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The Publication on AIDS Vaccine Research

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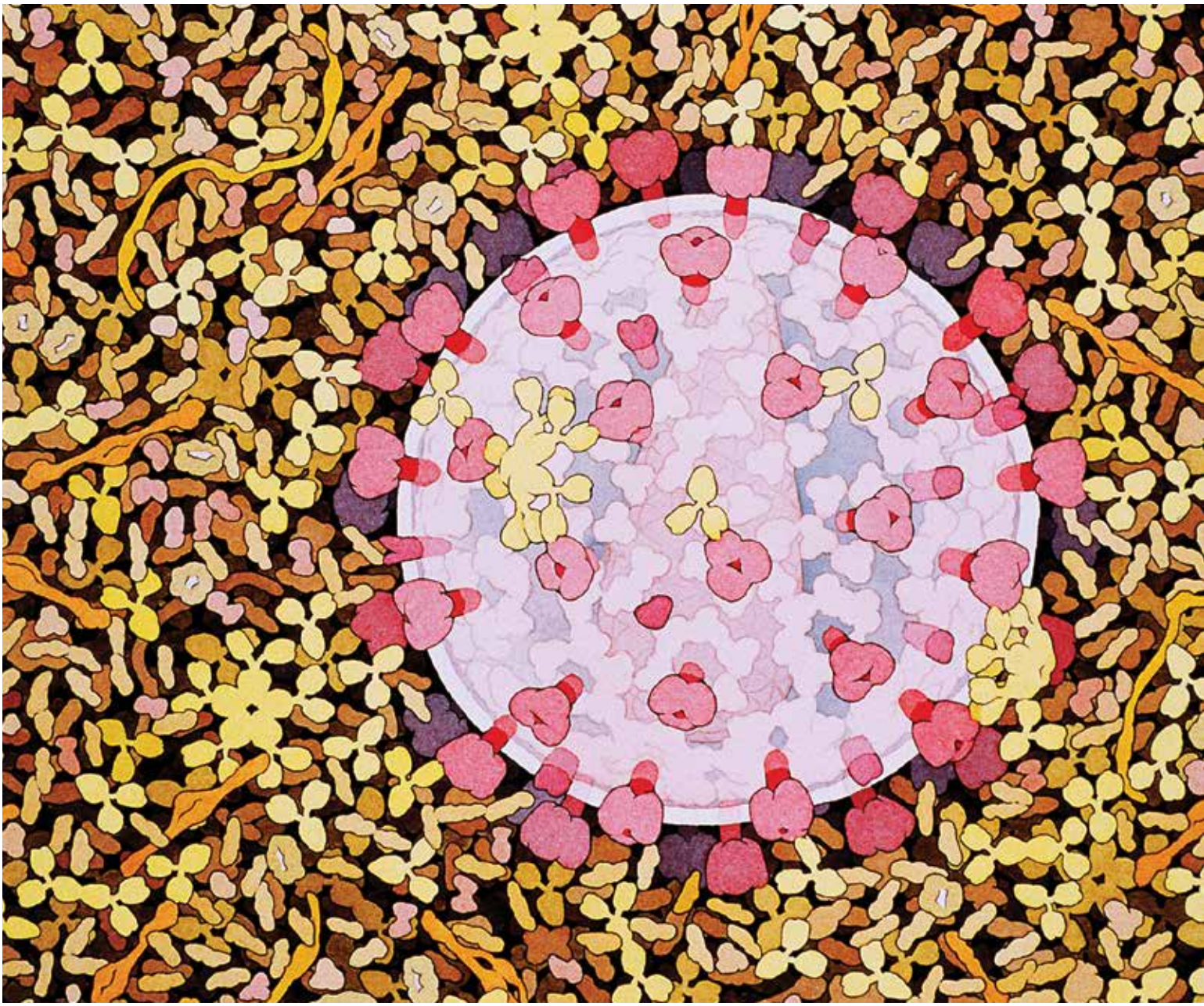

A new viral vaccine vector has broad potential

The history of vaccination: adapting to the times

A new efficacy trial of the mosaic HIV vaccine candidate

FROM THE EDITOR

"It was the best of times, it was the worst of times…" So begins Charles Dickens's famous historical novel *A Tale of Two Cities*, published in 1859. Dickens was describing the years leading up to the French Revolution, yet it is an oddly apt description of the current state of the vaccine field.

This October, the first Ebola vaccine was approved by the European Medicines Agency. This is by all accounts an important milestone in battling outbreaks of a lethal infectious disease that most often affects people in developing countries. This vaccine, known as Ervebo, was rapidly developed in 2014 during the deadliest outbreak of Ebola in history. It is estimated to be 97.5% effective. Approval of Ervebo also provides an important precedent for other vaccines in development that are using the same vesicular stomatitis virus (VSV) vector (see page 4).

Other exciting news was reported recently on potential new vaccination strategies against tuberculosis (TB), the world's deadliest infectious disease (see page 9). In November, a clinical trial began in South Africa to test the idea that revaccinating adolescents with the same BCG TB vaccine they received as infants could offer protection against sustained infection, as a previous study suggests it might. Final results were also published from a Phase IIb trial of GSK's M72/AS01$_E$ TB vaccine candidate. These results show the vaccine candidate was 50% effective at preventing individuals already infected with *Mycobacterium tuberculosis* from developing active pulmonary TB disease over a three-year period.

HIV vaccine research also took a step forward with the start of a new efficacy trial this October. Researchers from Janssen Vaccines & Prevention, part of the Janssen Pharmaceutical Companies of Johnson & Johnson, together with a consortium of public partners, began their second, and largest, efficacy study (named Mosaico) of Janssen's mosaic-based HIV vaccine candidate (see page 16).

Based on this, one might think it was the best of times. But amidst all this progress, global cases of measles are on the rise, a disease for which a highly effective vaccine has been available for more than 50 years. Last year more than 140,000 people worldwide died of measles, most of them children under five years old, and 100 million cases of this vaccine-preventable disease were reported, according to the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention. In a news release, WHO's Director-General Tedros Adhanom Ghebreysus said, "The fact that any child dies from a vaccine-preventable disease like measles is frankly an outrage."

In this issue, we present a brief history of vaccination that shows how scientific developments have led to the development of scores of vaccines, all of which have profoundly improved public health (see page 12). If only they are used. As the resurgence of measles shows, the challenge is not only developing vaccines against existing and emerging pathogens, but also ensuring the public health impact of existing vaccines is fully realized.

Starting next year, we will be migrating all online content for *IAVI Report* to the iavi.org website so you can find everything there. We hope you will continue to read, online and in print. Best wishes for the new year!

—Kristen Jill Kresge

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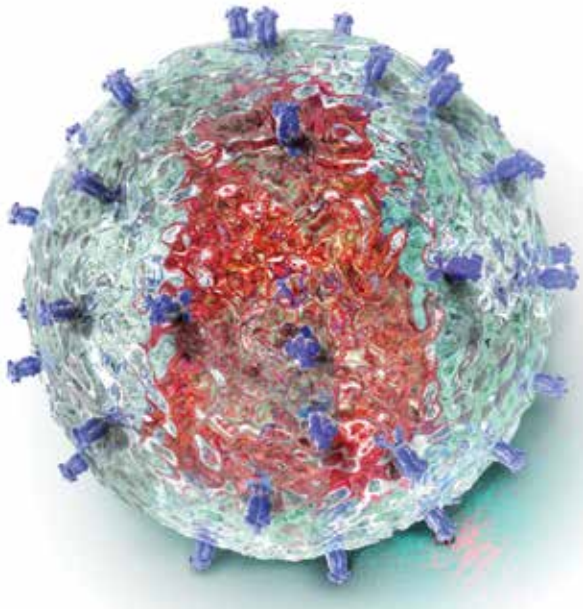

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ON THE COVER

This illustration shows HIV (the large spherical object in red) under attack by the immune system. Small Y-shaped antibodies are binding to its surface. Illustration by David S. Goodsell, Scripps Research

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Proven against Ebola, a vector shows its broader potential

Regulatory approval of a vesicular stomatitis virus-based vaccine paves the way for use of this viral vector in other vaccines.

by Michael Dumiak

In November, the European Union approved the first vaccine against the deadly Ebola virus. Having an effective vaccine that can be deployed whenever outbreaks occur—including the one that is still simmering in the Democratic Republic of Congo that has so far claimed the lives of 2,200 people—is a milestone.

That vaccine, now referred to as Ervebo, was developed at breakneck speed during the devastating Ebola outbreak in West Africa in 2014. The science that gave rise to it, though, was 20 years in the making. This science, and the stamp of regulatory approval, may open the door to other vaccine advances.

