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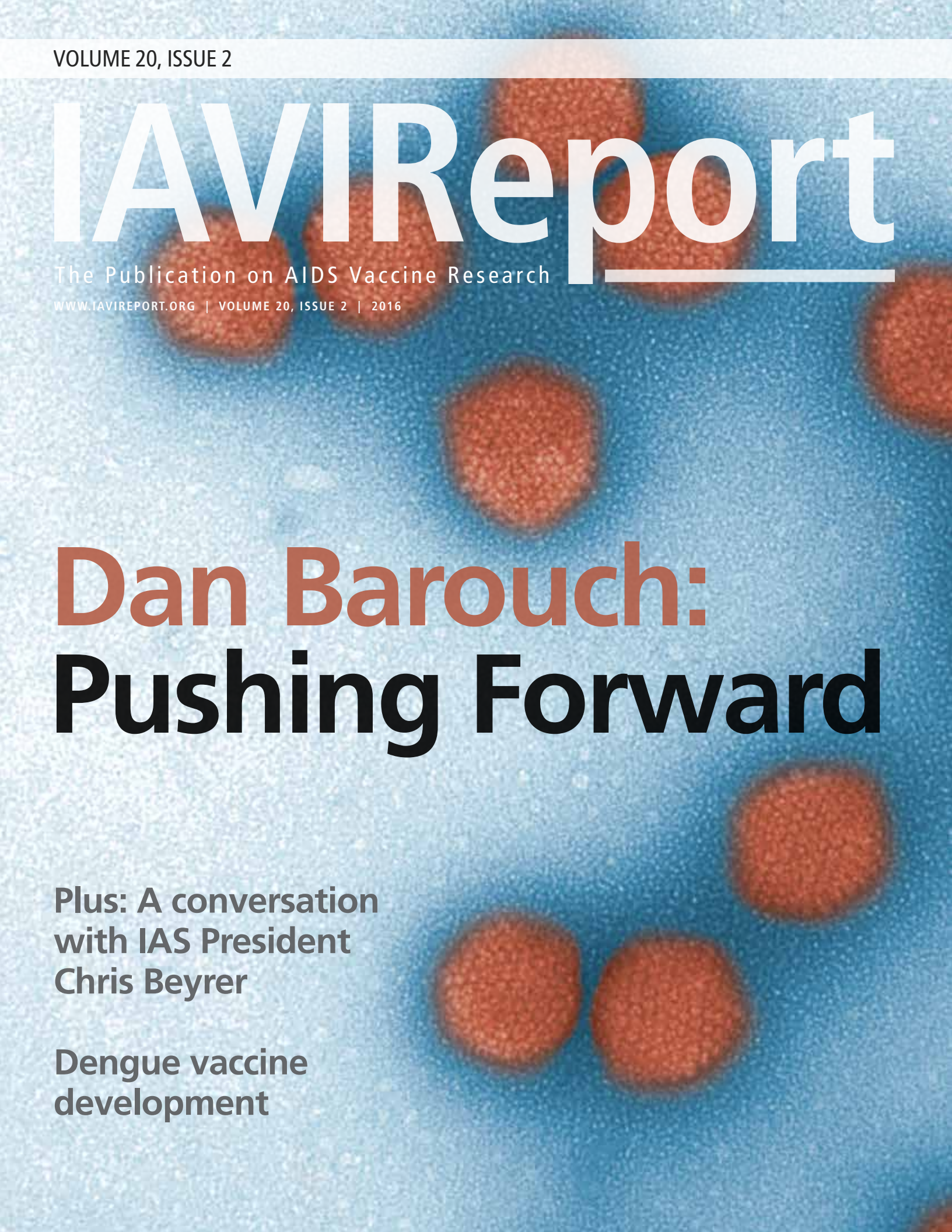

Dan Barouch: Pushing Forward

Plus: A conversation with IAS President Chris Beyrer

Dengue vaccine development

EDITOR'S LETTER

Next month, when thousands of HIV researchers, clinicians, and advocates descend upon Durban, South Africa, the situation will be dramatically different than it was 16 years ago. In 2000, when the International AIDS Conference was held in this coastal city, life-saving antiretroviral drugs were still not widely available in developing countries. AIDS was a death sentence in many parts of the world. But after that conference everything changed. A "movement" began, as current President of the International AIDS Society Chris Beyrer calls it. That movement has resulted in 17 million people receiving antiretroviral therapy, two million of whom were placed on treatment in 2015 alone, according to the latest statistics released recently by the Joint United Nations Programme on HIV/AIDS (UNAIDS). A success story, indeed.

But Beyrer warns against declaring victory too early (see page 4). In this issue, I spoke with Beyrer about the successes in battling HIV/AIDS as well as the multiple challenges that still remain before the end of AIDS can realistically be achieved. Despite significant gains in providing treatment to those in need, 20 million HIV-infected people remain without access. And although in certain places HIV incidence continues to decline, based on the latest UNAIDS data there are certain regions and within certain key populations that HIV is still very much on the rise. Beyrer has spent much of his career tracking HIV in these places and within these populations and he speaks about his experiences eloquently and emphatically.

Another virus that is on the rise is the mosquito-borne dengue virus. Several factors coincided since World War II that have led to the current explosion of dengue across multiple continents. But now, after years of research and development, the first dengue vaccine is being licensed in several affected countries (see page 9), and many others are in various stages of clinical testing. This offers hope that vaccines against dengue's relative Zika virus, which is of increasing global concern these days, may also have a clear development path.

We round out this issue with a profile of Dan Barouch—one of the most prolific young HIV vaccine researchers out there (see page 16). In just a short time since completing his medical and doctorate degrees, Barouch has amassed a large and varied portfolio of research projects on everything from HIV pathogenesis to vaccine and cure research. The story of Barouch's persistence, constant experimentation, willingness to collaborate, and focus on advancing his research agenda is inspirational to anyone considering a career in AIDS research today. And we hope there are many who are considering that career, because despite tremendous gains, there is a long way to go to ending AIDS.

– 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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SENIOR PRODUCTION MANAGER
Nicole Sender

MANAGING EDITOR
Kristen Jill Kresge

CONTRIBUTING WRITERS
Michael Dumiak
Mary Rushton

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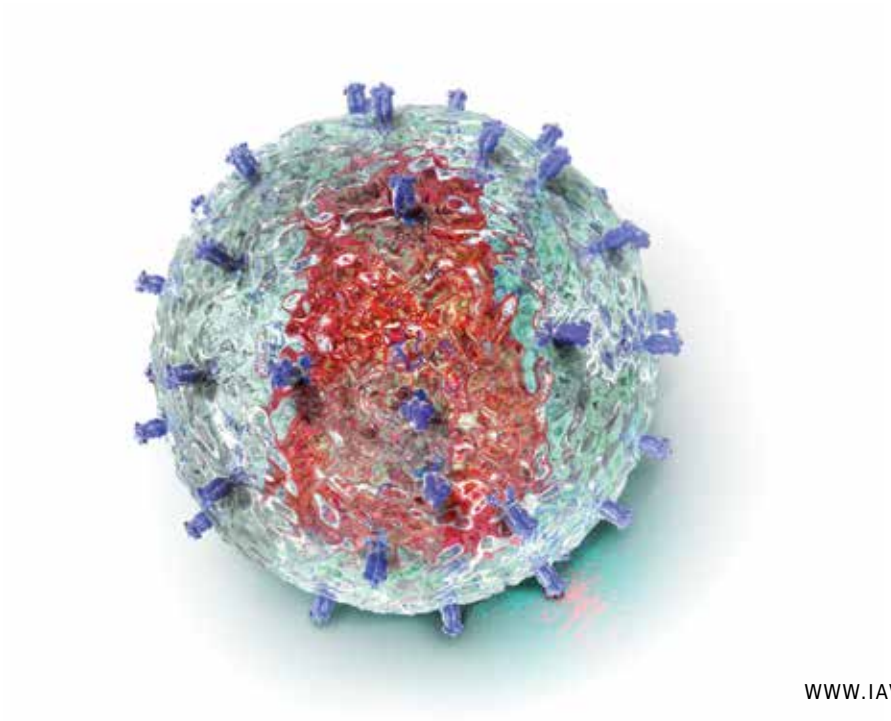


[ON THE COVER]

The cover image is a 100 nanometer-scale scanning electron microscope image of an adenovirus serotype 26 (Ad26) vector expressing a simian immunodeficiency virus (SIV) Gag antigen. Ad26 vectors developed by Dan Barouch and his team are being utilized in an HIV vaccine candidate currently in clinical trials.

Image courtesy of Peter Abbink, Center for Virology and Vaccines Research at Beth Israel Deaconess Medical Center in Boston.

"We're Not Done"

Chris Beyrer, current President of the International AIDS Society, talks about the remaining challenges to reducing HIV incidence in key populations and continuing the battle against AIDS.

By Kristen Jill Kresge

This July, HIV researchers, advocates, and policymakers will once again gather in the coastal South African city of Durban for an AIDS conference. Sixteen years ago the International AIDS Conference was also held in Durban. Then, people in developing countries, including South Africa, which remains the hardest hit by HIV/AIDS of any country in the world, were dying because they lacked access to the life-saving antiretrovirals that by that time were becoming a mainstay in most rich countries. In South Africa the situation was particularly troubling because of a history of AIDS denialism. But the conference in 2000 in Durban marked a sea change. It started a "movement," as Chris Beyrer, current President of the International AIDS Society (IAS) and professor of epidemiology at Johns Hopkins Bloomberg School of Public Health, recalls. "We changed the world and showed that you could treat millions of people with a complex disease in the poorest countries in the world and save lives."

