck to its role of raising large amounts of money and spending it as effectively as possible.

And raise money it did. Between 2001 and 2010, The Global Fund's top 20 government donors contributed US$18 billion, led by the US with a $5.4 billion contribution. For the period from 2002 to 2015, governments have pledged $28.3 billion to the Fund, $20.7 billion of which has actually materialized. The money spent by the Global Fund since 2001 has put 3.3 million people on antiretroviral therapy, helped detect and treat 8.6 million cases of TB, and has been used to distribute 230 million insecticide-treated bed nets to households across sub-Saharan Africa.

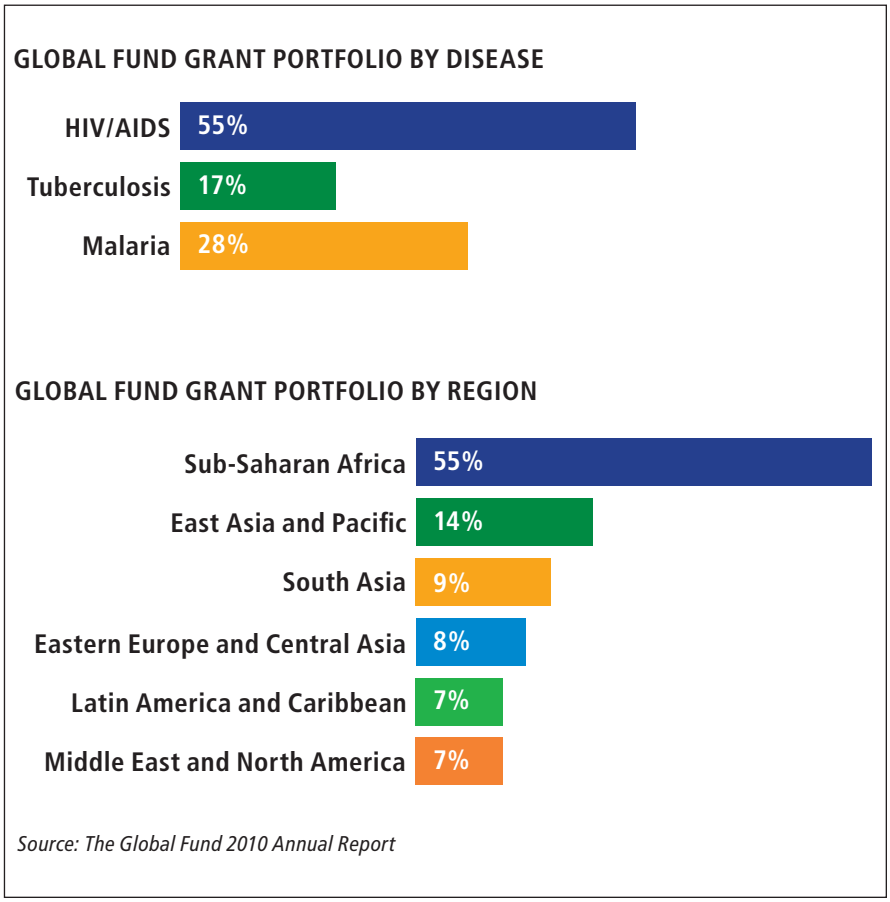
But after years of steady growth in both the number of donors and the amount of their contributions, The Global Fund now faces a $2 billion shortfall that Feachem says will carry devastating consequences for developing countries unless the fund is replenished. "A decade of massive investment produced spectacular progress," says Feachem, who now directs the Global Health Group at the University of California-San Francisco. "It would be unthinkable to let that progress be reversed. That is now a real possibility unless we can urgently mobilize additional resources."

The Global Fund's problems, both with funding and management, have played out very publicly. This has inspired many of its most ardent supporters to step up calls to rescue the Fund. A handful of Global Fund donors—notably the US and the UK—are considering hosting an emergency donors meeting in advance of the International AIDS Conference, which will be held in Washington, D.C., this July. At this meeting, they hope to raise $2 billion from the Fund's biggest government donors and lobby political leaders for reassurances that the pledges made by countries will be fully paid. In the interim, the Bill & Melinda Gates Foundation has stepped in to help fill the gap. The Foundation announced in January at the World Economic Forum in Davos, Switzerland, that it would make an emergency $750 million donation to help tide the agency over until it could raise more money. Japan also announced in Davos that the recent tsunami and nuclear disaster would not prevent it from meeting its $800 million commitment to The Global Fund.

Whether The Global Fund will rebound and continue to grow remains to be seen, though many of its allies are cautiously optimistic. "This is something we can easily reverse if the donors come together and we make a decision to mobilize additional resources and ensure donors fulfill pledges that they have made," says Joanne Carter, who sat on the Global Fund's board and is now a member of its strategy, impact, and investment committee.

US Global AIDS Coordinator Eric Goosby, who oversees international HIV/AIDS funding, agrees. "I firmly believe that The Global Fund will come through this period of transition stronger than ever."

FIGURE 1



Source: The Global Fund 2010 Annual Report

The economy of health

The current trend in development aid for health is brighter than might be expected, given the economic downturn. An annual report prepared by the Institute for Health Metrics and Evaluation in Seattle noted that development aid for health, estimated to be $27.7 billion in 2011, has risen steadily over the last decade, even during the recent global recession, albeit at much slower rates. Development aid flowing through the GAVI Alliance—a Geneva-based global health partnership launched in 2000 to increase access to immunizations—was an exception, increasing by 31% between 2010 and 2011, and GAVI could be on track to spend even more money in the near future. At its first pledging conference in London last year, donors committed $4.3 billion, exceeding the $3.7 billion goal, to help scale up immunization programs in developing countries.

But for HIV/AIDS, the funding picture isn't as bright. According to the Health Metrics and Evaluation report, the growth of development aid for HIV/AIDS, which is the primary reason foreign aid for health budgets ballooned over the last decade, slowed from 21% to 5% between 2007 and 2008, and increased at an even slower rate between 2008 and 2009 (the most recent years for which data is available in this report). The growth in aid for TB also slowed during the same period, though aid for malaria increased by 50%.