The candidate that would become Ervebo was first developed in the early 2000s by researchers at the Public Health Agency of Canada (PHAC). But it remained dormant there for many years: clinical data was very difficult to acquire and, until more recently, the effort drew no steady or adequate resources to support it.

It was the crisis sparked by the largest-yet outbreak of Ebola virus disease in 2014 that marshalled momentum decisive enough to bring the novel vaccine forward. By the time Ebola broke out that year in Guinea, Liberia, and Sierra Leone, the vaccine candidate had already been licensed from PHAC to a subsidiary of the biotech NewLink Genetics. With the West African outbreak growing dire, NewLink began a Phase I study of the vaccine candidate, forming a steering committee on development involving PHAC, the World Health Organization, and the U.S. National Institutes of Health (NIH). In November 2014, NewLink then licensed rights to the candidate to Merck. From there, development raced into high gear during the rest of the outbreak and through its subsiding in 2016.

The vaccine was deployed during the ongoing Ebola outbreak in Congo—now the second-largest in history—under compassionate use protocols, and delivered to thousands of people. A preliminary analysis of data from the field estimates its efficacy at 97.5%; a more detailed analysis is being prepared for peer-reviewed publication. Approval of the Ebola vaccine not only offers new hope for future outbreaks, it also lends researchers confidence for further development of vaccines using the vector in Ervebo.

Ervebo is built on the back of the vesicular stomatitis virus (VSV). VSV is sometimes called Indiana vesiculovirus or vesicular stomatitis Indiana virus. Gary Kobinger, director of the Research Center on Infectious Diseases at the Université Laval in Montreal and a key figure in Ebola research, is one of many who thinks VSV may have potential as a successful vector for other vaccines beyond Ebola. Ervebo's approval may just be the start. "It's going to increase confidence at all levels, not just from the scientific community, but more importantly, from the public and from regulatory agencies," Kobinger says.

More than 270,000 people in Africa have received Ervebo in the last five years as part of clinical trials or under emergency compassionate use protocols. This provides a significant amount of safety data. While every new vaccine application will need to be evaluated in the same ways, Kobinger says working on a licensed vaccine platform may ease that task.

"This is going to be very useful in the future. VSV would be one of a handful of new platforms in the last 20 years," he says. "These are exciting days for vaccinologists to see if the VSV platform can be used and applied to protect against other pathogens." Researchers are already employing VSV as a vector in vaccine research for other hemorrhagic fevers such as Lassa and Marburg, as well as against influenza, tuberculosis (TB), and HIV.

"While the Congo outbreak continues in a tragic way, it would have been far worse without

deployment of Merck's Ebola vaccine," says Mark Feinberg, IAVI's president and chief executive officer who, while at Merck, helped lead the collaborative effort that expedited Ebola vaccine development. "It's a tremendous public health accomplishment. But it's not only that there is a licensed Ebola vaccine that has a strong record of efficacy and tolerability, and the ability to be implemented. The fact that the vaccine was licensed by the European Medicines Agency, and will hopefully soon be licensed by the FDA [U.S. Food and Drug Administration], is a very positive precedent that makes it much more feasible to imagine developing additional vaccines based on the VSV platform."

VSV is an RNA virus in the rhabdovirus family that affects, among other animals, cows, pigs, and horses. Natural infection causes vesicular lesions of the tongue, teats, and hooves of livestock, a mild infection clearing within two weeks. VSV can infect humans but does so only rarely, causing mild flu-like symptoms, and is mostly inapparent, says Chris Parks, executive director of IAVI's Vaccine Design and Development Laboratory. His team is using VSV as a vector in developing experimental vaccine candidates against HIV, Lassa, and Marburg.

Yale's John Rose and his colleagues developed an experimental HIV vaccine using VSV back in 2001 and have worked with the virus for many years (*Cell* **106**, 539, 2001). Rose's work is a wellspring for research on VSV as a vector—including the work at PHAC that would eventually lead to the Ebola vaccine.

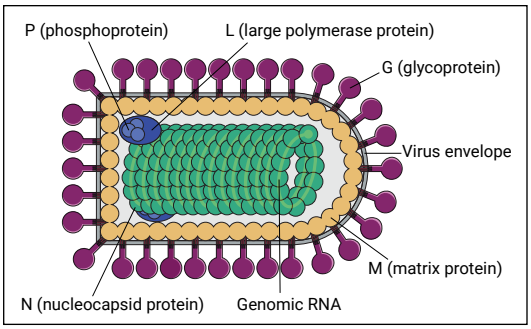
A vaccine vector is used to transport genes or proteins from another virus to trigger an immune response. The vector is the delivery system, and can be a live virus, an attenuated or weakened virus, or an inactivated or killed virus. There are very few licensed human vaccines that utilize viral vectors; among them are those for Japanese encephalitis and dengue. Both of these vaccines are based on a yellow fever viral vector. Now there is also Ervebo.

There are several features that make VSV an attractive vaccine vector. Its relatively small genome consists of a single molecule of RNA encoding for only five major proteins (see Figure), one of which, the glycoprotein (G), mediates its attachment to the host cell receptor, allowing it to enter the cell and hijack it in order to replicate and spread. The virus's small genome makes it easy to manipulate, says Andrea Marzi, a virologist and Ebola researcher at the National Institute of Allergy and Infectious Diseases' Rocky Mountain Laboratories in Hamilton, Montana. Cytomegalovirus, by comparison, another virus vector used in experimental vaccine development, has close to 200 genes, making it much more complicated to manipulate.

As a vector, VSV offers other advantages. It is a replicating virus, but its low prevalence in humans means there is no pre-existing immunity to VSV in the general population that could potentially limit its efficacy. Because VSV is replication-competent, Kobinger says, it is also a strong candidate to induce lasting responses to the antigen it is carrying. But because its RNA does not integrate into a host cell, there's less risk for oncogenesis or mutagenesis.

How VSV works its magic in stimulating a powerful immune response is still an open question, Kobinger says. "We don't exactly know all the details. Because it was used first for an Ebola vaccine, there was a very small community working on it. We don't know exactly the specific molecule or determinants that are involved in the immune stimulation," he says.

Lab work with VSV goes back a long time. Parks has seen papers from the 1930s detailing VSV research. But for decades it seemed like VSV might never be a vehicle for vaccines due to its potential neurotoxicity.



A bullet-shaped virus could hit the target for many future vaccines. Vesicular stomatitis virus, or VSV, is made up of five proteins, its viral envelope, and genomic RNA.

These concerns were what caused Kobinger to drop the virus for a time, figuring it was very unlikely that a vaccine using VSV would ever go into advanced clinical use. Injected directly into the brain of mice or other animals, VSV can cause neurotoxicity, Parks says. Researchers now know that the neurotoxicity caused by the wild-type virus probably has something to do with the G protein, which allows the virus to replicate extensively in the brain. That question is still not fully answered scientifically, but, as borne out by Ervebo's safety data, vaccine researchers eventually discovered how to neutralize it.

Starting with the non-clinical Yale work in 1999 and 2001 of Eli Boritz, Rose, and others in labs at Tulane, Duke, the University of California, San Francisco, and at Rockefeller University in New York, and then, finally, with a team including Michael Garbutt of PHAC's National Microbiology Laboratory and Heinz Feldmann (who supervised Marzi's postdoc there before both moved to Montana), researchers plotted a way around the neurotoxicity issue. They did it by knocking out the G protein in VSV to make chimeras (*J. Virol.* **78(10)**, 5458, 2004). The reputation that VSV had because of the toxicity in the brains of macaques—the virulence factor, Kobinger calls it—was defanged.

"If you remove the G protein, you really don't see those neurotoxicity effects," he says. This was quickly reflected in lab experiments done using VSV. Feldmann's group created VSV chimeras using glycoproteins from Lassa, Ebola, and Marburg viruses. Feldmann was once special pathogen chief at the PHAC's National Microbiology Laboratory (where he was succeeded by Kobinger). Along with Ute Ströher and other PHAC researchers, the group drew upon the previous work of Rose and others and knocked out the VSV G protein, replacing it with the Ebola virus glycoprotein (*CMAJ* **189**, E1326, 2017). The VSV-Ebola chimera Feldmann and his colleagues created is the origin of the vaccine developed by Merck.

Merck compiled safety and efficacy data for Ervebo from eight Phase I clinical trials and five Phase II and Phase III studies in a variety of countries and populations including Liberia, Sierra Leone, Guinea, Canada, Spain, and the U.S. These trials involved 15,996 people, including 234 children, 536 elderly people, 261 pregnant women, and 22 HIV-infected volunteers, showing limited local reactions—pain and swelling, for the most part—of mild to moderate severity.