Chris Beyrer

Since then, access to treatment has increased dramatically. According to the latest data released by the Joint United Nations Programme on HIV/AIDS (UNAIDS) this May, 17 million people worldwide are now receiving antiretroviral therapy. In eastern and southern Africa, the number of people on treatment has doubled since 2010, UNAIDS reports. When the AIDS conference returns to Durban in July, this time for AIDS 2016, it will be taking place in the country with the largest HIV treatment program in the world.

But, as Beyrer attests, the work is far from done. "We're less than halfway there on treatment and we're not implementing the prevention [options] we have," he says. "I think we've declared victory too soon. Nobody ever wanted the end of AIDS to be the end of the AIDS response," he says.

Beyrer lauds the goals set recently by the international community to combat HIV/AIDS but emphasizes that HIV prevention services must be implemented in key populations for these goals to be realized. These ambitious targets include a call to end the AIDS epidemic by 2030, as well as the scourges of malaria and tuberculosis, as part of the Sustainable Development Goals (SDGs) adopted by the United Nations to replace the Millennium Development Goals (MDGs). There is also the 90-90-90 target set by UNAIDS that calls for 90 percent of people living with HIV to know their infection status, 90 percent of all people with HIV to receive antiretroviral treatment, and for 90 percent of all people who receive treatment to achieve a suppressed viral load—all by 2020.

The "movement" that began in Durban 16 years ago faces several other challenges today. There are increasingly constrained budgets for HIV/AIDS programs globally, there are regions of the world that are locked in protracted periods of civil war, there is a devastating international refugee crisis, and there are many places where HIV incidence continues to rise in key popula-

tions. In 2015, 2.1 million people were newly infected with HIV, but behind this statistic are multiple disparities, according to the Global AIDS Update by UNAIDS. One of these disparities is that while new infections declined by four percent since 2010 in eastern and southern Africa, the number of new HIV infections in Eastern Europe and Central Asia actually increased by 57 percent over that same period. It is in these places that Beyrer has spent much of his career working with key populations. His focus on HIV/AIDS, epidemiology, and human rights has taken him around the globe. He has done extensive research in Thailand, Burma, China, India, and across Southeast Asia, as well as in Russia and Kazakhstan. He is as highly respected by scientists as he is by HIV and human rights advocates for his work with marginalized populations. Beyrer has served as president of IAS since 2014, a position he will hold until AIDS 2016 closes in Durban on July 22.

Beyrer talked with Managing Editor Kristen Jill Kresge about his two-year term as IAS President, his views on HIV prevention today, and what to expect when AIDS 2016 opens in Durban next month.

The field of HIV prevention has been buoyed recently by promising results, particularly with oral PrEP (pre-exposure prophylaxis). What is your take on the state of HIV prevention today?

The first place to begin is to say that indeed there's been a huge investment in new preventive technologies and approaches. Certainly oral PrEP, either daily or intermittent, but certainly daily oral PrEP with Truvada has turned out to be the most effective of the new prevention tools. And I've been impressed, as I think many have been, that the effectiveness data look even better than the efficacy data. That doesn't always happen, and it's really very striking.

But it seems that uptake of PrEP is slower than hoped for. Why do you think that is?

If you had said to me five or eight years ago that we'd have a new prevention technology that if adhered to has effectiveness levels approaching the high 90s or even higher, we would all have thought, I bet, that there would be a sea change. What we've seen instead is the US really leading the effort to implement this with FDA [US Food and Drug Administration] approval and CDC [US Centers for Disease Control and Prevention] guidelines, training for providers, and funding. Now in the US there are well over I think 34,000 or 35,000 people at risk of HIV on PrEP. So that's just incredibly encouraging.

Then we look at the landscape globally and we are looking at what I would say is lax implementation—slow and frustrating. So why is that? I would say first of all, for some of the G8 countries like the UK, there's no question that the price of Truvada has been a barrier. That isn't the case in much of the world—it's an inexpensive generic drug in Thailand, for example, where they've approved the use of Truvada. It's a generic formulation that costs about 30 cents a day or even less, and they have implemented it as part of their national program. So I don't think cost explains it all. There are certainly regulatory concerns and there's also been uncertainty, I think, on the part of many governments about where the international donors are. Is PEPFAR [the US President's Emergency Plan For AIDS Relief] going to support this? Is the Global Fund going to support it? That finally does seem to be happening.

But I think the other issue really gets to the challenge of who needs this. If a country has, for example, programs for youth and is worried about youth at risk, you look at PrEP and you think: we have millions of young people in this country under age 25, are we going to put a generation on PrEP?

But when we look at it from a public health perspective, that's not what we're talking about at all. In the US, for example, we have quite a small population of people who account for most of our new infections—63 percent are young gay men. And we're particularly worried about young gay men and transgender women of color. It is actually a very small population who are very heavily burdened and who could really benefit from this intervention, so the whole cost analysis becomes a very different thing. But it's very, very difficult still in so many parts of the world for people to do anything meaningful for people who are *really* at risk. What governments want to do is big, cheap, generic programs for people who are at very little risk.

Obviously a great deal of your work involves identifying and working with those at high risk, often marginalized populations. Should preventive approaches be targeted to these populations and how can that be done?

I think the epidemic is going to force us to do that. First of all, because we're just not seeing the resources we need. And secondly, because we've

passed an important milestone in 2015, which is that—this is UNAIDS data—the majority of new infections, over 50 percent, are in key populations worldwide. So this is the undone work of the response and this is where HIV is going to linger. Sadly, we see this in many countries including our own. Rates in heterosexual populations are in decline. Thailand has also achieved dramatic declines in heterosexual transmission and made tremendous accomplishments in preventing mother-to-child transmission, but has a hot epidemic in young gay men. I published on this more than a decade ago, saying these epidemics are going in different directions. And you hate to be proven right by human suffering, but in fact we were right.

I think the other discussion that is really important to have, which now is beginning to happen in some fora, is to be able to say we're actually talking about a relatively small number of people who really need these interventions. It's not about commitments to enormous numbers of people, which we all understand there are not the resources for. It's really about addressing where the epidemic is, and that is in relatively small pockets of very high-risk transmission.

It's really about addressing where the epidemic is, and that is in relatively small pockets of very high-risk transmission.

This is still a virus that's transmitted in a very specific number of ways. People are now all concerned about the fishing communities on Lake Victoria, but this is not something inherent about fishing. This is about sex workers, alcohol, and men with cash. It's an old story but it's one where, again, we need to have preventive interventions that fit with people's actual risks.

That is actually a perfect segue to the ring results, which is an intervention tailor-made for women at high risk of HIV who are unable to use other prevention strategies. What did you think of the recently reported results and the future licensure and implementation of the dapivirine ring?

I think many of us had hoped that there would be higher efficacy, and I think it's striking that both trials are so close in range of efficacy, that kind of 27 to 31 percent range, which is just right on the edge of making it worth fielding. That makes it challenging. The lack of efficacy for younger women and the clear adherence issues for younger women really is a challenge because they are the people we're obviously most concerned about in terms of incident infection.

I do think that this starts to move us toward the idea of a menu of options. It is an additional option for women right now who have relatively few. We know that we're pretty far along in the development and testing of a ring that will also have contraceptives and that would be a wonderful additional option, so we have to herald that.

I don't know how many settings the ring is really going to be relevant for, but there's no question that if you look at the epidemiology in East and Southern Africa, we have just been unable to reduce incidence. In the placebo arm of both of those trials, incidence is extraordinarily high—it's in the 6 percent range, and these are women who are getting counseling and their sexually transmitted infections are being treated. And of course we've already screened out everybody who's living with HIV infection, so that tells you that that epidemic is not under control. So even a tool of modest efficacy might really help make a difference.

There are also the long-acting antiretrovirals (ARVs) in development, which my be a potential way to eliminate the reliance on daily or intermittent dosing. Are you hopeful that this could be a promising prevention option in the future?

It is going to be a better fit for some. There are lots of places—a good example is India—where we know there's a strong preference for injectables over orals, but that varies. There are other contexts where people would rather have an oral than an injectable. I think there's a lot of hope and enthusiasm that the long-acting ARVs are going to matter more for adolescents and young adults. If you really look at the data from both of the dapivirine ring trials and also from ATN-110 [the adolescent trials network] study that looked at young MSM [men who have sex with men] on PrEP in the US, the youngest age groups just really have trouble adhering. So we have to explore if long-acting ARVs are going to help address that problem.

I think it is also important to keep in mind that it's not just age. In ATN-110, the Caucasian and Latino kids did just fine—they achieved basically measurable levels of Truvada, maintained them through the course of the trial, and they were protected. It was the African-American kids in the same age strata who brought down the overall curve because their uptake was so low and their adherence was so low. That tells you that it's not just age, and it's not biology. There are also probably socioeconomic, but certainly social and cultural barriers for subsets of people. Again, painfully, in the populations who need it most.

In the midst of all this new research, there are very ambitious goals being set by international organizations, including ending AIDS by 2030 and getting 90 percent of HIV-infected individuals on treatment. Are these goals achievable?

I'm a big believer in goals and I was turned around in some ways by this with the MDGs. I was somewhat of a skeptic early on and then I was just amazed how many ministers of health could quote for you where they were on the MDGs, if they were going to meet their targets, and which ones they were going to make and which ones they weren't.

I started to realize people actually pay attention to this and it's a motivator. That has a lot of value in public health, where in a world of many, many competing priorities, we're trying to keep ministers of health, finance, and education focused on AIDS. So that's welcome.

Beyrer giving a lecture at the Humphrey School of Public Affairs

I think the SDGs are beautiful. We're all worried, I think, that they are too vague and that they're going to be harder to measure. The MDGs of course were beautifully simple.