An HIV/AIDS report issued last year by the Kaiser Family Foundation (KFF) that analyzed the level of giving by donor governments suggests the funding of international HIV/AIDS programs actually declined in 2010, after flattening in 2009. The report noted that there were $6.9 bil-

FIGURE 2

Top Six Government Donors to The Global Fund

Country	Amount Pledged	Amount Given	Period of Pledge
USA	$9.5B	$5.1B	2001-2013
France	$3.8B	$2.4B	2002-2013
UK	$2.2B	$1.4B	2001-2015
Japan	$2.1B	$1.3B	2002-2013
Germany	$2.0B	$1.3B	2002-2013
European Commission*	$1.6B	$1.2B	2001-2013

**The executive body of the European Union, a confederation of 27 member countries located primarily in Europe*

B=billion

Source: The Global Fund 2010 Annual Report

lion in funds actually disbursed in 2010 compared to $7.6 billion in 2009. The KFF report also noted that the drop in funding in 2010 came after a nearly six-fold increase in disbursements by donor countries for international AIDS assistance between 2002 and 2008.

Despite the gloomy headlines, Feachem does not consider the Global Fund a "canary in the coal mine" with regard to the sustainability of international health agencies that have relied upon wealthy governments to support their causes, and says he does not foresee more Global Fund-like scenarios unfolding in the next several years. "It's a specific and special case," he says.

A sour economy

To a large degree, The Global Fund's financial headaches are a consequence of the 2008 financial crisis that brought about a worldwide economic recession, and the European sovereign debt crisis. The Global Fund gets 95% of its money from governments, most of them in Europe, and given the financial downturn, unsurprisingly, donations to The Global Fund began to slip. Spain, which has been especially hard-hit by the Euro zone crisis, froze contributions indefinitely in 2010. Iceland ended payments altogether, while the Netherlands and Ireland reduced their contributions. According to The Global Fund's 2010 annual report, Italy still owes the $172 million it pledged in 2009.

The Global Fund has also been hindered by the decline of the Euro, and the US's budgetary approval process, which gives the president the authority to submit a budget but grants the US Congress the power to approve spending limits and appropriate the money. Over the past two years, Congress has only appropriated about $2 billion for The Global Fund, and with only a year left in their commitment, there are concerns the US may come up short on its $4 billion pledge.

Seeking to alleviate those concerns, US President Barack Obama included in his administration's 2013 proposed budget about $1.7 billion for The Global Fund, though he also sliced $542 million from the bilateral President's Emergency Program for AIDS Relief (PEPFAR), the single biggest provider of ARV treatment for HIV-infected individuals in developing countries.

In the midst of the worldwide economic downturn, The Global Fund also found itself the unwanted center of a public relations disaster triggered after the agency posted on its website in late 2010 results of an audit that uncovered what the Fund later described as the "grave misuse" of some of the funds awarded to four recipient countries—Mali, Mauritania, Zambia, and Djibouti. The audit also prompted further investigations in nine other countries that the Fund supports. This resulted in a maelstrom of negative media coverage and prompted Sweden and Germany to temporarily suspend contributions. The Global Fund responded to the scandal by demanding an accounting of the missing money—estimated to be about $34 million of the $13 billion in total disbursements by the fund between 2002 to 2010—and commissioned an outside review of its procedures.

The review panel, led by former US Health and Human Services Secretary Michael Leavitt, reported in September that the organization lacked sufficient safeguards to prevent fraud and recommended widespread changes in how the Fund conducts its business. In January, the board appointed Gabriel Jaramillo, former chairman and chief executive of Sovereign Bank and a member of the panel assigned to review The Global Fund's accounting procedures, to the newly created position of general manager. Then, The Global Fund's executive director for the past five years, Michel Kazatchkine, announced soon after that he would be stepping down.

Gathering clouds

Signs that giving to The Global Fund had grown anemic surfaced in 2010—the same year the mishandling of funds was first reported and the Fund held its replenishment drive. The organization conducts the drive every three years, seeking higher pledges from public and private donors. In past cycles, the group had been extremely adept at building its support base. In 2010, however, The Global Fund was only able to raise $11.7 billion in pledges, and while the figure exceeded what it had raised during the previous drive in 2007, the amount was still

short of the $13 billion necessary to maintain its current programs in the 140 countries that The Global Fund has committed support to through 2014, let alone expand its presence in existing countries or add new countries to its recipient list.

Another problem that beset The Global Fund, as well as other non-profit organizations, is that even when government donors committed money, the full amount was not always disbursed. The Institute for Health Metrics and Evaluation report noted that prior to the recent recession, donor disbursements to the Fund were approximately the same as commitments. However, in 2009, donors disbursed only 94% of commitments, and only 78% of the amount pledged in 2010 was actually delivered. Preliminary data from the Fund suggest that donor disbursements continued to decline in 2011.

We are at some risk of seeing a noble experiment fall flat on its face.
– Alan Whiteside

The resulting shortfall led The Global Fund to announce last November that it would continue to support existing programs, but that it would not be accepting any new grant applications for its next funding cycle—round 11—and that it would be issuing no new grants until at least 2014. "That may be OK for those whose grants are carried through 2014, but for countries not successful in rounds 9, 10, or 11, it is a crisis," says Feachem. "Those countries will fall over the cliff with very serious potential consequences. With HIV/AIDS people will start dying within weeks."

Feachem says The Global Fund probably could have minimized some of its financial problems had it realistically projected lower levels of giving after the global recession hit four years ago. "It was pretty obvious from 2008 that this would have a big impact and The Global Fund should have adjusted," he says. "They did, but the adjustments came too late."

Feachem suggests in a recent commentary that The Global Fund also needs to do more to achieve a performance-based funding system that had been an important founding principle of the venture (*Lancet* 378, 1764, 2011).

The Global Fund has committed to prioritizing funding for low-income countries but acknowledged that middle-income countries, particularly those in Eastern Europe where the incidence of HIV is rising and the rates of TB are high, won't receive any additional funding and may even go unfunded. The Global Fund predicted that vulnerable groups such as men who have sex with men, injection drug users, and commercial sex workers would be particularly hard hit by the Global Fund shortfall because their needs are typically not covered by government-funded programs.