Now an Ebola vaccine is on hand with perhaps others on the way (see *When Ebola returns, will the world be ready?*, *IAVI Report*, Vol. 19, No. 4, 2015). Marzi says the lessons for vaccine development are clear: they show the need for basic research and ongoing vaccine design and development in advance of an epidemic and the need for vaccine platforms that could be swiftly applied to multiple pathogens. (*Ann. Rev. Microbiology* **72**, 423, 2018) Researchers hope this is what's in the cards for VSV.

There are many groups investigating VSV as a vector. Kobinger's lab in Montreal, the team Parks is leading at IAVI, the University of Manitoba, University of Texas, NIH's Rocky Mountain Laboratories, and the Medical University of Innsbruck are all part of collaborative efforts developing candidates against HIV, Ebola, and hemorrhagic fevers. Yale is experimenting with a VSV-based candidate against severe acute respiratory syndrome virus (SARS); the State Key Laboratory of Veterinary Biotechnology is experimenting with a candidate against Middle East respiratory syndrome coronavirus (MERS); and the University of Miami, which, along with Rocky Mountain labs, is pursuing candidates against Zika. The vector is also linked to candidates against flu, TB, and even plague (*J. Virol.* doi:10.1128/JVI.05991-11, and see survey *Hum. Vaccin. Immunother.* doi:10.1080/21645515.2019.1649532).

VSV may even prove useful as an oncology therapy, an avenue pursued by the Medical University of Innsbruck's Dorothee von Laer. The concept is that the virus would infect and replicate in tumor cells and lyse them, acting as a more benign alternative to chemotherapy. Von Laer's small biotech firm ViraTherapeutics was absorbed by Boehringer Ingelheim to further develop this approach.

Janine Kimpel is a virologist, speed chess champion, and one of von Laer's protégés in Innsbruck. She and her group are using the same VSV vehicle, developed by von Laer for tumor treatment and dubbed VSV-GP, as a vector for HIV vaccine candidates. VSV-GP is a replication-competent chimeric virus, using the backbone of VSV

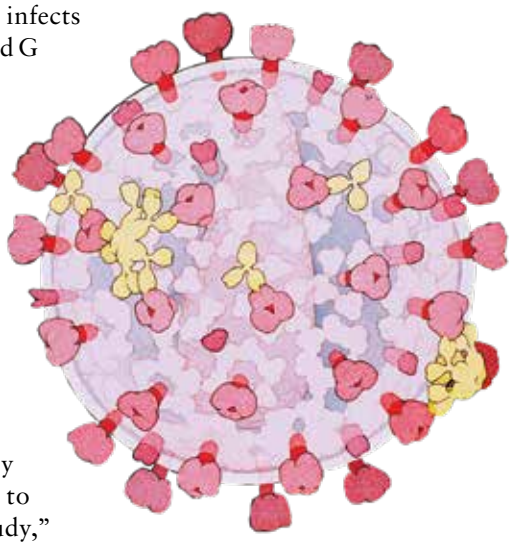
with its G protein exchanged for the glycoprotein of lymphocytic choriomeningitis virus (LCMV). The LCMV glycoprotein has a very broad cell tropism.

Kimpel's team is currently preparing VSV-GP candidates for preclinical trials in Paris as part of the effort under the European HIV Alliance (EHVA, www.ehv-a.eu). EHVA is an umbrella group with 41 partners working in discovery, immune profiling, and clinical trial platforms to develop novel HIV vaccine candidates. Kimpel's team has added an HIV Env protein to their VSV-GP vector and are hoping that they can take advantage of VSV's efficiency in incorporating foreign glycoproteins to boost the chances of inducing good antibody responses against HIV. The difference between Kimpel's approach and some of the other groups has to do with how Env is added to the chimeric vector, in this case adding a gene expressing HIV Env to a VSV chimera. As the LCMV glycoprotein is mediating replication in the vaccine formulation, the modified HIV Env does not need to be infectious, and the vector's target cell range can be more broad. As the team has shown, the antibody responses are not neutralizing to the vector itself (*J. Virol.* 88(9), 4897, 2014).

There are other concepts at play in the HIV field. Ma Luo of the University of Manitoba is running a group experimenting with expressed conserved HIV peptides carried by modified VSV; Jonathan Fuchs at the University of California, San Francisco, in collaboration with Profectus and U.S. National Institute of Allergy and Infectious Diseases, ran a safety trial with a VSV HIV-1 *gag* vaccine candidate four years ago (*Open Forum Infect. Dis.* doi:10.1093/ofid/ofv082). Kobinger and Parks are also both pursuing novel HIV vaccine candidates and employing different strategies to do so.

The concept in Kobinger's lab is to use an Ebola glycoprotein and show it as a target carried by the VSV vector to a sub-population of antigen-presenting cells. That vector will also carry an HIV protein. "There are spikes on top, which are normally the glycoprotein used by the viral particle for entry," Kobinger says. "Let's say for the sake of this that maybe 30% of the spikes are Ebola and 70% of the spikes are HIV. That 30% is enough to target the particle." Ebola glycoprotein should bring the VSV vaccine carrier in antigen-presenting cells, he says. The plan is to bring two or three candidates using this strategy into a large preclinical study.

Parks' lab is replacing the VSV G protein completely with HIV Env so it is the only glycoprotein expressed by the vector. As opposed to Kimpel's approach, Parks' team electroporates Vero-CD4-CCR5 cells with plasmid expressing VSV G protein, and then infects them with VSV with deleted G and HIV Env. The progeny virions incorporate Env. The team tested it in preclinical monkey studies, which Feinberg says showed a 70% protective effect against repeat, low-dose challenges using SHIV, the engineered virus containing both HIV genes and those of simian immunodeficiency virus (SIV). "We tried to repeat it in a second study," Parks says, but in that case the vaccine was notably less effective. "In both studies, we saw quite strong antibody responses." Parks and colleagues are now working to figure out why the VSV vector was efficacious in one preclinical study and not another. "We think we understand why: it's some technical issues with the vaccine production process. We hope to do another monkey study next year to try and sort it out, and hopefully it will work again."



The vaccine production in this case is complex. It starts with cell cultures, using lines of cells called Vero cells that were first extracted from green monkey kidney cells. Large quantities of Vero cells are grown in a manufacturing facility and these are infected with viral vector. The virus replicates under incubation, and then the virus that is released from infected cells is harvested, purified, and concentrated sufficiently for use in a vaccine. But standard Vero cells won't work for IAVI's HIV candidate because the cell line does not express the receptors recognized by HIV Env: they needed to modify the Vero line to fix this. Experiments using the first modified line were promising, but only a short-term solution, as the desired receptors gradually switched off expression. A second-generation

line provided higher immune response, but efficacy was diminished. The team's now experimenting with modifying the process to resolve this discrepancy.

IAVI is working with Batavia Biosciences of Leiden, the Netherlands, as a manufacturing partner, Feinberg says. "We're applying innovative manufacturing technologies that will hopefully be scalable and flexible, and make vaccine manufacturing—especially for outbreak-related pathogens—more simple and effective." Feinberg doesn't think viral vector-based vaccines are necessarily more complicated to manufacture than other vaccines.

Other pathogens are also under the microscope as potential targets for VSV candidates. IAVI is pursuing candidates against Lassa fever, supported by the Coalition for Epidemic Preparedness Innovations (CEPI), and Marburg. IAVI's VSV vectors for Lassa and Marburg, which were licensed from PHAC, are identical to the vector used in Ervebo. "That's an advantage because you know how the vector performs, and to the extent that the backbone can be one of the factors that influences your ability to produce and deliver it, that's a benefit," says Feinberg. "That's not the entirety of the vaccine, but at least these important elements of the vaccine are ones for which you will have set a precedent by licensure."

In 2018 CEPI also issued a grant to Profectus (which is currently in process of selling some of its research and development assets to Aurobindo Pharmaceuticals) and Emergent Biosciences to work on a Lassa vaccine based on its VesiculoVax vaccine delivery platform, which is also derived from VSV.

Feinberg says VSV's profile is well suited for outbreak-associated pathogens like Ebola, Lassa fever, and Marburg. "Others, though, are applying it for additional infectious diseases like Nipah virus or chikungunya. This demonstrates the tremendous flexibility of the VSV platform."

All told, it is the vector's viral structure that makes it flexible, says Trina Racine, a virologist, biotech consultant, and former colleague in Kobinger's lab. "We can pack in quite large antigens, and you can potentially express multiple antigens. In an ideal world, maybe one day we can produce a vaccine that is for Ebola and HIV at the same time." Marzi's group has experimented with a Zika-Ebola antigen in mice.

Researchers are also working with another VSV strain, VSV New Jersey, and other related vesiculoviruses like Alagoas, Maraba, and Chandipura. The idea of using alternate strains is to avoid potential effects of anti-vector immunity, especially if VSV Indiana becomes more widely used for vaccination in humans.