But let me speak to a couple of issues that I think we really have to deal with. First of all, the evidence on what we really need to do for 90-90-90 is not clear for a number of key populations. If you think about how much more treatment there is in South Africa than there was 10 years ago, there's just no comparison. We were in Durban in 2000 and there was essentially nobody on treatment except the rich. In 2016 when we go back to Durban, there are more people on treatment [in South Africa] than any other country in the world. It's the largest treatment program there is and it's enormously impressive. Yet we just talked about how high the rates of new infections are in young women and girls. So show me that this [expanded access to treatment] is really resulting in the declines in incidence that we want to see.

I think the same thing is really true when we look at the current epidemics underway in gay men. The men in the delayed PrEP initiation arm of the PROUD trial had a seroincidence of nine per 100 person-years. This is in the UK, where there's a national health system and 85 percent of people living with HIV are on antiretrovirals. So it just really is scientifically questionable to me. Unfortunately, we've embraced this without understanding how high incidence still is. That's my first critique.

The second is that this is a complex pandemic that has many different components, and all of the rhetoric about the end of AIDS and control of the epidemic presupposes that Eastern Europe and Central Asia are just taken off the table. The fact is they are taken off the table because they're in the "too difficult" box because the Russians are such a powerful force opposing evidence-based prevention in public health. Nevertheless, those are human beings. They're part of our world, they're part of the AIDS epidemic, and the epidemic is expanding there. There are plenty of good reports from the same agencies who are saying we're on the way to the end showing that the epidemic is expanding there in 2016.

The other regions where HIV is expanding is the Middle East and North Africa and that part of the world is going through a period that is really akin to World War I in Europe. It is multiple countries in rebellion, at war, and there is also a huge population displacement. There's a long history of these kind of contexts making it virtually impossible to control infectious diseases. So I think it really is questionable what effect the 90/90/90 goals will have. I would say that we've made remarkable progress in the parts of the world where we have really focused attention and resources. We've shown that it can be done. But that's not what much of the planet looks like.

What role do you see for an eventual HIV vaccine?

The fact that we are still seeing such high rates of incidence really speaks to the fact that primary prevention remains a challenge. And the fact that treatment is so good and this is a manageable disease, doesn't get around the fact that it is daily lifelong chemotherapy for the rest of people's lives. The other reality that we're seeing where care is really good is that the long-term chronic complications of life with treated HIV disease are many and are complex. Saying that something is a chronic disease to somebody

who has obesity, diabetes, hypertension, hyperlipidemia, and cardiovascular disease doesn't sound that great. When you talk to a lot of African clinicians, people who have been in this fight for a long time, they're thrilled that people are surviving, yet they have no idea how their health systems are going to deal with all of these aging, chronically ill people with multiple complications.

And we haven't even gotten to half of people worldwide who need treatment. We have 37 million people living with HIV and only 17 million on treatment. So I think we need a vaccine, but I also think we can't give up on looking for cure and remission. Even a five- or 10-years remission off therapy would make a huge difference.

Do you see a growing movement for cure research?

I think it's a long road, but nevertheless it's essential. I would say that it's very clear from the IAS perspective that the young investigators, the people who are really excited about HIV research, see cure research as a really exciting prospect. I think our last six out of seven new investigator awards went to people working on reservoirs and latency. That tells you that the intellectual fire power of the next cohort of researchers is going into cure.

It also turns out that once again HIV is providing insights into other disease systems. It turns out that there's probably a testicular reservoir with Ebola, which is why there's sexual transmission. There's also a reservoir with Zika, so there is also sexual transmission there. This is something we totally need to understand—how these immune-protected spaces in the CNS [central nervous system] and in the testes may really play a role in latency.

As you prepare for AIDS 2016, what do you see as the main themes for the conference?

I think the meeting in Durban in 2000 was the beginning of the treatment era. It really was a huge turning point. The world came together and really heard that it was unacceptable that the great majority of people living with this virus were going to be consigned to an early death. We came out of there with a commitment, and it took several years, but by 2003 things really began to move. And we changed the world and showed that you could treat millions of people with a complex disease in the poorest countries in the world and save lives.

In 2016, we're really at a new point, which is: we're not done. We have a remarkable achievement to be proud of as a movement, but we're less than halfway there on treatment and we're not implementing the prevention we have. We have harmful laws, policies, and practices that are aiding and abetting the virus. A proliferation of those laws in a number of countries, by the way, are going precisely in the wrong direction. We also have waning donor interest and a movement toward other priorities. I think we've declared victory too soon. Nobody ever wanted the end of AIDS to be the end of the AIDS response. That wasn't the plan. And so I think that Durban in some ways has an enormous burden for us as a movement, which is to reassert the importance of continuing this work and of doing the undone work of responding to this epidemic.

Happily, I think the science really looks wonderful. This is the most competitive scientific meeting we've ever had. We have more than 7,000 submissions and it's very interesting that the largest scientific component was in implementation science because so much of the field has moved toward implementation. There are a huge number of people working in that space, as there should be, and there really isn't another venue for people to put that kind of work forward. We're very gratified by that. We also have the largest scholarship program we've ever had. It's more than twice the size of the scholarship program for our last conference in Melbourne. It is critical to figure out ways to ensure civil society continues to have access to the science.

How would you describe your tenure as IAS President?

It's been profoundly rewarding. It's really an honor to try and serve our community. I love the people working on AIDS—I think they're some of the best people you can find. When I came into this leadership role there were some real challenges between governments, community, and science, and I think we've really worked hard to build bridges there and bring back that sense of all being in this together. That's been very positive.

I guess the other thing I would say is I hadn't realized how meaningful it would be for the community that I'm the first openly gay person to lead this organization. But it turns out that it really mattered to people, and so I think that that's important too.

And Linda-Gail Bekker is going to be amazing. ■

Working Feverishly to *FEND OFF DENGUE*

While the world is facing the growing threat of the Zika virus and its devastating consequences, a cousin to the vector-borne virus is wreaking even more havoc.

By Mary Rushton

When scientists isolated the first serotype of dengue virus in 1943—a mere four years before the Zika virus surfaced in a Ugandan rainforest—this mosquito-borne virus already had a lengthy history.

The first record of a disease that is clinically compatible with that caused by the dengue virus dates all the way back to 10th century China. Reports of outbreaks spread by the *Aedes aegypti* mosquito that caused high fever, headaches, severe muscle and joint pains, and a skin rash characteristic of dengue had been reported for centuries (*Trends Microbiol.* **22(3)**, 138, 2014).

Then World War II brought the virus to a whole new level. Millions of soldiers from Allied and Axis forces who had never been exposed to dengue were flooding the South Pacific and becoming infected. The situation was so dire that the Malaria and Epidemic Control Board of the South Pacific area classified dengue second only to malaria as a tropical disease of military importance (*Emerg. Infec. Dis.* **Vol. 18**, No. 4, 2012).

Around 90,000 US troops had been hospitalized for dengue infection by the time Japanese scientists Ren Kimura and Susumu Hotta identified the first serotype of the virus in 1943 while investigating an epidemic in Nagasaki (*Dengue Matters,* **Issue 11**, 2014). US scientists Walter Schlesinger and Albert Sabin (best known for his work on the oral polio vaccine) made the same discovery, independently, in Hawaii the following year.

The ecological disruption caused by World War II that encouraged dengue's spread was soon followed by decades of rapid urbanization and increased globalization due to more transient populations. Together, these factors encouraged the spread of dengue, including the emergence of multiple strains circulating simultaneously, which in turn contributed to more serious disease outcomes. The discontinuation or reduction in mosquito control programs also worsened the situation. What was once an occasional outbreak in a small number of tropical countries where mosquitoes persist year-round, became a pandemic with multiple serotypes co-circulating in the same regions (*Clin. Microbio. Rev.* **11**, 3, 480, 1998).

Today, dengue infects as many as 390 million people worldwide by some estimates (*Nature* **496**, 504, 2013). The World Health Organization (WHO) refers to dengue as the "fastest spreading vector-borne viral disease in the world." The virus is now endemic in over 100 countries, with the heavily urbanized countries of Brazil and Indonesia being the most affected, and the countries and regions impacted by dengue are growing. The US, for instance, battled a major outbreak of dengue in Hawaii last year that resulted in 260 cases, and cases occur in the state of Florida almost every year. The most dangerous form of dengue disease—a hemorrhagic fever that causes bleeding under the skin, frequent vomiting, abdominal pain, and in some cases death—is also occurring with greater frequency.

"Dengue is spreading steadily but consistently," says Oliver Brady, an epidemiologist with the London School of Hygiene & Tropical Medicine, who uses maps and models to evaluate epidemics, including dengue and malaria. Brady was part of the research study led by Oxford epidemiology professor Simon Hay, now with the Institute for Health Metrics and Evaluation in Seattle, who shocked the world with estimates that up to

10 percent of people living in the tropical world could be infected by dengue each year. "We're having real success in reducing malaria, but with dengue, no one has been able to stop it with any amount of resources," Brady says. "It's become a huge burden in middle-income countries in South America and some parts of Asia and is a huge drain on productivity."

The good news is that dengue finally made it onto the list of vaccine-preventable diseases with Dengvaxia, the first vaccine approved for a vector-borne virus since the yellow fever vaccine was introduced in 1937. In December, three tropical hotspots—Mexico, the Philippines, and Brazil—approved Dengvaxia, made by Sanofi Pasteur, the vaccine division of pharmaceutical giant Sanofi, for use in children and adults ages 9 to 45. A fourth country, El Salvador, has recently licensed it as well and the WHO endorsed it in April. The company has filed for regulatory approval of its vaccine in over 20 countries, including several in Europe, and expects to add another 15 countries to the list before the end of this year.