The decision by The Global Fund to cancel its latest round of new funding caught many public health advocates off guard. Alan Whiteside, who directs the Health Economics and HIV/AIDS Research Division at the University of KwaZulu-Natal in South Africa, says the situation there is not catastrophic. Nonetheless, he warned that "We are at some risk of seeing a noble experiment fall flat on its face."

And Jeffrey Sachs, who directs the Earth Institute at Columbia University in New York City, says, "Since the financial crisis, governments have cut back on spending in general, but many have found it convenient to cut back on spending on the world's poorest people," says Sachs. "This is, of course, a double tragedy. We have cutbacks on these highly effective, very inexpensive, but crucial life-saving interventions."

Goosby says The Global Fund investment has been critical in helping PEPFAR reach its goals. "We are jointly funding many country programs and specific service sites, and as we review our country PEPFAR programs, again and again we see that the success of The Global Fund grants is a critical factor in the success of our work."

And in his annual letter, Bill Gates, whose Foundation is the biggest non-government supporter of The Global Fund, discussed the impressive track record the organization has had. "I am confident that this is one of the most effective ways we invest our money every year," wrote Gates. As for the mishandling of funds, he wrote, "Given the places where The Global Fund works, it is not surprising that some of the money was diverted for corrupt purposes." ■

FIGURE 3

Pledges vs. Paid

Of The Global Fund's top 40 government donors, most paid what they pledged in 2009 and 2010. But a few did not, and the list of countries in arrears grew year to year.

	Amount Pledged	Amount Paid
2009		
Italy	$172M	$0
USA	$1B	$959M
2010		
Italy	$172M	$0
USA	$1.1B	$674M
France	$397M	$340M
India	$3M	$2M
Ireland	$46M	$11M
Portugal	$2.5M	$0
Spain	$250M	$136M

M=million; B=billion
Source: The Global Fund 2010 Annual Report

In Pursuit OF A CURE

An update on efforts to cure HIV from the 5th International Workshop on HIV Persistence during Therapy

By Richard Jefferys

From December 6-9, 2011, around 250 scientists assembled on the sunny island of St. Maarten for the 5th International Workshop on HIV Persistence during Therapy. The tropical location conjures up notions of escape and fantasy and until recently, the guiding philosophy behind this biannual meeting—that HIV persistence can be addressed and the infection cured—was widely perceived to belong in the realm of fevered dreams.

There are several parallels between cure research and the vaccine field, which for many years had to fend off criticism that there was no clear "proof of concept" to demonstrate that immunization against HIV was possible. That is until the results of the RV144 trial in Thailand finally quelled those arguments. Researchers pursuing a cure for HIV faced an even more vertiginous mountain of skepticism, but this mountain has now been moved; not by a large, randomized clinical trial, but by a single individual named Timothy Ray Brown.

Brown's case, which has understandably garnered considerable media coverage, first came to light in 2008 in a poster presentation at the 15th Conference on Retroviruses & Opportunistic Infections (CROI). After being on successful antiretroviral therapy (ART) for many years, Brown was diagnosed with acute myeloid leukemia (AML), necessitating a complex series of anti-cancer treatments and, ultimately, a stem cell transplant. The hematologist responsible for Brown's care, German doctor Gero Hütter, identified a donor homozygous for the CCR5Δ32 allele, which abrogates expression of the HIV co-receptor CCR5 on cells. After two stem cell transplants from this donor, along with a daunting panoply of chemotherapies and immune suppressive treatments, Brown has remained free not only of AML, but any sign of HIV in blood and tissues, despite being off ART for over four years and counting. In a paper published in 2011, Hütter and colleagues felt able to state: "From these results, it is reasonable to conclude that cure of HIV infection has been achieved in this patient," (*Blood* **117**, 2791, 2011).

In much the same way that the RV144 results invigorated the HIV vaccine field in 2009, the 2011 persistence workshop was suffused with a new optimism and sense of purpose as a result of this one-person proof of concept. Signs of the mainstreaming of cure research abounded: two large pharmaceutical companies—Gilead Sciences and Janssen Pharmaceuticals—described programs that aim to identify compounds capable of targeting the HIV reservoirs that persist despite ART. The ratcheting up of US National Institutes of Health (NIH) support was in evidence due to the participation of representatives from the Martin Delaney Collaboratory program, under which multiple groups of investigators have been funded, and the NIH-sponsored AIDS Clinical Trials Group (ACTG) network, which has recently made the search for a cure a top priority.

But for all the sunnier parallels between the vaccine and cure research domains, they are also both clouded by uncertainty. The mechanisms by which protection was achieved in RV144, and how the trial result might be translated into an effica-

cious vaccine for all populations, remain to be elucidated. Similarly, exactly how Timothy Brown's cure was obtained is not completely understood, and the challenges associated with attempting to convert this complex individual case into a scalable, accessible, curative therapy are gargantuan.

In broad strokes, current strategies discussed at the workshop focus largely on two main routes for curing HIV: rousting the virus from cells where its DNA has integrated into the cellular genome—latently infected cells—and/or enhancing the ability of the host to restrain viral activity, either via gene therapies or therapeutic vaccines that aim to bolster HIV-specific immunity. Two possible curative scenarios are envisioned: eradication, wherein all HIV is eliminated from the body, or what is termed a "functional cure," in which the body is able to tolerate the presence of some residual virus without ill effect.

An animal model

Another similarity between HIV vaccine and cure research is the ongoing quest for an optimized animal model. Jeff Lifson, director of the AIDS and Cancer Virus Program at the National Cancer Institute in Frederick, who has extensively studied SIV infection of macaques, in the context of both preventive vaccines and therapeutic interventions, provided an overview of this pursuit at the workshop. He explained that there is not yet an ideal system for studying viral persistence in macaques; rather, there are several published approaches that continue to be refined.