It also takes more than a vector to get vaccines to people. As the latest deadly outbreak illustrates, delivering Ervebo and other still-experimental Ebola vaccines into remote, conflict-stricken, and inaccessible areas while kept at -76 to -112 degrees Fahrenheit remains a challenge (see page 19).

When the outbreak hit Western Africa five years ago, Marzi went to Liberia to help the U.S. Centers for Disease Control and Prevention and Médecins Sans Frontières build a diagnostic lab and Ebola treatment unit in Monrovia. She saw the epidemic firsthand. "We have a great vaccine now, but people have to keep in mind what it was developed for," she says. "It is really developed to be used in an emergency situation, like an outbreak. For Ebola virus, we never thought it feasible until there was an epidemic that we would have to vaccinate the population of an entire country, which might now become necessary, particularly in Congo."

Marzi and her group just published results from a new set of dosing experiments, aiming to see if Ebola vaccine can eventually be stretched further by delivering it in lower, but still effective amounts (*EBioMedicine* **49**, 223, 2019). As a vaccine vector, though, VSV may end up stretching a lot further than that. ■

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

Research update

Turning the tide on TB?

A slew of potential advances in treating and preventing tuberculosis offers new hope in the battle against one of the oldest human afflictions.

by Kristen Jill Kresge

The last two years were a watershed moment in the fight against tuberculosis (TB). Encouraging results from vaccine trials, a newly approved treatment, and increased global attention suggest the world may be inching closer to tackling this ancient bacterial disease. And not a moment too soon.

According to the World Health Organization (WHO), TB is the deadliest infectious disease on the planet. Last year it killed 1.5 million people. Over the past 200 years, TB has claimed the lives of more than a billion people—that's more than malaria, influenza, smallpox, HIV/AIDS, cholera, and plague combined (*Nature* **502,** S2, 2013). In 2018, an estimated 10 million people acquired the disease, four million of whom don't even know they have it.

"Tuberculosis has been a huge burden on humanity," says Eric Goosby, the United Nations special envoy on tuberculosis and a professor of medicine at the University of California, San Francisco.

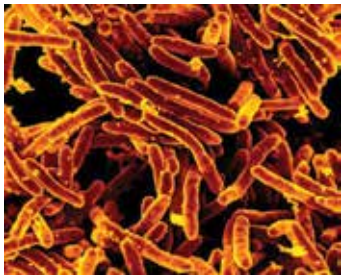
One reason TB is such a burden is that it is ubiquitous. A quarter of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (*M.tb*), the causative agent of TB. The infection can either progress to active TB disease or lie dormant, a state known as latent TB, indefinitely. People with latent TB infection cannot pass the airborne bacteria to others and are not sick, but they are at a 5%-10% life-long risk of developing active TB disease. "The association between exposure and disease is so delayed, it makes it very difficult to contain," says Goosby. For those who are immune compromised, including, among others, pregnant women and HIV-infected individuals, the risk of developing active disease is much higher, according to the U.S. Centers for Disease Control and Prevention.

Writing recently in *The Guardian*, Tedros Adhanom Ghebreyesus, director-general of the WHO, noted that the world has not made much progress against TB since 1993 when a third of the world's population was infected with *M.tb* and the WHO declared the disease a global emergency (*The Guardian*, *Why is the world losing the fight against history's most lethal disease?* Nov. 14, 2019). "Why, despite all the progress in medicine and public health over the past 150 years, is TB still the most common and lethal of all infectious diseases?" he asks.

It's a good question. Studies suggest that *M.tb* began spreading among humans nearly 6,000 years ago (*Nature* **514(7523)**, 494, 2014), making it one of the oldest human afflictions (*Clin. Microbiol. Rev.* **16(3)**, 463, 2003). And it was back in 1882 that Nobel Prize winning scientist Robert Koch first identified the bacteria as the causative agent of TB. Yet the only licensed TB vaccine was developed nearly a century ago and many of the drugs used to treat TB infection were developed more than 50 years ago, need to be taken for a period of six months, and are less effective against the increasingly prevalent drug-resistant strains of the bacteria that are now in circulation. This is why the WHO identifies "intensified research and innovation" as one of the pillars of their "End TB Strategy," which aims to end the TB epidemic by 2035 (www.who.int/tb/strategy). While this may seem an ambitious goal, recent progress may help turn the tide against TB.

The only existing TB vaccine was developed by French scientists Albert Calmette and Jean-Marie Camille Guérin and is referred to as BCG (Bacille Calmette-Guérin). This live attenuated TB vaccine is still given to infants and children in endemic countries, and while it is effective at preventing life-threatening cases of TB in infants, protection is variable against pulmonary TB in all age groups. Pulmonary TB is the most common form of the disease and the one that is contagious.

But a recent study is breathing new life into this old vaccine. Results of a Phase II study published in 2018 show that adolescents who were re-vaccinated with the same BCG vaccine they received as infants were 45% less likely to have a sustained TB infection (*N. Engl. J. Med.* **379(2)**, 138, 2018). This has sparked interest in re-vaccinating adolescents as a strategy for TB control. The Bill & Melinda Gates Medical Research Institute, a non-profit biotechnology organization funded by the Bill & Melinda Gates Foundation, launched



Scanning electron micrograph of *Mycobacterium tuberculosis*. Credit: U.S. National Institute of Allergy and Infectious Diseases, National Institutes of Health

a follow-up study in October to try to replicate these findings. The Phase II trial is enrolling 1,800 BCG-vaccinated, healthy adolescents between the ages of 10 and 18 at five study sites in South Africa (clinicaltrials.gov/ct2/show/NCT04152161). The trial is designed to determine the efficacy, safety, and immunogenicity of this revaccination approach.

In November, researchers, policymakers, public health workers, and advocates who gathered in Hyderabad, India, for the 50th Union World Conference on Lung Health had other reasons to be encouraged. The final results from a Phase IIb trial of GSK's adjuvanted TB protein vaccine candidate, referred to as M72/AS01$_E$, were presented there. These results showed that the vaccine candidate was 50% effective at reducing the incidence of pulmonary TB disease over a three-year period (*NEJM* doi:10.1056/NEJMoa1909953). These efficacy findings are consistent with an earlier analysis of the data that was published in September 2018 (*NEJM* **379**, 1621, 2018). Although only partially effective, this vaccine candidate is the first to show protection against the development of active disease in people who are already TB infected, and many in the TB field were buoyed by these findings.

The Phase IIb trial of M72/AS01$_E$ was conducted in three sub-Saharan African countries—Kenya, South Africa, and Zambia—and involved over 3,500 volunteers between the ages of 18 and 50 with latent TB. The trial was sponsored by GSK and conducted in partnership with IAVI.

The vaccine candidate contains an M72 recombinant fusion protein that was derived from two *M.tb* antigens combined with GSK's AS01 adjuvant, which is also a component of both their malaria vaccine, Mosquirix™, and their shingles vaccine, Shingrix™. The mechanism of protection for M72/AS01$_E$ is unknown, but samples collected during this study may help identify immune markers that correlate with protection against the development of active TB disease. Such findings could help advance development of this vaccine, and others.

"We really haven't had anything that was very promising the last several decades though we've had other vaccine candidates, so it was very encouraging to see the results of the M72 trial," says Soumya Swaminathan, chief scientist at the WHO. "It was the first time that a significant amount of protective efficacy was seen, and in a post-infection population, so I think it gives everybody a little hope. Although there are still many questions around this vaccine and how it could be used and in what populations, this at least it gives us enthusiasm for moving ahead."

In fact, when the WHO outlined the preferred product characteristics, or PPCs, for new TB vaccines, the agency specified that although mathematical modelling studies suggest that a vaccine with relatively low efficacy could still be cost effective, a vaccine that was at least 50% effective for a period of two years would help achieve the ambitious END TB Strategy goals (who.int/tb).

Swaminathan and Goosby were among dozens of TB experts on *The Lancet* Commission on tuberculosis that published a report in March 2019 detailing what they see as a path to a tuberculosis-free world (*Lancet* **393**, 1331, 2019). "These encouraging results need to be validated and extended, particularly in different geographic situations, but despite challenges, the scientific prospects for developing a safe and effective vaccine to prevent tuberculosis are promising. Long-term and sustained investments will be necessary … but the returns even from a partially effective vaccine would be very great," the commission's members wrote.

Swaminathan says the WHO convened two meetings soon after the initial M72/AS01$_E$ results became known to try to quickly advance the field. "We really wanted to bring a sense of urgency to this," she says. "The global research community really needs to come together and prioritize further work on this vaccine and work with the developer and other stakeholders, particularly the countries with the highest burden of TB."