It also has filed a fast-track designation for its vaccine with the US Food and Drug Administration—an option for drug and vaccine makers that allows them to have portions of their application considered before the full application is submitted, which in Sanofi's case is expected to occur by early 2017. The fast-track designation helps expedite the approval process and is reserved for experimental products that address unmet medical needs.

Sanofi already had a track record in making vaccines against flaviviruses, namely yellow fever and Japanese encephalitis, so scientifically it made sense for them to focus on dengue. But tropical diseases don't always attract a lot of commercial investment, and dengue, in particular, was challenging. The brewing public health crisis drew Sanofi in.

"Dengue is a major and growing public health issue threatening almost half the world's population and cases have been reported in the US and Europe," says Guillaume Leroy, vice president of Sanofi Pasteur's Dengue Business Unit. "The fact that dengue is so well adapted to spread in urban centers of the tropical and sub-tropical world makes it a real threat to global growth and economic stability in these emerging countries."

Sanofi invested an estimated €1.5 billion to develop its vaccine. The company is not alone in making substantial investments in this area. Another promising vaccine candidate is undergoing efficacy trials in Asia and Latin America that was developed by the US National Institutes of Health (NIH), and Merck and GlaxoSmithKline both also have candidates in early clinical trials. There are also discussions underway to develop a combined dengue and Zika vaccine candidate. And while there are no antiviral drugs to treat dengue on the horizon, work is being conducted in earnest to develop therapeutics that could help quell symptoms.

"Zika and Ebola are teaching us that infectious diseases know no borders and can rapidly become global public health threats that require innovative solutions in terms of both vaccine development and timely access to curb further geographic spread," says Leroy.

With so much attention fixated on what to do about Zika—a virus once thought to be relatively benign but now, almost overnight, linked to severe fetal birth defects (primarily microcephaly) and the rare autoimmune disorder, Guillain-Barré Syndrome (*NEJM* **374**, 1981, 2016; *Lancet* **387**, 1531, 2016)—dengue provides important insights, some fleshed out below, into how quickly vector-borne viruses can spread and how challenging it can be to control and prevent them when they do.

Dengue's family

Considering how much damage they cause, viruses are pretty simple creatures. The retrovirus HIV's genome encodes for consists of a mere nine proteins; Ebola, a filovirus, encodes for seven. Dengue, a flavivirus, consists of a single strand of RNA that is referred to as positive-sense RNA because it can be directly translated into proteins. The viral genome is translated as a single, long polypeptide that is cut into ten proteins (*Cell* **108**, 717, 2002).

There are four confirmed serotypes of dengue. In 2013, a researcher from the University of Texas Medical Branch reported on the discovery and characterization of a possible fifth serotype in Malaysia—the first new subtype in over 40 years—however, the work has not been published yet, and so its existence remains a topic of some debate (*See sidebar, page 11).*

Flaviviruses belong to the *Flaviviridae* family, which got its name from yellow fever—flavus being the Latin word for yellow. At least 53 flaviviruses have been identified, a third of which are medically important human pathogens (D. Gubler, K. Goro, L. Markoff, *Flaviviruses*. Fields Virology, 4th Edition. Eds. B. Fields, D. Knipe, P.

Howley, Philadelphia: Walters Kluwer Health/ Lippincott Williams & Wilkins, 2007). West Nile Virus, also transmitted by mosquitoes, is asymptomatic in most people but can cause fatal neurological disease. Yellow fever virus got its name because in the most severe forms it causes jaundice and hepatitis. Dengue, thought to be named for the Swahili term "Ka-dinga pepo"—cramp-like seizures caused by an evil spirit—does indeed cause debilitating joint pain that takes weeks and even months to recover from.

But in more serious cases dengue can also damage the overall vascular system, leading to increased vascular leakage and abnormal blood clotting, and interfering with the body's ability to repair itself. Widespread bleeding may accompany this condition, which is why it is referred to as dengue hemorrhagic fever (DHF). The most severe form of DHF is Dengue Shock Syndrome (DSS), characterized by severe vascular leakage, multi-organ failure, and a complete breakdown of the circulatory system. People with DHF, and particularly DSS are at risk of dying from dengue.

The culprit

Many viruses that cause tropical diseases are transmitted to humans by mosquitoes. About 3,000 different species of mosquitoes have been described in the scientific literature, according to the Entomological Society of America, but only a small percentage are vectors for pathogens that sicken and kill humans. Female *Anopheles* mosquitoes spread malaria, and various species of *Culex* mosquitoes are the primary chauffeurs for West Nile virus, and Japanese, Eastern Equine, and St. Louis encephalitis.

The *Aedes aegypti* mosquito spreads a number of different viruses, but its reputation as a vector seems to hinge mostly on the transmission of a quartet of viruses that include dengue, yellow fever, Zika, and chikungunya, which is an alphavirus. *Aedes albopictus*, also known as the Asian Tiger mosquito, may also spread these viruses, though *Aedes aegypti* is the most common carrier.

Many mosquitoes live and feed outdoors. According to the US Centers for Disease Control and Prevention (CDC), the *Anopheles* mosquito lays its eggs in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers, and small, temporary rain pools. They generally breed outdoors, in open, sun-lit pools or shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants. They are active at dusk, dawn, and at night.

Aedes aegypti mosquitoes are different. They are highly domesticated insects that like to lay their eggs in flower vases, automobile tires, rain buckets, cisterns, and other containers in and around homes, and prefer to feed on humans during daylight hours. They weren't always this "friendly," says Duane Gubler, Founding Director of the Emerging Infectious Diseases research program at the Duke-NUS Medical School in Singapore and formerly chief of the CDC's dengue branch. Gubler, who has studied dengue since the 1970s, says the yellow fever and dengue-spreading mosquitoes used to be feral insects that lived in forests and didn't mix much with humanity. "But thousands of years ago, *Aedes aegypti* started moving into the villages of Africa and over time have become highly adapted to their human surroundings."

The feeding habits of *Aedes aegypti* have, inadvertently, also made the spread of disease more efficient. Gubler notes that the female mosquitoes tend to be very nervous feeders that are easily distracted by the slightest movement. This peripatetic behavior means that females often feed on several individuals during a single blood meal, greatly increasing the rate of transmission when their hosts are infected with dengue or other viruses.

A Fifth Serotype?

Designing vaccine candidates that effectively target four separate serotypes of dengue virus is difficult enough, but a 2009 outbreak in Malaysia suggests there may be a fifth serotype. Nikos Vasilakis, a virologist at the University of Texas Medical Branch in Galveston, reported three years ago at the Third International Conference on Dengue and Dengue Hemorrhagic Fever in Thailand that a fifth serotype had been discovered and characterized in an adult male with acute dengue fever. The man lived on the Malaysian island of Borneo. The four recognized serotypes of dengue are genetically similar—about 65% of their sequences are homologous—while the virus identified in the Malaysian male, though 40% similar to dengue serotype 4, was thought to be phylogenetically distinct (*Med. J. Armed Forces India* **71**, 67, 2015).

Vasilakis reported at the time that he thought the virus isolated in the Malaysia man may have been circulating in nonhuman primates (the only known animal reservoir of Dengue) and made its way into humans (*Science* **345**, 415, 2013). However, Vasilakis and his colleagues who identified the new serotype have not formally described or published their work. "Official ratification of a separate serotype awaits the recovery of an isolate, which should be characterized by performing a series of rigorous identification tests to confirm, or indeed conversely to refute, its uniqueness," writes Andrew Taylor-Robinson, a virologist with Central Queensland University in Australia, in a commentary earlier this year (*Int. J. Clin. Med. Microbiol.* **1**, 101, 2016). —*M.R.*

Man-made calamity

Scientists generally agree that dengue would not have spread so rapidly in recent years were it not for a succession of events that began during the Second World War. Prior to WWII, dengue viruses circulated throughout the tropics, but the relatively small urban populations and the fact that viruses and mosquitoes relied primarily on boats to move around the world meant that epidemics were sporadic (*Trop. Med. Health* **39** (Suppl), 3, 2011). Most regions had only one or two dengue serotypes circulating at any one time.

This changed when millions of soldiers descended on the South Pacific—US and Japanese enforcements alone totaled around 10 million. These soldiers transported viruses and their vectors across the region. By the end of the war, many countries in Asia were endemic with all four circulating serotypes of dengue, says Gubler.

Aedes Aegypti Mosquitoes. Female (left) and male (right). Credit: National Institute of Allergy and Infectious Diseases

Major shifts in population from rural areas to cities during and after the war further fueled dengue's spread. Rapid industrialization began in earnest in post-war Asia, causing millions of people to move into cities ill-prepared or too poor to accommodate them. As an example, the mean population in the Asian cities of Dhaka, Bangkok, Jakarta, Manila, and Saigon grew from about 1 million to over 12 million between 1950 and 2010. Families lived in overcrowded houses with poor sanitation and poor mosquito control. This enabled dengue to thrive.

It was in the 1950s and 1960s that epidemics of DHF began occurring across southeastern Asia, the first one being in the Philippines in 1953-54. It is still not entirely clear what mechanisms provoke DHF, which seems to occur slightly more often following infection with serotype 2 of the virus but can occur, nonetheless, following infection with all four serotypes. Gubler says co-circulation of multiple serotypes doesn't just increase the probability of infection. "It increases genetic mutations, which in turn increases the probability of a more virulent strain of virus emerging.