One approach is to use rhesus macaques infected with reverse transcriptase (RT)-simian immunodeficiency virus (SIV)/HIV hybrids known as SHIV, that encode the reverse transcriptase gene from HIV in order to render the virus susceptible to a wider array of antiretrovirals (*J. Virol.* **84**, 2913, 2010). This strategy is reminiscent of the creation of SHIVs for vaccine research, but in that case Env from HIV was inserted to facilitate the study of antibody-based vaccines.

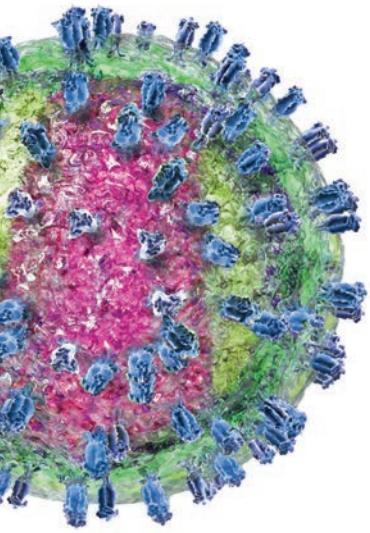
Another approach is to study rhesus macaques infected with SIVmac251 and treated with multidrug antiretroviral regimens, including integrase inhibitors (*Retrovirology* **7**, 21, 2010). And yet another is to study pigtailed macaques dually infected with SIV 17E-Fr and SIVdeltaB670 treated with the ARVs tenofovir, the integrase inhibitor L-870812, saquinavir, and atazanavir (*Curr. Opin. HIV AIDS* **6**, 37, 2011).

Lifson invoked the metaphor of "building the boat as we sail" to characterize the current state of play in studying persistence in nonhuman primates (NHPs). But, he argued that the diversity of current models was not necessarily a bad thing, citing the adoption of the SHIV89.6P challenge virus by the vaccine field as a cautionary tale of premature standardization (the virus initially appeared to have a number of advantages but turned out to have essentially no relevance to HIV infection).

Part of the difficulty with studying persistence in NHPs is identifying ARV regimens that can mimic the viral control these drugs exhibit in humans. Lifson outlined work conducted by his group to select a multi-drug regimen capable of suppressing the highly virulent SIVmac251 strain, noting that while suppression of viral load to <30 copies/ml blood could be achieved, it took considerably longer than is seen in HIV infection. It could be, Lifson suggested, that SIV infection of Indian rhesus macaques is too virulent. Chinese rhesus macaques appear to have a slower disease course, and Lifson speculated that this subspecies could be better suited for cure-related studies.

During his talk, Lifson also introduced a relatively new technology that is now being employed in both vaccine and viral persistence research: digital polymerase chain reaction (PCR). Unlike traditional PCR, which exponentially amplifies DNA or RNA from a single sample then attempts to back-calculate how much was originally present, the digital version divides a sample into multiple tiny wells and then performs PCR on each well in order to give a binary readout as to whether the target sequence is present or not. The total amount of DNA or RNA in the sample is then calculated by summing the results from the wells using Poisson distribution, allowing for far more accurate quantification of small amounts of genetic material. The approach is ideally suited to measuring very low levels of viral RNA and DNA in people or animals on suppressive ART.

The vagaries of current macaque methodologies for studying viral persistence were further illustrated by the subsequent presentations. Paul Luciw, a professor in the Department of Pathology and Laboratory Medicine at the University of California at Davis, described an experiment involving an RT-SHIV that, unlike SIV, is susceptible to the nonnucleoside reverse transcriptase inhibitor efavirenz. Animals were treated with a triple drug combination comprising efavirenz (fed in peanut butter sandwiches), tenofovir, and emitricabine for 32-35 weeks, then randomly assigned to either receive two additional drugs targeting the latent viral reservoir (prostratin and valproic acid), or remain on antiretrovirals for an additional eight weeks. Prostratin



and valproic acid are among the compounds that have emerged from basic science research into the molecular mechanisms regulating HIV latency. Prostratin is a protein kinase C activator that is reported to stimulate latent virus by activating the cellular enzyme nuclear factor kappa B (NF-κB) (*J. Biol. Chem.* **279**, 42008, 2004). Valproic acid belongs to a class of drugs called histone deacetylase (HDAC) inhibitors, which have emerged as leading candidates for coaxing latent HIV into action because they interfere with cellular factors that package genetic material and prevent its active transcription. Luciw showed that receipt of prostratin and valproic acid were associated with significant reductions in viral RNA and DNA in tissues, but when ART was subsequently interrupted there were no differences in viral load rebounds between the groups. Luciw acknowledged that the activity of valproic acid against latent virus has been called into question in the time since his experiment was launched, and said follow-up studies with more potent HDAC inhibitors are planned.

Andrea Savarino, program director of HIV Eradication Strategies at the Istituto Superiore di Sanità in Rome, and his graduate student Iart Luca Shytaj were able to report more salutary findings from studies involving Indian rhesus macaques infected with SIVmac251. Savarino's research group has previously published work suggesting that two existing drugs, buthionine sulfoximine (BSO) and auranofin, have the capacity to deplete latent virus (*Retrovirology* **6**, 52, 2009; *AIDS* **25**, 1347, 2011). BSO is thought to work by inhibiting the synthesis of the antioxidant glutathione, thereby creating an intracellular environment favorable to HIV transcription. Auranofin is a gold-based rheumatoid arthritis drug that inhibits the proliferation of central memory CD4+ T cells, a major reservoir of latent HIV. At the workshop Savarino presented analyses indicating that combining these drugs with multiple antiretrovirals may have allowed three macaques to maintain SIVmac251 viral loads below the limit of detection (<40 copies/ml) for several months after ART was suspended. Savarino and colleagues believe this finding may be an augury that anti-reservoir approaches can contribute to a functional cure.