The collaborative model Swaminathan sees as best to drive TB vaccine development involves multiple stakeholders. "We must explore a new model where there is broader participation than just the traditional funders. Traditional funders are definitely needed to back up this development process, but I think there's an opportunity to explore other options, including bringing other manufactures on board that could produce a product at an affordable cost, and involving ministries of health and patient groups to discuss how

this vaccine would be deployed." She also thinks that middle-income countries such as Brazil, Russia, India, China, and South Africa, the so-called BRICS nations, which have a high burden of TB disease, could provide significant investments, whether it be in research, development, or implementation science. "These countries have the capacity to contribute," she says.

While the next steps for the M72 vaccine candidate are being formulated, other institutions are also stepping up efforts to support TB vaccine development. At the end of September, the U.S. National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, announced US$30 million in funding to establish new centers for TB immunology research. These three Immune Mechanisms of Protection Against *Mycobacterium tuberculosis* (IMPAc-TB) Centers will work to develop a better understanding of the immune responses required to prevent initial infection with *M.tb*, establishment of latent infection, and transition to active TB disease. The goal of this work is to guide the design and development of new and improved vaccine candidates. The three centers are the T.H. Chan School of Public Health at Harvard, the Infectious Disease Research Institute (IDRI), and the Seattle Children's Hospital.

"Without an effective vaccine we are always going to be chasing TB," says Goosby. "It is essential for eradicating TB from the planet." He also notes that combatting TB is integral to expanding access to health care through the Universal Health Coverage (UHC) agenda. "You will not be able to implement UHC if you don't address TB and HIV."

Better TB drugs are also urgently needed and are finally starting to be approved. In August, the U.S. Food and Drug Administration (FDA) approved a new drug called pretomanid, developed by the not-for-profit TB Alliance, as part of a three-drug combination for the treatment of extensively drug-resistant or multi-drug-resistant TB. The other two drugs in the so-called BPal combination are Johnson & Johnson's bedaquiline and linezolid.

Pretomanid was only the third new TB drug to reach the market in half a century, and it is the first to be developed and registered by a non-profit organization. Following the FDA approval, the TB Alliance announced an agreement with generic drug manufacturer Macleods Pharmaceuticals Limited to manufacture pretomanid as part of the BPal combination. Macleods intends to commercialize the medicine in 140 countries and territories. The drug is also currently being considered for approval by the European Medicines Agency and is being reviewed by the WHO for inclusion in its policy guidelines on TB treatment. The big question then will be implementation.

These advances in drug and vaccine development, together with a renewed sense of urgency from the global public health community, are offering new hope for defeating the world's deadliest infectious disease. As Goosby and Michel Kazatchkine, special adviser to the Joint United Nations Programme on HIV/AIDS (UNAIDS) for Eastern Europe and Central Asia, wrote in a *STAT* op-ed in March 2018: "This is doable, but by no means easy." ■

> "Without an effective vaccine we are always going to be chasing TB."

The first and, so far, only TB vaccine

By today's standards, development of the nearly 100-year old BCG vaccine may seem hard to imagine (see page 12). French scientists Albert Calmette and Camille Guérin started by passaging *Mycobacterium bovis* on potato slices soaked with gallbladder cells from an ox 230 times to effectively attenuate the bacteria (*Front Immunol*. 8, 1203, 2017). After testing the efficacy and safety of the attenuated bacteria in animals, they attempted the first vaccination of a human neonate born in a household with someone who had tuberculosis (TB). Risk of TB transmission was high among household contacts at that time, but this first baby vaccinated with the BCG vaccine did not develop TB. Between 1921-1924, 20,000 neonates in households with someone with TB received this vaccine. Of those vaccinated, 5% of the babies died, but only 1% of deaths were from TB. The expected death rate for non-vaccinated newborns at the time was 25% and many of those deaths were expected to be TB-related. This vaccine is still the only one available and is given to infants in TB-endemic countries. Now, a new study suggests re-vaccinating adolescents who receive the BCG vaccine as infants could reduce sustained TB infections by 45% (*NEJM* **379(2)**, 138, 2018).■

Vaccines: adapting to the times

Over hundreds of years, researchers have matched contemporary scientific tools to address the infectious disease threats of their times.

by Karie Youngdahl

Vaccines are widely recognized as one of the greatest medical advances in human history.

Their discovery has roots at least as far back as the 1500s, with the practice of inoculation taking hold in the Western world in the early 1700s. Even before English physician Edward Jenner (1749-1823) formulated his idea to use cowpox to prevent smallpox, and before French scientist Louis Pasteur (1822-1895) demonstrated germ theory, ideas and practices circulated both in everyday life and in medicine that revealed a basic understanding of the nature of infectious disease and the concept of sterilizing immunity. This concept evolved along with the scientific advances that supported new and better vaccine design, a process that continues today (see page 4).

1720s-1870s: smallpox and measles

The earliest insights into vaccination were based on observations and reactions to smallpox. Pre-Jennerian smallpox inoculation, also called variolation, involved introducing a small amount of infectious smallpox matter—from pus, scabs, or sometimes fomites—into a smallpox-naïve recipient with the intent of producing a mild disease that would prevent future severe illness. The practice was based on the observation that smallpox survivors didn't become ill with smallpox a second time.

British physician Arthur Boylston (*J. Roy. Soc. Med.* **105**(7), 309, 2012) provides some evidence that the practice of inoculation might have emerged independently in China and on the Arabian Peninsula some time before 1550 and spread along trade routes. By as early as the 1700s, some Westerners were aware of inoculation.

Inoculation in the American colonies was widespread enough just 15 years after its introduction that Benjamin Franklin (1706-1790) considered it for his son Francis during a 1736 smallpox epidemic in Philadelphia. Given the risk of inoculation—between 1%-3% of inoculees died from smallpox infection in the immediate post-variolation period—Franklin declined to pursue it. He grieved bitterly when the four-year-old subsequently died from smallpox.

Successes with smallpox variolation likely enabled another demonstration of an early understanding of infection and protection. Constant Huygelen (1929-2001), in a chapter on measles in veteran vaccinologist Stanley Plotkin's (b. 1932) *A History of Vaccine Development*, traces more than a century of occasional and inconclusive experiments with measles inoculation beginning in the mid-1700s. During a measles epidemic in 1758, Francis Home (1719-1813), a Scottish physician, used a mixture of blood and scrapings from a measles rash to inoculate about a dozen children via incision in the arm.

Jenner's well-known experiments in 1796 to induce smallpox immunity by inoculating his subjects with cowpox, a related disease, were also based on observation. Milkmaids had an apparent immunity to smallpox, which led Jenner to test his theory that cowpox, a relatively mild disease that dairy workers contracted from cows, could potentially offer protection against smallpox, a much more serious disease. This was a critical milestone in the history of medicine. But virologist and self-described aficionado of vaccine history José Esparza says that while Jenner's accomplishment was exemplary, he wasn't necessarily aware that he was immunizing his subjects against a specific pathogen. "Jenner had no concept that he was inoculating against a germ—he was inoculating against disease. It was very unclear what caused diseases," says Esparza.

Jenner was insightful and a careful experimenter, but he lacked specific knowledge about the nature of pathogens and infection that wouldn't emerge for decades.

1880s-1920s: homing in on germs

It was up to French national hero Louis Pasteur to demonstrate to the medical world, if not the layperson, that disease is spread by agents too small to be seen with the naked eye.

Pasteur soon applied this understanding to a specific infectious disease. In 1879 he produced the first lab-developed vaccine for the bacterial disease chicken cholera (caused by *Pasteurella multocida*) (*J. of Appl. Vir.* **4**(2), 11, 2015). He

quickly followed that with a veterinary anthrax vaccine in 1881, relying on Robert Koch's (1843-1910) seminal 1876 demonstration of the causative agent of anthrax (*Beiträge zur Biologie der Pflanzen* **12**, 277, 1876).

Pasteur was not just applying the new understanding of disease and immunity, but also the understanding of the need to attenuate or weaken microbes to induce an immune reaction strong enough to prevent disease, but not strong enough actually to cause disease. This is the balance a live vaccine must strike to be effective.

For his chicken cholera vaccine, Pasteur exposed the bacteria to oxygen for a prolonged period to attenuate the bacteria. The story of his attenuation of anthrax is murkier—it is now thought that he appropriated the technique of another French scientist, Jean Joseph Henri Toussaint (1847-1890), who used potassium bichromate to kill the bacteria (*Med. Imm.* **4(5)**, 2005 doi:10.1186/1476-9433-4-5).

Pasteur also introduced the first rabies vaccine in 1885, marking another important innovation—a therapeutic vaccine for post-exposure prophylaxis (*PNAS* **111(34)**, 12273, 2014).

Pasteur's accomplishments sparked wide interest in vaccination. This interest, coupled with an explosion of advances in microbiology tools, techniques, and knowledge, set off a remarkable era in scientific history. Soon scientists began setting their sights on isolating pathogens and devising vaccines to target them.