Epidemiological evidence suggested that DHF was provoked by an immune response. Many of the individuals infected with DHF had a secondary antibody response to dengue and lived in regions where there were two or more serotypes of the virus in circulation. Researchers concluded that the course of infection with a second dengue virus of a different serotype was worse because it was adversely affected by the immune response against the first infection (*Yale Journal of Biology and Medicine* **42**, 262, 1970).

Steven Whitehead, a dengue researcher at the National Institute of Allergy and Infectious Diseases (NIAID)—part of the NIH—says antibodies elicited to the virus serotype in the primary infection are able to bind a second infecting virus, "but they do not effectively neutralize the new serotype and, in fact, may enhance entry of the new dengue serotype into susceptible cells, such as monocytes, through Fc receptors," says Whitehead. "This, in turn, leads to increased viral replication, increased viral load, and enhanced disease." Whitehead says cross-reactive antibodies from one's first dengue infection are therefore more problematic than helpful during the next encounter with a different virus serotype. The WHO now warns that cross-immunity between serotypes following recovery from dengue is temporary and subsequent infections increase the risk of developing DHF or DSS. "Of course, you always have good protection against infection with the same serotype," says Whitehead.

People do gradually build up immunity as they are progressively infected with other serotypes. Young children are much more vulnerable to infection and severe disease because they haven't been around dengue long enough to be exposed to different serotypes.

Mosquito control

Along with urbanization and globalization, inconsistent and sporadic mosquito control operations also fostered the spread of disease. Concerned more with yellow fever than dengue, an effort led by the Rockefeller Foundation's International Health Division (IHD) sought to destroy mosquito breeding grounds in key communities

or "seedbeds," where the *Aedes aegypti* mosquitoes thrived. During the early 1900s, the IHD established campaigns in South America and Africa, with the aim of reducing house infestation of *Aedes aegypti* to 5 percent, enough to break the virus' transmission cycle.

The Pan American Sanitary Organization (later re-named the Pan American Health Organization) followed up in the late 1940s with a successful campaign that actually eradicated *Aedes aegypti* from 18 countries in Latin America and several Caribbean nations. The campaigns were carried out by semiautonomous groups who sprayed urban buildings, including residential homes, with the inexpensive but controversial insecticide DDT that would later be banned for health reasons. These efforts and the introduction of the vaccine helped eliminate urban yellow fever from the Americas.

But even before the DDT ban, some countries wouldn't participate in the eradication efforts, and those that did eventually lost interest, political will, or lacked funds. Surveillance efforts were scaled back, which helped mosquito populations to return and re-establish habitats.

Robert Tesh, a pathology professor at the University of Texas Medical Branch who studies the epidemiology, pathogenesis, and natural history of arthropod-borne and zoonotic viral diseases, says this door-to-door spraying would likely be pretty unpopular today, even in countries that once embraced such tactics. "When I was living in Panama they used to come door to door and spray the walls with insecticide in a kerosene base. The house would reek of it," says Tesh. "Can you imagine trying to do that in a residential neighborhood in the US?"

More innovative approaches to stop mosquitoes from transmitting viruses are now being pursued. Researchers at the University of Melbourne have developed two strains of the bacteria *Wolbachia*—an organism that infects arthropods—that reduce the ability of *Aedes aegypti* mosquitoes to transmit dengue and Zika (*PLoS Pathogens* 2016, doi:10 1371/journal.ppat.1005434 2016). In superinfected mosquitoes, pathogens appear to lose their ability to replicate, and if abundant enough are able to out-compete the disease-bearing mosquitoes until the cycle of transmission is broken. Studies are ongoing in Australia, Columbia, Vietnam, and Brazil using Wolbachia-infected mosquitoes to control dengue. Several other efforts to alter moquitoes to inhibit their ability to transmit viruses are also being explored to control mosquito populations and prevent diseases such as malaria.

The promise of vaccines

One of the most promising technologies for stopping dengue is an effective vaccine, which thanks to years of effort is now available. The earliest dengue vaccine development efforts date back to during and just after World War II when pioneering microbiologist Albert Sabin used mouse brains to passage wild-type dengue viruses to develop a live-attenuated dengue vaccine containing two different serotypes. Sabin found that as the virus became adapted to the mice, it became less pathogenic. He later gave the vaccine candidate based on this live-attenuated dengue virus to humans to show that the virus was indeed attenuated and that it caused only mild symptoms. Subsequently, the volunteers were found to be protected following challenge with wild-type virus, and the protection was shown to be generally due to neutralizing antibodies (*Antiviral Therapy* **14**, 739, 2009). Further testing of the candidate was not pursued, however, over concerns that the mouse-brain preparations might be contaminated.

Since then, multiple vaccine candidates have been developed and tested, with Sanofi Pasteur's Dengvaxia vaccine the first and thus far only one to cross the finish line. The live-attenuated recombinant tetravalent vaccine was designed by scientists from Acambis, a vaccine company acquired by Sanofi Pasteur eight years ago. Dengvaxia uses the licensed yellow fever vaccine YF-17D as a backbone, but replaces certain genes that contain neutralizing epitopes for yellow fever with homologous regions of the four different dengue serotypes. This novel approach was pursued because previous live-attenuated vaccine candidates were associated with a high rate of adverse events, and inactivated vaccine candidates didn't induce broad enough or durable enough responses.

The Acambis scientists produced four live-attenuated vaccine viruses based on the yellow fever 17D strain, one per dengue serotype. Each recombinant virus was constructed by swapping yellow fever genes with dengue genes, a strategy made possible because of the similarities in the virus genus (*Vaccine* **29**, 7229, 2011). "Because dengue is also a flavivirus, it made it easier for molecular biologists to take out the pre-membrane and envelope genes of yellow fever and insert the corresponding genes from dengue fever," says

Stanley Plotkin, a vaccinologist and emeritus professor at the University of Pennsylvania who serves as an executive advisor to Sanofi Pasteur. "The same strategy also works for Japanese encephalitis, and possibly may work for Zika."

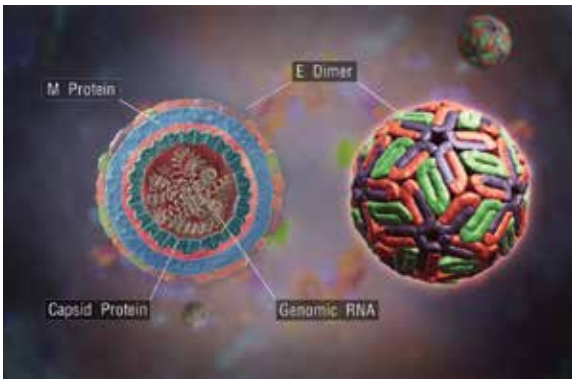
Two large international studies conducted in children and adolescents found that a three-dose regimen of Dengvaxia administered over 12 months was safe and effective in reducing severe disease, and that the vaccine candidate induced neutralizing antibodies against all strains. However, efficacy data by age was mixed.

The first study was conducted in about 20,900 healthy children ages 9-16 from Brazil, Colombia, Puerto Rico, Honduras, and Mexico. The second was conducted in 10,000 healthy children ages 2-14 from Malaysia, Vietnam, Thailand, the Philippines, and Indonesia. Overall, efficacy was around 60 percent in the Latin America trial and 57 percent in the Asia trial, though the vaccine provided greater protection against serotypes 3 and 4 than against serotypes 1 and 2 (*NEJM* **372 (2)** 113, 2015; *Lancet* **384**, 1358, 2014). The studies also found that efficacy increased with age and that previous exposure to dengue prior to vaccination also increased efficacy. The results also varied considerably by country. An exploratory analysis found that in the Latin America study, vaccine efficacy was 83.7 percent among those with a prior exposure to dengue, but as low as 43.2 percent among seronegative participants. Efficacy was as high as 78 percent in Brazil, and as low as 31.3 percent in Mexico.

Pooled results of both studies found an efficacy of around 65 percent for ages 9 and older, but only 44 percent for those under age 9, the group most vulnerable to infection. Long-term follow-up of vaccines recipients found that the risk of hospitalization among individuals aged 9 years or older was less than 1 percent three years after the start of the study. Among vaccinated children under 9 years old it was 1.5 percent and among 2-5 year olds it was as high as 7.4 percent.

Why the hospitalization rates were so high among this group isn't entirely clear, though some believe the candidate isn't balanced enough. If the immunization in very young children elicits only partial or transient immunity, it predisposes them to infection later on for which hospitalization is required, wrote Cameron Simmons, a microbiologist and immunologist at the Peter Doherty Institute for Infection and Immunity in Melbourne, who was not involved in the study (*NEJM* **373**, 1263, 2015).

Peter Hotez, president of the Sabin Vaccine Institute in Houston, Texas, and Washington, DC, and a leading advocate for the treatment and prevention of neglected tropical disease (NTDs), says designing vaccine candidates for dengue is so challenging because it has four serotypes that are co-circulating. "What this means is that in a vaccine you have to get equal immunity to all four serotypes at the same time."



Cross section of a dengue virion, showing viral components.

Sanofi is still evaluating the value of vaccinating children under 9 against dengue as part of its long-term follow-up to the two Phase III studies. This analysis will be finalized in 2018. "We can, however, anticipate that large-scale dengue immunization programs in endemic countries could provide indirect protection for unvaccinated younger age groups by lowering the pool of infected individuals and, thus, the transmission risk for all," said Leroy.

Another live-attenuated, tetravalent vaccine candidate, this one developed by scientists at the NIH, is now being tested in Phase III clinical trials in Brazil. The TV003 vaccine candidate was recently licensed to the Butantan Institute, a Brazilian research organization in São Paulo, which is also sponsoring the trial. The vaccine candidate is a mixture of all four dengue serotypes. The study is enrolling three age groups: 18-59, 7-17, and 2-6 years old. Each age group will have at least 5,000 volunteers.