Shifting to the mechanisms by which latent HIV reservoirs are maintained, Vicente Planelles, professor in the Department of Pathology, Microbiology, and Immunology at the University of Utah School of Medicine, explained how immunological memory—the biological blessing that allows vaccines to work—can be a curse in the context of viral latency. Specifically, HIV integrates into the DNA of central memory CD4+ T cells, a population endowed with the capacity for long-term persistence and self-renewal by proliferation. Planelles documented that when latently infected central memory CD4+ T cells undergo mitosis, the integrated HIV DNA is copied along with the cellular genome. Some proposed therapies that stimulate central memory CD4+ T-cell proliferation, such as the cytokine interleukin (IL)-7, might therefore increase rather than reduce the size of the viral reservoir. Conversely, drugs that inhibit the division of central memory CD4+ T cells, such as the rheumatoid arthritis treatment auranofin used in Savarino's experiment, may be able to reduce the amount of latent HIV, but could also potentially risk impairing immunological memory.

The power of ART

For many years now, the most controversial question in the cure research field has been whether ART fully suppresses HIV replication in the majority of individuals. The answer to the question continues to be a point of contention and several talks at the workshop offered differing perspectives. Sarah Palmer, a professor in the Department of Microbiology, Tumor, and Cell Biology at the Karolinska Institute, debuted results from a detailed evaluation of viral genetics prior to ART initiation and after long-term treatment (up to 12 years) in 12 individuals, seven of whom started therapy during acute infection and five during the chronic phase. Palmer was unable to find any evidence of HIV evolution suggestive of ongoing replication in samples from blood and multiple tissues. In a dramatic illustration of the scenario outlined by Planelles, Palmer highlighted a case where a large expansion of HIV DNA was seen that was clearly non-functional (it contained a deletion of the entire protease gene), a finding that could only be explained by the proliferation of the cell containing the defective viral DNA.

Palmer's conclusion that little or no HIV replication occurs in most people on ART—at least at the sites sampled—was supported by a number of other presentations at the workshop involving intensification of standard ART with additional drugs such as the integrase inhibitor raltegravir and the CCR5 inhibitor maraviroc. Martin Markowitz, professor and clinical director at the Aaron Diamond AIDS Research Center, gave an overview of one such study, comparing ART combinations involving three versus five drugs administered during acute infection. Through follow-up of up to 96 weeks, no significant differences in various measures of the HIV reservoirs in blood and tissues could be documented between the two regimens.

However, a countervailing view was offered by a triple-header of scientists studying ART penetration into lymphoid tissues. The work centered around 12 treatment-naive individuals starting combination ART. Pharmacologist Courtney Fletcher, dean of the College of Pharmacy at the University of Nebraska Medical Center, assessed levels of each component of their ART regimens in blood and lymphoid tissues and reported that, in some cases, suppressive levels were not reached in gut-associated lymphoid tissue (GALT) and lymph nodes. Mario Stevenson, chief of the Division of Infectious Diseases at the University of Miami, then described the virology results, showing that in some of the study participants, levels of HIV DNA forms called 2-LTR circles increased in lymphoid tissue during follow up, indicating ongoing replication was occurring (2-LTR circles are circularized forms of unintegrated HIV DNA produced during viral replication). Stevenson argued that this could occur without evidence of viral evolution if the majority of the events involved just one round of replication. The third co-investigator, Timothy Schacker, professor of medicine and director of the Infectious Disease Clinic at the University of Minnesota, then outlined his ongoing work to correlate the findings with another measure of viral persistence, the trapping of HIV RNA on follicular dendritic cells (FDCs) in lymph nodes, as measured using a technique called *in situ* hybridization. The data were very preliminary and only derived from a subset of individuals, but he suggested that there was a link between poor drug penetration and the markers of persistent replication. The study is now being expanded, and more data from additional volunteers along with more follow-up time should help shed light on whether this is a broadly applicable phenomenon, as well as the extent to which it might contribute to sustaining HIV reservoirs in the face of ART.

Drug development

Until very recently, the hunt for compounds that might be capable of depleting HIV reservoirs was confined to academic laboratories. But, at the persistence workshop, Romas Geleziunas, director of clinical virology at Gilead Sciences, and Roger Sutmuller, principal scientist at Janssen Pharmaceuticals, explained how these companies are now conducting these searches on an industrial scale. While these are the only two companies to have publicly discussed their research programs so far, rumors were flying that several others have quietly started similar efforts.

Geleziunas explained that Gilead decided to eschew cell-line based models of HIV latency as a screening tool due to concerns that their artificiality can produce misleading results. Instead, a primary $CD4^+$ T cell assay developed by Planelles and Alberto Bosque, research assistant professor at the University of Utah (*Methods* **53**, 54, 2011) has been adapted to allow high-throughput screening of drug libraries. It is early days, but several new HDAC inhibitors have been identified, one of which is undergoing preliminary toxicology testing. Geleziunas offered an example of how the approach can be used to identify interesting compounds. A pilot screen of a subset of Gilead's drug library produced a 1% hit rate of active drugs, one of which belonged to a class called kinase inhibitors. Because this was an unexpected finding, a library of kinase inhibitors was then evaluated, producing a much higher hit rate of 20%. Geleziunas noted that this is an example of how drug screening can feed back into basic science research on mechanisms of HIV latency—the identification of kinase inhibitors suggests a previously unappreciated role they may play in HIV latency that can now be investigated.

Gilead is also interested in immune-based therapies, based on the evidence that simply reactivating latent HIV may not be sufficient to kill the infected cell. Tae-Wook Chun from the National Institute of Allergy and Infectious Diseases (NIAID) discussed this issue, highlighting the fact that in his laboratory, viral reactivation by HDAC inhibitors has not induced a notable amount of cell death. Geleziunas stated that Gilead is looking at an agonist of toll-like receptor (TLR) 7 as a potential candidate, based on data indicating it can enhance immune responses to hepatitis B and an encouraging safety profile in a Phase I trial.

Janssen Pharmaceuticals is taking a slightly different approach, outlined by Sutmuller, which starts with a Jurkat cell-line based assay for high-throughput screening and then uses an in-house primary cell testing system for further evaluation of promising candidates. A humanized mouse model of HIV infection will be employed to assess the best leads that emerge from this process (*Proc. Natl. Acad. Sci.* **103**, 15951, 2006). Some 35,000 compounds have gone through the preliminary screen to date, with another 480,000 waiting in the wings.