Most vaccines developed during this early era of microbiology were for bacterial diseases. Bacteria could be easily grown and attenuated or killed through a variety of methods. Cultivating viruses in living cells—a necessity before advances in molecular biology—was a hurdle that researchers would not clear until the mid-20th century.

The 1880s and 1890s were a fertile time for bacterial vaccinology, though not all of it was successful. In 1884, Spanish bacteriologist Jaime Ferrán (1852-1929) developed and tested the first live bacterial vaccine against cholera. Koch worked fruitlessly on a TB vaccine that would remain elusive until Albert Calmette (1863-1933) and Camille Guérin (1872-1961) developed the BCG vaccine in the early 1920s. That vaccine is still in wide use today, though researchers are attempting to devise alternatives (see page 9). Wilhelm Kolle (1868-1935) developed a killed cholera vaccine in 1896, Almroth Wright (1861-1948) and Richard Pfeiffer (1858-1945) developed killed typhoid vaccines separately in the 1890s, and Waldemar Haffkine (1860-1930) produced a killed plague vaccine in 1896. Wright also experimented with killed pneumococcal vaccines, though the diversity of pneumococcal serotypes remained unknown, and so his vaccines had limited effect.

Killed whole-cell pertussis vaccines were developed and used from about 1914, but their effectiveness was variable. In the 1930s, Michigan bacteriologists Pearl Kendrick (1890-1980) and Grace Eldering (1900-1988) began to apply a more systematic approach to developing what turned out to be an effective, widely used vaccine. The two Michigan State Department of Health researchers made critical improvements to the pertussis vaccine and conducted a large efficacy trial in the mid-1930s that introduced more rigorous clinical trial methods that would serve as a model for the large poliovirus vaccine trial in 1954 (*James Lind Library Bulletin*, 2006).

The late 19th and early 20th centuries brought some advances in virology, but advances in cultivating and attenuating viruses for study and vaccine development were hampered by the nascent science. There was some progress, however, in passaging viruses in cows or other large animals such as sheep to grow stock for smallpox vaccines.

Esparza and others have been collecting late 19th- and early 20th- century smallpox vaccine samples and performing genomic analysis on them. So far, their findings show that the closest ancestor to many of these vaccine viruses, and to the standard smallpox vaccine virus developed in the late 1800s by the New York City Board of Health, is horsepox, not cowpox. Esparza's findings perhaps shouldn't be much of a surprise, as even Jenner suspected that the material he harvested from Blossom the cow for his 1796 experiments was actually from horsepox (*Vaccine* **35(52)**, 7222, 2017).

Large mammals were also widely used to produce antitoxin. Diphtheria antitoxin was produced by inoculating horses, sheep, and sometimes other animals with diphtheria toxin. In response, the animals produced large quantities

Important dates in the history of vaccination
1700–Present

1721

Variolation introduced in England
Lady Mary Wortley Montagu returned to England from Turkey and had her child inoculated to protect her from smallpox.

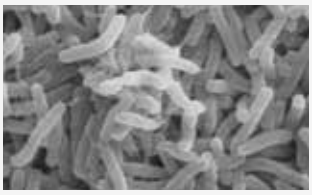
1796

The first vaccination
Edward Jenner inoculated a boy with cowpox and later challenged him with smallpox. The boy remained healthy.

1840

Variolation banned in Britain
An act of parliament outlawed variolation and provided free smallpox vaccine to the poor.

1859



Germ theory of disease
Louis Pasteur demonstrated the existence of airborne germs in his famous swan-neck flask experiment.

1879

First lab vaccine created
Almost 100 years passed between the use of the first vaccine and the second, Pasteur's vaccine for chicken cholera.

1921

BCG tuberculosis vaccine
French scientists Calmette and Guérin used attenuated bovine tuberculosis bacteria as the basis for their vaccine.

1938

Yellow fever vaccine
Max Theiler grew yellow fever virus in mouse embryo cultures and in chick eggs. The vaccine is still used today.

1963

Oral polio vaccine
Albert Sabin's trivalent OPV, seen being dropped on sugar cubes, was approved and replaced inactivated polio vaccine.

1970

Rubella vaccine approved
A rubella vaccine developed in fetal lung cells was approved in Europe. U.S. approval occurred in 1979.

of antibodies. Purified animal serum was then used to treat patients ill with diphtheria. This process was also used for other bacteria.

Research into the exotoxin-producing bacteria eventually led to experiments with preventives that included toxin-antitoxin mixtures and finally to the production of toxoids (in the case of diphtheria, formalin-treated diphtheria toxin, later administered with alum to boost immunogenicity).

1930s-1950s: tissue and cell culture advances

As scientists began to focus on viruses in the 1930s, they looked for alternatives to large animal production of vaccine material. Max Theiler's (1899-1972) approach to propagating and attenuating yellow fever virus was an important advance on this front. He began by growing the yellow fever virus in mice, which provided a convenient, easy-to-handle animal model, and also led him to develop a method for assessing mouse antibody responses to inoculation, which he was able to apply to humans (*J. Exp. Med.* **204(12)**, 2779, 2017).

Mouse passage of the yellow fever virus attenuated the virus somewhat, but it took 100 passages through chicken embryos to render it safe (*Singapore Med. J.* (**58)4**, 223, 2017). First used in Brazil in 1938, his vaccine using the attenuated yellow fever virus 17D proved safe and highly effective for use in humans. It provides lifelong protection with just a single dose and continues to be used even now for global yellow fever virus vaccine production (*Yale J. Biol. Med.* **83(2)**, 77, 2010). Theiler won the Nobel Prize in Physiology or Medicine in 1951 for his innovations in virus adaptation.

As research methods for working with viruses began to mature, some scientists turned their sights on poliomyelitis. But a poliovirus vaccine trial that occurred in the 1930s had a chilling effect on the field. A chemically attenuated polio vaccine developed by John Kolmer (1886-1962) at Temple University in Philadelphia killed five children and paralyzed 10 others (*Am. J. Pub. Health* **26(2)**, 143, 1936).

Unbeknownst to scientists at the time, any vaccine that didn't cover all three serotypes of polio was destined to fail. It wasn't until 1949 that David Bodian (1910-1992) from Johns Hopkins University in Baltimore, Maryland, showed that three different antigenic types of poliovirus exist and that an effective vaccine would have to block all of them. That same year, John Enders's (1897-1985) discovery that he could use primary human and simian non-nervous cell cultures to grow polioviruses was the breakthrough that finally allowed safer, more reliable, and more productive cultivation of poliovirus (*Science* **109(2822)**, 85, 1949). The tissue and cell culture methods resulting from Enders's work, and other advances in viral cultivation, helped propel the field forward. Enders, Thomas Weller (1915-2008), and Frederick Robbins (1916-2003) were given the Nobel Physiology or Medicine in 1954 for their contributions.

Jonas Salk (1914-1995), Albert Sabin (1906-1993), and Hilary Koprowski (1916-2013) were quick to incorporate these new findings and methods into their poliovirus research. Salk's inactivated trivalent vaccine was advanced into a large field trial in 1954 and approved a year later, while Sabin and Koprowski continued working on their live, attenuated viral strains. Sabin, of course, developed the strains that were selected for use in the live oral vaccine, and Koprowski moved on to lead a team that developed, among many other vaccines, an improved rabies vaccine at the Wistar Institute in Philadelphia.

1960s: crisis in cell culture

The development of new methods of vaccine production also necessitated new approaches to assessing vaccine safety. At the U.S. National Institutes of Health (NIH), vaccine safety researcher Bernice Eddy (1903-1989) had discovered in 1955 that some samples of Salk's supposedly killed poliovirus vaccine retained virulence. Though she passed her findings up the chain of command at the NIH, authorities took no immediate action. They didn't intervene until the virulent vaccine from Cutter Laboratories was given to the public, causing dozens of cases of paralytic polio and five deaths. Soon, more stringent methods of poliovirus deactivation were instituted.

In 1959, as part of her new focus on the relationship between viruses and cancer, Eddy tested the monkey kidney substrate used for growing Sabin's vaccine viruses. Hamsters exposed to extracts of the cells developed tumors at a much higher rate than control animals. Eddy suspected a viral contaminant, but once again her findings were suppressed. Prolific vaccine developer and

virologist Maurice Hilleman (1919-2005) at the pharmaceutical company Merck soon identified the contaminant as simian virus 40 (SV40), and the discovery prompted a shift to the use of cells from African green monkeys, not a natural host of SV40, for poliovirus vaccine production (*Proc. Soc. Exp. Biol. Med.* **105(2)**, 420, 1960).

Though researchers widely agree that SV40 is not associated with disease in humans (see the extensive bibliography at Children's Hospital of Philadelphia, Vaccine Ingredients: SV40, 2016), researchers at the time worried in general about the risks of using non-human cells for human vaccine production.