An unusual clinical trial—a human challenge study—conducted by Johns Hopkins already suggests that the vaccine candidate may perform well against at least one dengue serotype. The study enrolled 48 healthy adult volunteers from two college campuses—the University of Ver-

mont College of Medicine in Burlington and Johns Hopkins Bloomberg School of Public Health in Baltimore—and randomly assigned them to receive either a vaccine or placebo injection. Six months later, 41 of the volunteers returned for the dengue challenge. The challenge virus used in the trial was a genetically modified version of the serotype 2 virus isolated in the Kingdom of Tonga in 1974. All 20 placebo recipients were infected and 16 of them got a rash. None of the 21 vaccine recipients showed any signs of infection after challenge (*Sci. Trans. Med.* 8, 330, 2016).

"We chose a strain of dengue 2 that was historically associated with less disease," says Whitehead, who developed the TV003 candidate. "People who received placebo and who were then challenged did not get ill, but they did have a good virus load and a rash."

Whitehead said the human challenge studies were conducted to help ask questions that in this case can't realistically be answered by models. In endemic regions, where there is more than one circulating strain, one inevitably finds large populations with a high degree of partial immunity, which can make it difficult to assess vaccine efficacy against a particular serotype. Challenging animals isn't feasible because they don't develop symptoms to dengue. A second human challenge study with the TV003 vaccine candidate is now underway to assess efficacy against serotype 3.

Until the Zika outbreak, Whitehead's group was planning on adding a Japanese encephalitis component to their TV003 vaccine candidate. Instead, they are now developing several Zika components, which they plan to test in nonhuman primates and humans in the coming months. Then they will select the best one to add to the tetravalent vaccine candidate and test the combination dengue/Zika vaccine candidate. "I think we can have efficacy data in three to four years," he says.

They have also licensed the TV003 to three different companies in India and a company in Vietnam. Brazil acquired an exclusive license, which means Butantan Institute has sole rights to distribute the vaccine there. India did not, which gives the three companies based there the right to compete for market share in the South Asian country and also export the vaccine candidate to other countries that do not have exclusive licenses. Vietnam also doesn't have an exclusive license.

Other vaccine candidates in early clinical testing include Illinois-based Takeda Pharmaceuticals' chimeric tetravalent candidate based on an attenuated dengue 2 serotype backbone, and a purified, inactivated tetravalent dengue candidate containing the adjuvant alum. GlaxoSmithKline has since signed a research agreement to develop the latter vaccine with Brazil's Oswaldo Cruz Foundation. Meanwhile, Merck is developing a tetravalent recombinant envelope protein vaccine candidate also using an alum adjuvant that was originally designed by Hawaii Biotech.

This is a much different environment than 15 years ago when companies were disinterested in dengue vaccine development. Whitehead likens it to the "little red hen," who couldn't get anyone to help her turn wheat into bread until it was baked. "Large pharmaceutical companies looked at it but they didn't think it would be profitable," he says. "They weren't excited about it. But after Sanofi found a path forward for a dengue vaccine candidate and we developed ours and were able to show that you could administer it in one dose and that it wouldn't be expensive to produce, then it became a little more attractive."

Sanofi says both governments and physicians in the countries where Dengvaxia is now licensed are excited about the prospect of finally having a biomedical tool to fight the virus. In the Philippines, for instance, over 200,000 public school children have been vaccinated against dengue, with a target to initiate the vaccination of one million school children by the end of the year.

Unfortunately, this scenario is not playing out with other tropical diseases, including the seven most common NTDs (ascariasis, hookworm infection, trichuriasis, schistosomiasis, lymphatic filariasis, onchocerciasis, and trachoma) that are the focus of the non-profit Sabin Vaccine Institute. Hotez says it might be due to the fact that dengue is not just a disease of poverty. "Dengue is widely occurring among wealthy and middle-class populations around the world—it's become a huge problem in Singapore, Rio de Janiero, São Paulo, all over Jakarta—and so the market is not as depleted as it would be for hookworm or schistosomiasis," he said. "We are in the midst of a dengue explosion." ■

Mary Rushton is a freelance writer based in Cambridge, Massachusetts.

Dan Barouch's lab is a bustling hive working on complex initiatives in many areas of HIV research. On the brink of a **big vaccine study,** Barouch reflects on the painstaking steps required to build a **formidable investigative operation**—and to feel sure about decisions in a field known for uncertainty.

THE CONFIDENCE BOOSTER

By Michael Dumiak

A cutting wind has Boston commuters in scarves, but in a gleaming glass tower on Blackfan Circle, the 10th floor laboratory is orderly and calm. This is where Dan Barouch, in just a short time, has built a formidable investigative operation. Barouch oversees a 57-person research group active in HIV pathogenesis and basic science, vaccine research and development, and cure research, as well as working on emerging pathogens such as Ebola and, more recently, Zika.

Barouch is a clinician and Harvard Medical School professor. He's director of the Center for Virology and Vaccine Research (CVVR) at Beth Israel Deaconess Medical Center and a founding member of the Ragon Institute, which is a joint research endowment of Massachusetts General, Harvard, and the Massachusetts Institute of Technology.

"He's built a very large group that covers all the bases in terms of taking things from the concept stage to early testing and all the way forward into clinical trials," says Bruce Walker, Ragon Institute director. "I don't really know of another example of someone who's done this in such an independent way. Certainly nobody at that young age." Barouch just turned 43.

Barouch's always been on the young side among his peers. It's a topic that comes up often, though, he chuckles, it won't always be like this. "I've often found myself to be younger than a lot of the people I sit with in various conference rooms." He didn't get to this point overnight. It just feels that way.

Barouch finished his clinical fellowship as an infectious disease specialist 14 years ago at Brigham & Women's Hospital. Becoming a doc-

Center for Virology and Vaccine Research

Barouch in his office at the Center for Virology and Vaccine Research (CVVR) overlooking Blackfan Circle and the Harvard Medical School campus.

tor was a lifetime goal—he still does hospital rounds once a month. Before this Barouch went to Oxford to pursue a doctorate in microbiology. He finished it in two years, then turned to medical school.

While doing his medical school residency Barouch began working with Norm Letvin, the late Harvard HIV scientist renowned for research with nonhuman primates. With Letvin, Barouch was able to publish papers and establish a research record. Barouch applied for career development grants from the US National Institutes of Health (NIH) and the Doris Duke Charitable Foundation. He got them both. Letvin gave Barouch space in a fusty prewar building that Beth Israel was renting from Emmanuel College. He was able to hire two technicians with the grants. Just like that, Barouch started his own research lab at 29 and began working toward developing an HIV vaccine.

Rare air

When he's home in suburban Newton, Barouch begins every day practicing the violin with his two young daughters. When he's traveling, Barouch's wife, Fina, an ophthalmologist and retinal surgeon at Lahey Hospital & Medical Center, plays music with the girls. Eight-year-old Susanna is learning Beethoven's Minuet in G minor, while five-year-old Natalie is learning "Twinkle Twinkle Little Star." As someone who started playing the violin when he was four, Barouch can relate.

He arrives at work on this windblown March day in a good mood. "It's all fun. Every morning I look forward to coming to work. There's not a single day that goes by uneventfully. Every day something new happens. It's exciting," he says, with a slight sniffle, sipping coffee at eight in the morning. Bustling and cluttered inside, the 10th-floor CVVR sits in an arching glass building designed by Boston architects Tsoi / Kobus & Associates. The sleek complex also houses Pfizer's research and development facilities and the Wyss Institute for Biologically Inspired Engineering. Barouch's broad desk at the CVVR comes with a view over the Harvard Institutes of Medicine. The Ionic columns of Harvard Medical School's Gordon Hall are just visible. With close-cropped dark hair and strong eyebrows, Barouch is focused and crisp. He is youthful and friendly, but not overly so; he seems intent on keeping composure at all times, but is not anxious.

A little anxiety wouldn't be surprising given the amount of activity Barouch is keeping track of. Over the spring he and his team readied for what could be some of its biggest exploits yet. The CVVR recently initiated an in-house Phase I HIV vaccine clinical trial, showing the lab's capability to take its own basic science work into translational human research. Barouch is also part of a collaboration that's just been awarded a US$42 million grant for HIV vaccine and cure research that will make extensive use of the team's nonhuman primate program, with Barouch and Louis Picker of the Oregon Health & Science University as co-primary investigators.

Then there is the lab's role in bringing its leading vaccine candidate into efficacy trials in collaboration with Johnson & Johnson (J&J), the National Institute of Allergy and Infectious Diseases (NIAID), the HIV Vaccine Trials Net-

"Every day something

work (HVTN), and multiple clinical partners. The vaccine candidate is already being tested in a series of Phase I/IIa clinical trials, and pending the results, Phase IIb efficacy trials could start next year. If effective, the vaccine candidate is on a path to potential development and production, given J&J's industrial manufacturing capabilities. This would be a rarified stage—only a handful of HIV vaccine candidates have made it this far.

Barouch's weekly team meetings begin in a boisterous atmosphere. But he quickly turns to business. The schedule of ongoing studies run down every Friday represents a broad amount of scientific effort and dozens of precisely tracked ongoing experiments. On shelves next to Barouch's desk are hundreds of plain manila folders. "These are just the data from primates," Barouch says offhandedly.