Human trials

While these drug screens are a long way from clinical testing, the final day of the workshop did feature a number of discussions related to planning for interventional trials in humans. Dan Kuritzkes,

professor of medicine at Harvard University, is also co-leader of the ACTG network that now has a committee on HIV Reservoirs and Viral Eradication charged with developing trial protocols, and Kuritzkes outlined multiple issues that will likely arise in the design of clinical trials of eradication or functional cure strategies. Looming large among them is the complex ethical conundrum of early stage studies, which may have little prospect of benefiting an individual participant and considerable risks—the primary benefit would be to advance the science and hopefully contribute to the longer term development of curative strategies. Kuritzkes stressed that in an era when effective ART can extend the lifespan of HIV-infected people close to that of their HIV-uninfected counterparts, it will be particularly important to involve potential participants in discussions relating to the adjudication of risks along with regulators, investigators, funding agencies, and institutional review boards.

David Margolis, director of the School of Medicine at the University of North Carolina and a recipient of one of the NIH's Martin Delaney Collaboratory awards, reported very preliminary results from one of the first major cure-related human studies to be launched, a Phase I trial of the HDAC inhibitor vorinostat (also known as SAHA). The trial is an example of a potentially high-risk endeavor, as the drug is used as a cancer treatment and has a host of potential toxicities, including testing positive on the AMES test for mutagenicity. Margolis acknowledged that it took considerable time and patience to obtain US Food and Drug Administration approval to open the study. So far, four participants (all healthy HIV-infected individuals on stable ART) have received three doses of vorinostat (the first to assess safety, the second for pharmacokinetic analysis, and the third to measure activity). Margolis presented evidence of a mean 4.4-fold increase in HIV RNA expression associated with receipt of the drug. While encouraged by the data, he emphasized that many questions remain, including whether induction of HIV RNA expression leads to clearance of latently infected cells. No serious toxicities were seen in any trial participant.

Data from another clinical trial came from Carl June, professor of pathology and laboratory medicine at the University of Pennsylvania. He provided an update on a gene therapy approach that aims to mimic the knockout of the CCR5 receptor achieved in Timothy Brown, but in a kinder, gentler way. Developed by Sangamo BioSciences, the treatment uses enzymes called zinc finger nucleases to target and disrupt the CCR5 gene in $CD4^+$ T cells extracted from HIV-positive individuals. These modified cells are then expanded in the laboratory and re-infused into the original donor, in hopes of creating a large population of CCR5-negative, HIV-resistant $CD4^+$ T cells. As was reported at the 2011 CROI, participants in two Phase I trials (all receiving ART) have experienced significant $CD4^+$ T cell increases after a single infusion. One of the trials involves a 12-week analytical interruption of ART to gauge whether there is any impact on viral load parameters. So far six individuals have undertaken the interruption, and June noted that the results are extremely interesting.

Perhaps of greatest importance, June has been able to demonstrate a significant correlation between the proportion of modified $CD4^+$ T cells detected and the extent of the diminution in viral load levels documented prior to ART reinitiation. One participant in particular is drawing attention because his viral load declined to undetectable levels (<50 copies/ml) just before the end of the ART interruption. June explained that this individual is heterozygous for the CCR5Δ32 mutation and therefore has the highest proportion of modified $CD4^+$ T cells because the zinc finger nucleases only have to disrupt one CCR5 allele in each $CD4^+$ T cell for expression of the co-receptor to be completely abrogated (as opposed to having to knock out both alleles). Sangamo BioSciences is now investigating methods to increase the proportion of modified cells and attempting to confirm this intriguing observation by recruiting a large cohort of CCR5Δ32 heterozygotes into the trial.

At the end of the three days in St. Maarten, while no earth-shattering breakthroughs had been reported, the sense of a major sea change in the field remained. To close the workshop, the lead organizer Alain Lafeuillade, head of the department of infectious diseases at General Hospital in Toulon, France, introduced Françoise Barré-Sinoussi, director of the Regulation of Retroviral Infections Unit at the Pasteur Institute, Nobel laureate, and incoming President of the International AIDS Society (IAS). Barré-Sinoussi called on attendees to sustain and accelerate the momentum that has gathered behind the pursuit of a cure for HIV and reported that, to this end, IAS is developing a Global Scientific Strategy "Towards an HIV Cure," and sponsoring a symposium on the subject to take place in Washington, D.C., in July, immediately ahead of the International AIDS Conference. ■

Richard Jefferys is Coordinator, Michael Palm Basic Science, Vaccines & Prevention Project at the Treatment Action Group.

Vaccine BRIEFS

Trial Evaluating DNA/Ad35 Prime-boost Regimen Commences in Africa

A Phase I trial that began in December will evaluate the safety, tolerability, and immunogenicity of a DNA-based HIV vaccine candidate with or without co-administered recombinant interleukin (IL)-12 delivered via electroporation in a prime-boost regimen with a recombinant adenovirus (Ad) serotype 35 vector-based candidate delivered by intramuscular injection.

The randomized, double-blind, placebo-controlled trial known as B004 will enroll 75 healthy HIV-uninfected men and women ages 18-50. Vaccinations are underway in Rwanda and Uganda. A third clinical research center in Kenya will also enroll volunteers once the trial receives regulatory approval.

Both the DNA vaccine candidate, known as HIV-MAG, and the adjuvant, known as GENEVAX IL-12, were developed by Profectus BioSciences. The Ad35-GRIN/ENV candidate was designed by IAVI, which is also the sponsor of the trial. By using Ad35, a rare serotype of adenovirus, researchers are hoping to circumvent the issue of pre-existing immunity to the viral vector; however, a recent study suggests this may not be entirely straightforward (see *Adenovirus Vectors: Promise and Possible Pitfalls,* page 4).

Investigators are evaluating five different heterologous prime-boost regimens in the B004 trial. Four of the five groups will receive the DNA vaccine candidate first (in three of the groups the DNA will be co-administered with IL-12 and in the other group no IL-12 will be given) followed by the Ad35 boost. The other arm will receive the candidates in reverse order. Two different doses of IL-12 will be evaluated, as well as two different dosing schedules of the prime and boost (for details, go to clinicaltrials.gov).