In the wake of this controversy, Stanley Plotkin set up a rubella virology laboratory at the Wistar Institute in 1963. He had studied rubella in London, where the disease was epidemic in the early 1960s. By 1964-65, rubella had caused about 13,000 pregnancy losses and infant deaths in the U.S., as well as about 20,000 cases of congenital rubella syndrome in infants whose mothers had been infected during pregnancy. Leonard Hayflick (b. 1928), a biologist and cell culture expert, also had a lab at the Wistar Institute where he had recently developed a cell line from human fetal lung cells that was free from contaminants (fetuses growing in the sterile environment of the uterus were likely to be less contaminated than other sources). "Rubella virus could be cultivated in monkey cells, but it was a natural thing at the time to try to use fetal cells, particularly because they were human and should be sensitive to infection in the lab by human viruses. And they were free from contaminants," says Plotkin. Plotkin's rubella vaccine, still used today in the measles-mumps-rubella vaccine, was the first of several vaccines to be developed with WI-38. Another human cell line developed in the U.K. has been the source of many others.

In the case of rubella, the virus was isolated in 1962 and Plotkin's vaccine was licensed in several European countries just eight years later.

For contemporary vaccinologists, working at this speed is inconceivable. Paul Offit, co-developer of a widely used rotavirus vaccine and author of several books on vaccines, says, "It was the same with mumps—Hilleman isolated mumps virus from his daughter in 1963 and there was a vaccine just four years later. It was a different time. You could do trials with just a few thousand children for one. The consent form was an index card that said, 'I allow my child to participate in blank trial,' and then the parent signed it. It was a less litigious, less cynical time, so you could make a vaccine that quickly then. You can't make a vaccine today in less than 20 to 25 years."

1980s and on: the recombinant revolution

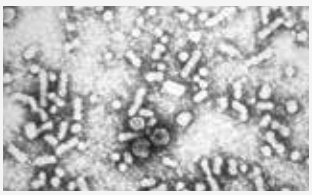
Advances in molecular biology that occurred in the 1980s and 1990s led to a significant shift in vaccine development and production. In 1981, Hilleman, still at Merck, developed a hepatitis B vaccine from antigen isolated from the blood of infected donors. Though the antigenic material was carefully purified and not thought to have caused disease in any vaccine recipients, the emerging HIV/AIDS crisis meant that using human blood products for vaccine production was not advisable. Hilleman found a solution in recombinant DNA technology. The hepatitis B antigen could be produced at high yields and in a native-like state by yeast cells genetically altered to construct the target protein (*Stud. Hist. Philos. Biol. Biomed. Sci.* **64**, 11, 2017). The episode, Esparza says, is an example of Hilleman's unique gift. "His genius was not to invent new vaccines but to identify anywhere in the world what new scientific knowledge was being developed that could be applied to vaccines." The recombinant hepatitis B vaccine was approved by the U.S. Food and Drug Administration (FDA) in 1986.

When HIV was identified in 1983, some thought that the task of developing a preventive vaccine would be relatively straightforward. U.S. Health and Human Services Commissioner Margaret Heckler made the infamous prediction in 1984 that a vaccine could be ready for production in two years.

One avenue of HIV vaccine research that initially seemed promising combined traditional and novel scientific approaches. Live-attenuated vaccines had always been more immunogenic than killed vaccines, so some researchers began to develop and investigate live, attenuated simian immunodeficiency virus (SIV) vaccines in animal models. The new technology involved creating gene-deleted mutants of SIVs, following on the observation that humans infected with HIV with certain gene deletions did not experience disease progression, and that macaques infected with similar SIVs had persistently low viral loads. But when macaques infected with the attenuated

continued on page 18

1986



Recombinant hepatitis B vaccine
Replacing a vaccine made from blood of HBV-infected people, the recombinant vaccine was approved in 1986.

1987

First HIV vaccine trial
A vaccine based on vaccinia vector carrying a gene encoding HIV's envelope protein was evaluated in a Phase I trial.

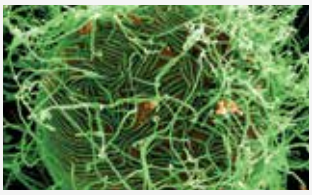
2003

Thai RV144 trial begins
The prime-boost HIV vaccine regimen in this clinical trial would eventually be shown to have 31% efficacy.

2016

HVTN 702 trial begins
The vaccine evaluated in the RV144 trial was modified in an attempt to boost efficacy. A new trial began in 2016.

2019



Ebola vaccine approved
Innovative science and clinical trials led to the approval of a highly effective VSV-vectored Ebola vaccine.

Interview

Taking the next step with the mosaic HIV vaccine candidate

Maria Grazia Pau, senior director, compound development team leader for HIV vaccine programs at the Janssen Pharmaceutical Companies of Johnson & Johnson, talks with *IAVI Report* about the start of the company's Phase III Mosaico trial.

by Kristen Jill Kresge

Maria Grazia Pau

This October, Janssen Vaccines & Prevention, part of the Janssen Pharmaceutical Companies of Johnson & Johnson, together with a consortium of public partners, took the next step in advancing its HIV vaccine candidate. The company launched its second, and largest, efficacy study of Janssen's mosaic-based HIV vaccine candidate—a Phase III trial, aptly named Mosaico. The trial (HPX3002/HVTN 706) will enroll 3,800 men who have sex with men and transgender volunteers at 56 clinical sites in North and South America and Europe.

The trial is being supported by a public-private partnership involving Janssen, the National Institute of Allergy and Infectious Diseases at the U.S. National Institutes of Health, the HIV Vaccine Trials Network, and the U.S. Army Medical Research and Development Command. It is the second efficacy trial for Janssen's mosaic-based vaccine candidate. The first, an ongoing Phase IIb trial known as Imbokodo (HPX2008/HVTN 705), has enrolled 2,637 women in South Africa, Mozambique, Zambia, Zimbabwe, and Malawi.

The goal of the mosaic vaccine is to overcome the vast genetic diversity of HIV, making it what the company refers to as a "global vaccine." Many vaccine candidates, including the one that is being tested in another ongoing efficacy trial (HVTN 702; see *HVTN 702 Efficacy Trial Ready to Launch in South Africa*, *IAVI Report*, Vol. 20, No. 3, 2016), are constructed specifically to match the predominantly circulating clade of the virus in the region where the vaccine is tested. But the potential advantage of a mosaic is that it is computationally derived to provide coverage against all circulating strains.

The vaccine regimen being tested in Mosaico involves four vaccinations over a year-long period. The first two deliver a mixture of four Ad26 vectors containing the globally relevant mosaic HIV antigens (Ad26.Mos4.HIV). These are followed by two more vaccinations of the Ad26.Mos4.HIV candidate administered along with a protein-based vaccine component that contains a combination of mosaic and clade C trimeric HIV gp140 soluble proteins.

Mosaic vaccine regimens were previously tested by Janssen in Phase I/IIa trials—known as APPROACH, TRAVERSE, and ASCENT—as well as in a similarly designed non-human primate study (*Lancet* **392(10143)**, 232, 2018). These vaccine regimens produced strikingly similar immune responses, both in type and magnitude.

Although there are no efficacy data in humans, in the animal study a mosaic-based vaccine afforded 67% protection against infection with SHIV, a hybrid virus that combines simian immunodeficiency virus and HIV. The immune responses correlated with this protection were binding antibodies against clade C HIV Env, as measured by ELISA, and HIV Env-specific T-cell responses, as measured by an interferon-γ enzyme-linked immunospot (ELISPOT) assay.

Initially, the mosaic vaccine candidate was designed to stimulate primarily T-cell responses, but this regimen also appears to induce binding, non-neutralizing antibodies that may act against the virus through a process called antibody-dependent cellular phagocytosis. The only vaccine regimen to provide any protection against HIV so far (a prime-boost strategy tested in the RV144 trial) also appeared to induce non-neutralizing, binding antibodies (see *Overflowing with Antibodies and Optimism*, *IAVI Report*, Vol. 22, No. 3, 2018).

The results of the APPROACH study were compelling enough—having met the pre-defined criteria—to convince the company and its partners to advance the vaccine. Data from the Phase I/IIa studies also helped to determine the specific mosaic regimens that are now being evaluated in the Imbokodo and Mosaico efficacy trials.

IAVI Report spoke recently with Maria Grazia Pau, senior director, compound development team leader for HIV vaccine programs at the

Janssen Pharmaceutical Companies of Johnson & Johnson, about the Mosaico and Imbokodo trials, Janssen's commitment to HIV vaccine development, and how the company is preparing for potential success. Below is an edited version of our conversation.