Barouch did not set out to build a big research group, but the work required it. NIH funding provides the bulk of money to the lab. Barouch has administrative staff to help manage these large and complex grant budgets, but he still does his own grant writing. In the last five years the Barouch lab has received more than $80 million in NIH funding, averaging about $15 million a year. The group also gets substantial funding from the Department of Defense, amfAR, the Ragon Institute, and other industry and philanthropic sources such as the Bill & Melinda Gates Foundation.

Relationships

Down the narrow hallway from Barouch's office, past a shared shotgun office dubbed 'Techtopia' with a half-dozen lab techs poring over data, the wing opens up into lab space. In one corner the protein group is assembling. In another area, two researchers from the virology group, Peter Abbink and Michael Boyd, are opening up the vacuum manifold on a Promega filtering unit. Boyd will use the unit along with a Zymo research kit to purify RNA for the assays used to measure HIV viral loads in experiments and clinical trials. Boyd is a research assistant; Abbink, a Dutchman, is the virology lab manager, the Barouch lab's longest-serving member, and is introduced, somewhat jokingly, as 'The Master of the Vectors.'

This refers to his work in developing the viral vector used in the Barouch lab's vaccine candidate now in clinical trials. Abbink and the virology group make vectors based on adenovirus serotype 26 (Ad26), a strain of the common-cold virus. Barouch's experiments with Ad26 are one of the defining stories of his lab and career so far.

Interest in adenovirus vectors took him to the Netherlands in the summer of 2004. He'd just finished his medical studies and Letvin knew that Barouch was interested in adenoviruses and their potential in HIV vaccine research. Letvin introduced him to Jaap Goudsmit, who at the time was chief medical officer of a biotech company called Crucell, located in the old Dutch university town of Leiden.

"I said, 'you want to work with adenoviruses? Come spend a couple months with us,'" Goudsmit recalls. So started what would become a key partnership even when Crucell was eventually bought by J&J's pharma subsidiary, Janssen. Janssen is now a vital partner of Barouch's and the network of collaborators testing their Ad26-based candidate in combination with other candidates in clinical trials. "Janssen is directing late-phase manufacturing and clinical development of this vaccine. That's what we need. In the HIV vaccine field, we need more industry involvement, not less. There is no way an academic group can conduct all the activities for late-phase development. It requires industry," Barouch says.

Of course back when Barouch first went to Leiden he didn't know what was coming. He just wanted to learn. "I learned how to grow and clone adenovirus vectors with my own two hands," he says. It was a productive stay. Crucell had an intern that helped Barouch that summer. By the time Barouch went back to Boston, he'd made an agreement with Crucell: they would provide the viral particles, cell lines, and the DNA plasmids necessary for producing new vectors. Barouch also offered the Crucell intern a job in Boston. That's how Peter Abbink became the Vector Master.

Lab toys. A stuffed monkey atop perhaps one of the few adenovirus plush toys ever produced.

new happens. It's exciting."

"He was in a hurry. Very bright, wanted to get on to do things. To make a difference. He was always pushing forward."

– Sir Andrew McMichael

An arduous time for adenovirus

For many years now researchers have harbored hopes of utilizing attenuated viruses as vectors to deliver HIV antigens. Using a live-attenuated version of HIV itself is not feasible as there's too much risk that the virus could mutate and regain its pathogenicity. Several different viral vectors are being investigated, including adenovirus, and there are many different strains or variants of adenovirus, called serotypes. Adenovirus serotypes 5 (Ad5) and 26 are two strains that have been tested extensively as vectors.

A replication-deficient Ad5 vector developed by Merck was first tested in the STEP trial in partnership with NIAID and the HVTN. The study involved 3,000 volunteers from a diverse population at high risk of HIV infection, including men who have sex with men and female sex workers between ages 18 and 45. A companion to the STEP trial called Phambili tested the same Ad5 vaccine candidate in a different population of high-risk men and women in South Africa. At the time these trials started, Barouch had just returned from his sojourn in the Netherlands. Researchers were upbeat about the prospects for Ad vectors, including Merck's Ad5 candidate.

"It was *by far* the *most* immunogenic vaccine, and to this date still one of the best vaccines for inducing T-cell responses," says Nicole Frahm, associate director for laboratory science at the HVTN. Previous efficacy trials focused on stimulating antibody responses, but the Merck candidate was designed to stimulate only cellular immune responses. The logic was that even if a strong T-cell response couldn't protect against infection, it might help reduce the severity of disease in vaccinated individuals who still became HIV infected.

But three years into STEP its safety committee stopped immunizations. Phambili was halted too. The data indicated that in STEP there was a higher infection rate in the vaccinated group than in placebo recipients.

Frahm, who had left studying HIV pathenogenesis as a postdoc in Bruce Walker's lab to take up vaccine research at the HVTN just as STEP was closing, remembers the sadness that pervaded the field at that time. "It was depressing," she says. "This was one of those vaccines where everybody thought you would see a positive signal because it was so immunogenic."

A detailed analysis of the STEP results showed that the higher risk of HIV infection in the vaccinated volunteers seemed to come from a subgroup of uncircumcised male volunteers, who, due to being previously exposed to the virus, were already sero-positive for Ad5.

After STEP, a troubling hypothesis gained currency: that Ad5 vectors may recruit activated CD4 T cells to mucosal tissues, thereby increasing the number of target cells for the virus to infect and increasing the chance of HIV infection occurring.

So far no definitive data supporting this hypothesis has come to light. Frahm's group, which is about to publish findings from studying Ad5, has found no evidence for this either. "We have looked extensively in the mucosa," she says. The bottom line is that researchers still don't know what happened.

While Frahm's lab and others were racing to understand what had happened in the STEP trial, another large-scale Ad5 vaccine trial began. HVTN 505 tested a DNA prime/Ad5-based vaccine candidate boost that was similar but distinct from the one tested in STEP study. This trial also limited itself to circumcised men who have sex with men and whom did not have pre-existing Ad5 immunity at the time of enrollment. Even so, four years into the study, 505 ground to a halt because there was no difference in the HIV infection rate between the vaccine and placebo groups.

The results of these trials caused researchers to question the use of adenovirus vectors altogether. In the summer of 2013, NIAID held a meeting to discuss their future.

Through all this, Dan Barouch and his rapidly growing team watched closely. "We all took a long, hard look at the vaccine field and what made sense to do and what did not," he says. "At that moment in time it was not entirely obvious that we were going to continue to develop Ad vectors."

What Barouch and his team did was start investigating alternate Ad serotypes. They researched how different the various Ad serotypes looked to see if there was any evidence that the Ad26 vector the lab was zeroing in on was any better than Ad5. They also tested whether Ad26 vectors would recapitulate the safety concerns seen with Ad5.

From 2007 on, Barouch's group published paper after paper outlining experiments with Ad vectors. These were summarized in a perspective co-authored by Picker in which, drawing on the previous experiments, they outlined

the differences between Ad5 and Ad26 *(Nat. Rev. Immun.* **12**, 765, 2014*)*. The review outlines biological differences between the two serotypes, differences in human exposure to adenovirus strains, and differences in the innate and adaptive immune responses they induce, including in the mucosa. Based on these findings, Barouch pushed forward with Ad26.

Going viral

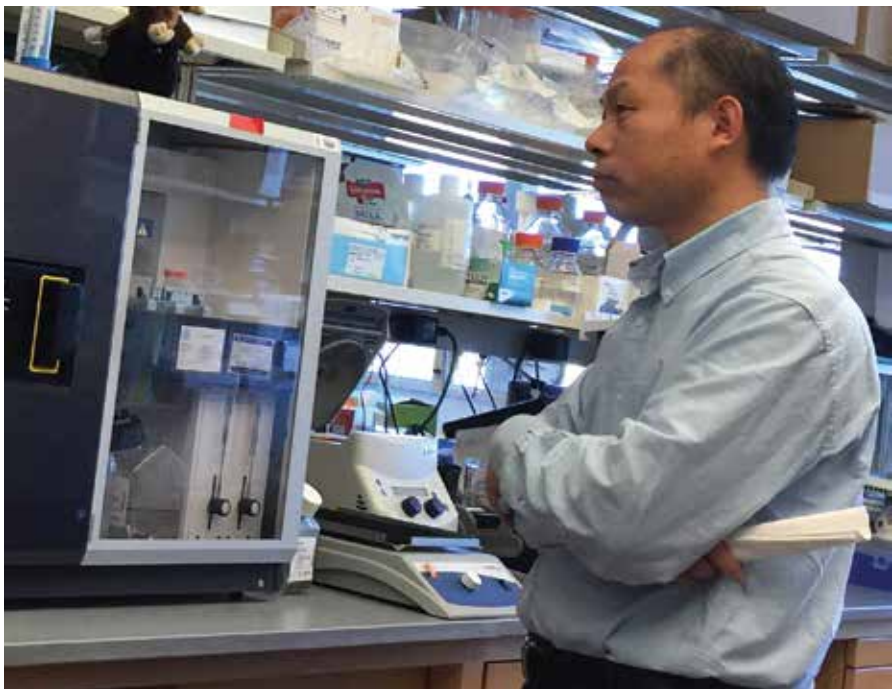
Colonel Nelson Michael was drinking coffee in a hotel lobby in Seattle's South Lake Union neighborhood getting ready to attend a meeting at the Gates Foundation. It was 2010 and Michael, director of the US Military HIV Research Program (MHRP), had begun to work with Barouch and a few others on monkey experiments testing the combination of Barouch's Ad26 candidate with another viral-vector based candidate developed by MHRP scientists. Barouch was in Seattle for the Gates meeting too, staying at the same hotel as Michael. He came down from his room, laptop in hand, excited, Michael recalls. The data Barouch shared with Michael showed that together the two vaccine candidates could protect monkeys against a stringent challenge. "It was so exciting! I'll never forget how pumped up we were," Michael says. "In those moments Dan has a childlike excitement about data that I find endearing. He's a fun person to work with. You don't have to like people you collaborate with, but it's a joy when you do."