Both the DNA and IL-12 will be administered intramuscularly via an electroporation device developed by Ichor Medical Systems that is meant to enhance immune responses. The small hand-held electroporation device uses a needle to inject the vaccine and four thin wires to administer electrical pulses that are milliseconds in length. This device was previously tested in a Phase I trial, sponsored by Rockefeller University, of a DNA candidate known as ADVAX involving 40 HIV-uninfected adults in the US. The study found that the candidate and delivery method were safe and well tolerated, and the magnitude of immune responses, as measured by interferon (IFN)-γ ELISPOT, were up to 70-fold higher in volunteers who received the DNA candidate via electroporation as compared to vaccinees who received the vaccine candidate via standard intramuscular injection (*PLoS One* **6**, e19252, 2011). The same device is also being used in a Phase I therapeutic vaccine trial involving 60 HIV-infected adults. The trial, which is ongoing, is comparing volunteers who receive the DNA vaccine intramuscularly via electroporation to those who receive it through standard intramuscular injection. —*Regina McEnery*

Trial Testing Mucosal and Systemic Delivery of DNA Vaccine Candidate Begins

A Phase I trial launched in December will examine three different routes of vaccination of a DNA-based candidate containing trimeric gp140 from a clade C virus, the dominant strain circulating in southern and East Africa, administered in combination with two experimental adjuvants.

The trial, known as MUCOVAC2, is funded by the Wellcome Trust and is being conducted at two clinical research centers in the UK. Twenty women will receive either a high or low dose of the DNA candidate administered via intramuscular injection, along with glucopyranosyl lipid adjuvant (GLA), which was developed by the Seattle-based non-profit Infectious Disease Research Institute (IDRI), a product-development partnership that is part of a vaccine consortium—including St. George's, Imperial College, Hull York Medical School, and the Medical Research Council Clinical Trials Unit—that developed the vaccine candidate.

GLA is a synthetic glycolipid based on monophosphoryl lipid A, a lipid form of detoxified lipopolysaccharides (LPS), a component of the outer membrane of gram-negative bacteria, which is used as an adjuvant in licensed vaccines. Scientists have found that GLA induces antibody and cellular immune responses and speculate that it works by activating toll-like receptor 4, which senses bacterial LPS (see *A Vaccine's Little Helper*, *IAVI Report*, May-June 2011 and *An Immunological Rationale for Vaccines*, *IAVI Report*, Nov.-Dec. 2010).

An additional arm of the trial includes six women who will receive the DNA vaccine candidate delivered nasally in the form of drops, along with the adjuvant chitosan, a compound derived from the outer skeleton of shellfish and insects that is thought to improve the immunogenicity of vaccines administered mucosally. Chitosan is not used in any licensed vaccines as this time but is used as a dietary supplement.

An additional 10 women will receive the DNA vaccine candidate both systemically and mucosally. This group will receive an intramuscular injection of the vaccine candidate and GLA, followed by vaginal application of nine doses of a microbicide gel, each containing 100 micrograms of the gp140 protein. Vaginal

application of the DNA gp140 vaccine candidate was previously tested in a Phase I trial involving 22 healthy women ages 18-45 from the UK. The study found the candidate to be safe, though it did not induce local or systemic immune responses (*PLoS One* **6**, e25165, 2011). In pre-clinical studies, the gp140 recombinant protein has been shown to be immunogenic when administered systemically in mice and intravaginally in rabbits.

"This is the first time the [candidate] is being used intranasally or intramuscularly," says Catherine Cosgrove, honorary consultant in infectious disease and general medicine at St. George's, University of London, who is leading the study. Cosgrove says the intramuscular injection combined with vaginal application aims to induce a more focused mucosal stimulation of the immune system. —*Regina McEnery*

Polio Eradication: One Step Forward, One Step Back

A multi-decade effort to rid the world of polio took a major step forward in February after the World Health Organization (WHO) announced that it was removing India from a list of countries where the disease is still endemic. However, there has been an alarming surge in new cases in neighboring Pakistan and Afghanistan (see table, below).

India reached this crucial milestone because of a US$2 billion campaign that the south Asian country largely self-financed. During national immunization days throughout India's 35 states and union territories, public health workers vaccinated more than 170 million children under age five annually—70 million of them multiple times. They then followed up with seven smaller immunization days aimed at hard-to-reach populations. On Jan. 13, 2012 the country had gone a year without a single new case of wild poliovirus infection. The last case of wild poliovirus was reported in the West Bengal region of India on Jan. 13, 2011.

No. of cases by year in countries where polio is endemic

	2010	2011
Afghanistan	25	80
Nigeria	21	60
Pakistan	144	198

Source: Global Polio Eradication Initiative

Three years ago, India led the world with polio cases (741), but signs the country was turning the corner became evident the following year when transmission of the most dangerous strain of wild poliovirus and the cause of 95% of India's polio cases until 2006, dropped to record low levels, led by the absence of cases in Uttar Pradesh. If three years elapse with no new polio cases, India will be declared polio-free.

"India's success is arguably its greatest public health achievement and has provided a global opportunity to push for the end of polio," according to a statement by Margaret Chan, the director-general of the WHO, a collaborator in the Geneva-based Global Polio Eradication Initiative that was launched in 1988.

The Global Polio Eradication Initiative is a public-private partnership led by national governments and spearheaded by the WHO, Rotary International, the US Centers for Disease Control and Prevention, and the United Nations Children's Fund. The Initiative has spent $8.2 billion so far toward establishing a polio-free world, focusing on 200 countries, and immunizing 2.5 billion children over 23 years.

India's struggle against polio has been focused largely on the states of Uttar Pradesh and Bihar in northern India, where high population density and poor sanitation have complicated efforts to break the transmission cycle. The trivalent oral polio vaccine, which contains weakened versions of three types of wild poliovirus, has had a lower efficacy (74%) in these northern regions compared to the remainder of India (85%), possibly because substandard living conditions make children more prone to diarrheal diseases that can prevent the vaccine from working effectively. Additionally, the strains in the trivalent vaccine can interfere with each other, producing immunity to one strain but not another.