Michael first got to know Barouch in 2008 at a scientific meeting run by immunologist Barton Haynes at the Duke Center for HIV/AIDS Vaccine Immunology (CHAVI). Barouch was still working with Ad5 at the time and Michael was in the midst of RV144, which tested a canarypox virus candidate known as ALVAC along with a gp120 boost. The RV144 trial would go on to show modest efficacy.

Michael was also working with another poxvirus vector: modified vaccinia Ankara (MVA). "You get more CD8 responses from adenoviruses," Michael says, "and more CD4 responses out of MVA." According to Michael, the MVA vector itself, even just with repetitive vaccinations, can also generate antibodies.

The two began talking about combining their appraoches, that is priming with Barouch's Ad vector and boosting with Michael's MVA-based candidate. The collaboration proved fruitful. First, monkey experiments that Barouch previewed with Michael back in 2010 showed protection *(Nature* **512**, 74, 2014). Now, the combination of Barouch's Ad26-vectored mosaic Env/Gag/Pol prime, MHRP's MVA vectored candidate expressing the same mosaic antigens, and an HIV gp140 protein boost are being tested in the Phase I/IIa study called APPROACH. The Env protein boost was added because of the RV144 efficacy data and monkey studies showing the protective efficacy of an Ad26/gp140 regimen (*Science* **349**, 320, 2015). This study is a big effort. It involves researchers from multiple institutions—Beth Israel, MHRP, the HVTN, the International AIDS Vaccine Initiative (IAVI), and Janssen. With the trial now fully enrolled, these institutions are collecting immunogenicity data on the experimental multivalent HIV vaccine candidates from 400 healthy volunteers at 14 sites in the US, Rwanda, Uganda, South Africa, and Thailand.

APPROACH is also a complex study: It involves eight arms, each receiving different regimens of the vaccine candidates or placebo with immunizations at 0, 3, 6, and 12 months. The regimens include: Ad26 mosaic Env/Gag/Pol candidate alone, or the Ad 26 candidate boosted by the MVA vectored candidate expressing the same antigens followed by either a high- or low-dose clade C gp140 boost with alum adjuvant. The mosaic antigens were developed as a result of another collaboration, this one between



Immunologist Jinyan Liu eyes an incubator at one of the lab benches at the Center for Virology and Vaccine Research.

Center for Virology and Vaccine Research

THE BAROUCH TEAM: A varied and large group, Barouch's Center for Virology and Vaccine Research at Beth Israel Deaconness Medical Center in Boston marshals an impressive array of brainpower.

Barouch and Bette Korber of the Los Alamos National Laboratory. Mosaics are bioinformatically engineered HIV protein sequences optimized to cover the diversity of HIV around the world.

The Phase I/IIa results are expected sometime later this year, but even before the results are in, Barouch's group has an eye toward simplifying this seemingly complex vaccine regimen.

APPROACH tests what would be a year-long, four-shot vaccine regimen, which would be challenging to administer in the parts of the world where HIV is most prevalent. This is why Barouch's group is conducting IPCAVD 010. This is the first clinical trial the group is conducting wholly in-house. It is funded by the Ragon Institute, which provides flexible funds to support pre-clinical and clinical HIV research. The goal of IPCAVD 010 is to test whether the APPROACH regimen can be simplified: the same vaccine candidates will be administered over three or six months in IPCAVD 010 instead of the year-long administration being tested in APPROACH. If comparable immunogenicity is seen with this shorter regimen, it would be cheaper and easier to implement should the vaccine prove effective.

Mustering monkeys

The APPROACH collaboration is just one example of how Barouch operates so effectively within an often competitive field. Barouch is also working with other companies and collaborators to advance efforts to test broadly neutralizing monoclonal antibodies for their ability to prevent, treat, or even help cure HIV infection.

A few years ago Barouch was talking with Dennis Burton, a professor of immunology and microbial science at The Scripps Research Institute (TSRI) in La Jolla, CA, and head of the IAVI Neutralizing Antibody Center. Burton had done protection studies with the broadly neutralizing antibody (bNAb) PGT121, isolated from an IAVI cohort, in 18 rhesus macaques. But as they talked, the two began wondering whether the antibody could be utilized in a therapeutic context. "Instead of wondering what it would do in an uninfected animal, what would it do to an infected animal? That's what we were asking," Burton says.

At the time there was a lot of skepticism, based on prior research results, that antibodies could be effective against established infection. But Burton and Barouch wondered whether the

new crop of more potent bNAbs being isolated at breakneck speed since 2008 would do better. The two decided to give PGT121 to a group of monkeys infected with a simian immunodeficiency virus/HIV hybrid. What they saw was a dramatic therapeutic effect: virus levels in the animals were suppressed up to three logs a week after antibody administration. Even after the antibody was gone, some of the animals with the lowest viral loads at the start had undetectable viral loads. "The antibody appeared to reduce virally infected cells as well as free virus," Barouch says. The experiment showed viral suppression for a median of 56 days even after the antibody was gone. Three of the monkeys never did rebound with infection (*Nature* **503**, 224, 2013).

Now Barouch has a call every two weeks with the contract manufacturer Catalent, which is nearly finished manufacturing PGT121 for clinical trials funded by the Gates Foundation to test the safety, pharmacokinetics, and efficacy of the antibody in both HIV-infected and uninfected volunteers. This work is also a collaborative effort, involving researchers from Beth Israel, the Ragon Institute, TSRI, Theraclone Sciences, Gilead Sciences, and IAVI. The Ragon Institute provided the initial funding for the PGT121 manufacturing. Another bNAb, PGDM 1400, is also being manufactured. It was more recently isolated by researchers at IAVI's Neutralizing Antibody Center at TSRI.

Rising to the top

Michael recalls something else about getting to know Barouch at the CHAVI meeting. Barouch was wondering whether he'd have an impact: there were a lot of big-time scientists in the HIV field there, and the young researcher said he didn't know if the adenoviruses would work or not. "Even if they do," Michael recalls him saying. "I don't know that there will ever be room for someone as junior as me."

Michael laughed. "I said if your data is good, there's always going to be room."

Barouch's not only had good data, he's made a point of communicating it clearly. "What's critical for running a research group is clarity. So much about science and research is communicating the findings to others," he says. That includes publications, grant applications, and public speaking. Barouch says he was once quite shy but made the effort to learn how to speak in front of people (mostly, by speaking in front of people). Barouch presents on his work in one form or another once a week at least. He takes teaching seriously, spending time with the graduate students in his lab. On a Monday morning at nine o'clock they're chatting and queueing outside his door as though it was a bagel truck.

He also spends much of his time travelling—in the last month he's been to Portugal, Marseille, Paris, San Diego, and Washington, DC. He also attended two conferences in Boston. It's a punishing schedule, but he doesn't stay in the lab late at night as he once did. "I leave here at six, most of the time. I spend time with my family," he says. They ski. They go to Hawaii on vacation. He does work every evening for several hours after the girls are in bed, and also in those hours he spends travelling. "I do my best work on the plane. A good six-hour plane trip, I do like that."

Barouch comes from a bright family: his mother Winifred was a homemaker until the children went to school, then she went back for her PhD in biochemistry and worked for the NIH. Barouch's father, Eytan, is a math lecturer and flow lithography engineer, teaching at Clarkson University in Potsdam, New York, and started his own company, Vector Technologies. Barouch's sister, Lili, is a cardiologist at Johns Hopkins. It's likely they stood out growing up in Potsdam, which is so far upstate it's possible to bike to the St. Lawrence River, the rolling border with Canada and a lumberjacking lodestar. Barouch found a place in Potsdam, though, starring in the public Potsdam Senior High School's math and science competitions.

"The striking thing about Dan was that his experiments always worked," says Sir Andrew McMichael, a pioneering HIV researcher who was Barouch's doctoral supervisor at Oxford, and who introduced Barouch to Norm Letvin. "Some students run into problems here or there. They need to work things out. Three months go by."

McMichael says some researchers are laid back and then have a rash of results. Others bash away all the time. He counts Barouch as more of a basher.

"He was in a hurry," he says. "Very bright, wanted to get on to do things. To make a difference. To do, and push forward. He was always pushing forward." ■

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

Jeffery Totaro, courtesy of Tsoi / Kobus & Associates

The Center for Life Sciences on Blackfan Circle in Boston. Designed by Tsoi / Kobus & Associates, the LEED-certified center opened in 2008 and became a home for, among others, a growing Barouch team.

Upcoming HIV-Related Meetings

JULY 2016

8th International Workshop on HIV Pediatrics
July 15-16; Durban, South Africa
More information: www.virology-education.com/event/upcoming/hiv-pediatrics-workshop-2016

21st International AIDS Conference
July 18-22; Durban, South Africa
More information: www.ias2016.org

SEPTEMBER 2016

5th Annual Rural HIV Research and Training Conference
September 9-10; Savannah, Georgia, USA
More information: academics.georgiasouthern.edu/ce/conferences/ruralhiv-2

OCTOBER 2016

5th Latin American Meeting on Hepatitis & HIV
October 14-15; Rio de Janeiro, Brazil
More information: www.virology-education.com/event/upcoming/latam2016

HIVR4P: HIV Research for Prevention
October 17-20; Chicago, Illinois
More information: www.hivr4p.org

HIV Glasgow 2016
October 23-26; Glasgow, UK
More information: hivglasgow.org

DECEMBER 2016

National HIV PrEP Summit
December 3-4; San Francisco, California
More information: hivprepsummit.org