A child from Uttar Pradesh receives the oral polio vaccine. *Photo courtesy of the Global Polio Eradication Initiative.*

The success in India was heralded by the Bill & Melinda Gates Foundation, which has made polio eradication its top priority. The Foundation has committed close to $1.4 billion to polio eradication efforts. "The challenge in India was mind-boggling," remarked Bill Gates in his annual letter describing the Foundation's priorities for the coming year. "It's hard to imagine how you would design a polio campaign that reached every Indian child…But the government kept raising awareness and improving the quality of its campaigns, even in the toughest locations."

Oliver Rosenbauer, a spokesman for the Global Polio Eradication Initiative, says while the news from India is encouraging, the increase in polio cases in Pakistan, Nigeria, and Afghanistan is of grave concern. "There is very real recognition that things need to be scaled up rapidly and concretely, in particular in the remaining endemic areas," he says.

Also worrisome are Angola, Chad, and the Democratic Republic of Congo, where the circulation of imported viruses has persisted for more than 12 months, and a handful of countries in Africa and Asia, including China, which have been grappling with outbreaks triggered by imported cases.

Last month, the WHO's executive board declared the completion of its polio eradication efforts a programmatic emergency for public health, and urged the handful of countries where polio still exists to declare a national public health emergency. The WHO board also called for certification-standard surveillances to identify the emergence of circulating vaccine-derived polioviruses, and adequate funding to interrupt wild poliovirus transmission globally, which they believe can be achieved by the end of 2013. —*Regina McEnery*

Research BRIEFS

Researchers Analyze CD8+ T Cell Types in Unprecedented Detail

A new technology that can simultaneously measure about twice as many different cellular markers than flow cytometry has allowed researchers to analyze the different types of CD8+ T cells in healthy individuals in unprecedented detail. Evan Newell, a research associate in Mark Davis' lab at Stanford University, and colleagues analyzed CD8+ T cells from six healthy individuals for 16 cell surface markers, nine functional markers such as cytokines, and six antigen specificities for three viruses: Epstein-Barr virus (EBV), flu virus, and cytomegalovirus (CMV). This showed, the authors say, that CD8+ T cells exhibit a much greater degree of complexity than previously appreciated (*Immunity* **36**, 142, 2012).

Simultaneous analysis of this many markers has only become possible recently with the development of a new method called cytometry by time-of-flight (CyTOF), or mass cytometry, which allows for the simultaneous analysis of up to 40 different markers in living cells, Newell says. With flow cytometry, researchers can simultaneously analyze up to about 20 different markers, but according to Newell, even that is difficult with that technology. "Above 10 it gets really dicey," he says.

Both methods use antibodies that bind to different markers, but CyTOF differs from flow cytometry in that the antibodies are tagged with heavy metal atoms that are usually not found in living cells, rather than with fluorescent markers. The labeled cells are then analyzed by mass spectrometry, which can distinguish between more markers at the same time than flow cytometry because their signals overlap less than fluorescent signals.

A study last year used CyTOF to analyze human bone marrow immune cells in unprecedented detail (*Science* **332**, 687, 2011), but Newell and colleagues used this approach to take a detailed look at CD8+ T cells. To visualize the many different marker combinations, the researchers projected their 25-dimensional data set into a three-dimensional data set, choosing as the three dimensions the parts of the data that accounted for most of the variation. In other words, they projected the data in such a way that they would cast the biggest shadows in each of the three dimensions.

In the resulting data cloud, cells that are closer to each other are more similar in the levels and kinds of markers they express than cells that are further apart. Although the analysis is unbiased, the shape of the cloud was similar in all six healthy individuals analyzed in the study. Newell compares it to a Y with a stem that's bent (see cover image).

Many of the clusters of cells in the cloud correspond to known types of CD8+ T cells. For the first time, the analysis showed that some of these previously known cell types, such as the central and effector memory CD8+ T cells, are not separate cell types, but connected with each other, suggesting the existence of intermediate cell types between them, according to Newell. "The memory cells are all one big continuum," he says. The cloud might also include the possible location of stem cell-like CD8+ T cells, a new type of CD8+ memory T cell recently identified that can multiply and regenerate better than other CD8+ memory T cells (see *Research Briefs, IAVI Report*, Sep-Oct. 2011). These "stem cell memory" cells might be located between the cluster of naive and central memory CD8+ T cells in the cloud, Newell says.

Cells specific for EBV, CMV, or flu virus occupied different parts of the cloud, but the clusters they occupied were surprisingly large, Newell says, suggesting that the CD8+ T-cell response to a certain type of virus is surprisingly variable.

Even though CyTOF can analyze more markers than flow cytometry, traditional flow cytometry still has its place. CyTOF is much slower and kills the cells during the analysis, while flow cytometry keeps the cells alive, making it possible to sort the cells for additional experiments, Newell says.

Nicholas Haining, an assistant professor of pediatrics at the Dana-Farber Cancer Institute and Harvard Medical School who wrote a commentary on the study in the same issue of *Immunity* (*Immunity* **36**,10, 2012), says it shows that the CD8+ T cell compartment is even more complex than previously thought. The study is also one of the most compelling pieces of evidence yet, he says, that there is a continuum of CD8+ T cell types rather than separate "buckets" of cell types. "That was very clearly demonstrated in this paper," says Haining, who wasn't connected to the study.

At the same time, he adds, the paper shows that the CD8+ T cells don't just randomly express all possible combinations of functional markers. "There is an enormous amount of heterogeneity, but the fact that fewer than all possible permutations of those markers [are expressed], implies that there is some programming that drives the heterogeneity, and that it's not just a random arrangement of cell functions."

One question now is whether all the cells that can be differentiated from each other using CyTOF truly have different biological functions. "It will be even more challenging to try and disentangle what all of these cells are doing than we previously thought," Haining says. —*Andreas von Bubnoff*

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