What is the status of both the Mosaico and Imbokodo trials?
The Imbokodo trial was fully enrolled as of the end of May 2019. We enrolled 2,637 women in sub-Saharan Africa, with most of the sites being in South Africa, however, we also have sites in Mozambique, Zambia, Zimbabwe, and Malawi. Vaccinations are still ongoing. All 2,637 women have received at least the first vaccination and about 1,000 women have already received the full vaccine regimen as of December 2019. If things go well, vaccinations will be completed by May of 2020.

And at the very end of October we officially opened the first site in the Mosaico trial and began enrollment. The first vaccination in that trial occurred in November in the U.S.

Before the HVTN 702 trial began in South Africa, the ALVAC-HIV/gp120 protein vaccine regimen was reformulated based on clade C virus, which is the predominantly circulating strain in South Africa. But Janssen is testing its mosaic-based vaccine regimens in efficacy trials in sub-Saharan Africa, the Americas, and Europe, despite clade variation. Will that show whether the mosaic is a global vaccine?
That is the goal. The mosaic antigens were designed in such a way that we should be able to induce immune responses against many globally relevant clades. And, what we indeed saw in our Phase I and IIa clinical studies is that we do induce immune responses against many different clades, including clades B and C. With Imbokodo and Mosaico, our fingers are crossed that the immune responses are found to be protective, and that we will confirm that we don't need a so-called regional vaccine with our approach.

What data from the Phase II trials make you the most optimistic that the Mosaic vaccine candidate has the potential to protect against HIV?
The optimism comes from the fact that what we have seen from the very first Phase IIa study—the one that we call APPROACH, which involved almost 400 volunteers in the U.S., Africa, and Thailand—is so similar to what we saw in a similarly designed study in non-human primates that tested the same vaccine regimen and identical vaccine components.

We first saw the data from the non-human primate study, but we didn't know yet what the outcome would be in humans. And when we first saw that the immunogenicity data from APPROACH were so similar to what we saw in non-human primates, I can tell you we were thrilled. We could not have been expecting a better outcome. It confirmed exactly what we saw in non-human primates in terms of immune responses and in terms of which vaccine regimen was superior. It was all the same. Again, we have no idea about efficacy yet, but that's what we find encouraging.

How would you characterize Janssen's commitment to HIV vaccine research, and why don't you think more companies are involved to the same extent?
Well, I'm a little bit biased, of course, but to your point, the commitment is quite unique. The first reason is that there is a commitment from the senior leadership of the company. For example, our chief scientific officer and our chief executive officer are both champions of HIV research and the company's campaign to "make HIV history." Equally important are the science and data supporting the mosaic vaccine concept, as well as the incredible global partnerships we've established. There are also legacies in companies like Janssen, where our 25-year involvement in the HIV area spurs us on; we'd like to finish the job.

If the mosaic-based vaccine is found to be effective, do you think there is potential to simplify the regimen, or do you think it will be feasible to introduce a vaccine that requires four immunizations over a year-long period?
It will be challenging, there is no doubt about it. But if the data shows that the vaccine is efficacious, I think we will have to join forces. We will need not one village, we will need many villages to get it done. I think about it in terms of one of my favorite mottoes from Nelson Mandela: "It always seems impossible until it's done." We are completely aware that it's going to be challenging and we will have to work together with many stakeholders.

However, we are also fully aware that regimen simplification will be something that will need to be assessed if we have a good efficacy signal from the ongoing studies.

What plans, if any, is the company considering around access to the vaccine should it prove effective?

There are definitely proactive discussions and planning taking place. We have, together with our colleagues in the company's global public health division, initiated discussions around access, and we already had our first advisory board one year ago, at which major stakeholders from developing countries were involved, as well as global stakeholders, including the Bill & Melinda Gates Foundation, IAVI, affected communities, and other groups as well. Access is a high-priority issue and we will continue to discuss it with our partners.

One other critical thing is that we already scaled up our manufacturing process, assuming the large volumes that may be required for global access to this vaccine. We invested, at risk, in manufacturing for all the components because if the vaccine is successful, we don't want to have to wait years to make it. Supply, even for the world's long-established vaccines, is always one of the major issues.

Earlier this year I was in Zambia, and I was able to visit the Zambia-Emory HIV Research Project site in Lusaka, which is participating in the Imbokodo trial. It was truly remarkable to see the dedication of the clinical trial staff and the volunteers.

I totally understand because if there is something that inspires me enormously, it is visiting the sites. I had the pleasure to do that with Johan Van Hoof, who is the global head of infectious diseases and vaccines for Janssen, and Paul Stoffels, vice chairman of the executive committee and chief scientific officer of Johnson & Johnson, and the energy and the inspiration that you get from the people there, from the staff and the investigators, is incredible. Every site has a different idea and a different approach that makes them unique and the staff and volunteers are truly heroes. ■

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SIVs eventually developed disease due to the virus regaining its virulence, the live-attenuated HIV vaccine concept was shelved.

The first HIV vaccine given to humans was based on a *vaccinia* vector—*vaccinia* being the virus used throughout the 20th century for smallpox vaccination. French scientist Daniel Zagury inserted the gene for gp160, HIV's envelope protein, into vaccinia's extensive genome, and evaluated the resulting vaccine in a controversial Phase I trial in 1986 (*Nature* **326**, 249, 1987). He followed that with a small study using gp160 as a boost after immunization with the vectored candidate, but neither prevented HIV infection.

It wasn't until 2009 that an HIV vaccine candidate showed any efficacy. The Phase III RV144 trial tested a priming immunization with a canarypox vector containing inserts of HIV *gag, pol,* and *nef* genes, followed by a gp120 protein boost. This regimen was about 31% effective at preventing infection (*NEJM* **361(23)**, 2209, 2009).

Researchers are now evaluating a modified version of this vaccine regimen to see if they can boost its efficacy and the duration of the immune responses in an ongoing Phase III clinical trial in South Africa (HVTN 702). The only other HIV vaccine approach being tested in efficacy trials involves a mosaic vaccine candidate—one that is computationally derived to provide maximum protection against the many circulating strains of HIV (see page 16).

Taking advantage of new vaccine development technologies, Doug Lowy (b. 1942) and John Schiller (b. 1953) managed to produce self-assembling virus-like particles (VLPs) by infecting yeast cells with a viral vector encoded with a gene for the human papillomavirus (HPV) surface protein (*PNAS USA* **89**, 12180, 1992). The first HPV vaccine was approved by the FDA in 2006.

Other advances in chemistry and protein science enabled the development of conjugate vaccines that chemically link polysaccharide antigens to protein carriers to provoke an immune response in young children. This conjugation technique was first used in 1990 in the *Haemophilus influenzae* type b vaccine and has been applied to meningococcal, pneumococcal, and typhoid vaccines.

The most recently authorized vaccine is the Merck Ebola virus recombinant vaccine (see page 4). The Ebola vaccine is just one example of the sophisticated science that is allowing scientists to continue developing vaccines against existing and emerging pathogens (see page 9). ■

Medical drones

Could vaccines reach remote areas … remotely?

Drone delivery may help overcome the challenge of getting medicines and vaccines to hard-to-reach populations.

by Michael Dumiak

Some of the longstanding obstacles to improving access to vaccines are often as basic as desert, jungle, or mountain. Small aircraft that are piloted remotely, or drones—those as simple as the ones taking Instagram video, or the more sophisticated machines that look like oversized flying spiders—have made enough progress to at least capture the imagination of public health workers trying to get over these obstacles and get vaccines, medicines, and other health supplies to people who need them.

At the World Health Summit in Berlin in October 2019, the World Health Organization's (WHO) chief information officer, Bernardo Mariano, outlined a vision of digital health—bolstered by a new WHO department of digital health, which he heads—by giving a mention to Ghana's ongoing experiments with using Zipline drones to quickly deliver blood for transfusion in the more remote parts of the country (see photo).

Drone delivery in public health is also drawing some criticism, the argument being that Silicon Valley-backed remote tech solutions are a distraction from building basic infrastructure. But it's a complicated issue. Even in a country with more advanced infrastructure, such as Rwanda or South Africa, the distances between health outposts can be vast and the supply logistics very challenging. Only 25% of Rwanda's mountainous roads are paved. These obstacles are a real part of what Seth Berkley, chief executive of Gavi, the Vaccine Alliance, outlined on a panel in Berlin as the "last mile" that keeps people from needed medical treatment and basic vaccinations. Keeping vaccines refrigerated at subzero temperatures and getting them through heat and rugged terrain is a problem drawing many ideas, from solar-powered refrigerators in Yemen to camel-carried passive coolers.

Zipline is among many companies looking for new ways to get medicines and vaccines to inaccessible areas. Credit: World Bank Photo Collection licensed under CC BY-NC-ND 2.0.

And drones are now becoming part of the picture. This summer the Ghanaian drone pilot program started running out of the first of what will be four distribution centers in the country, ferrying blood north of the capital Accra. A year ago, the island nation of Vanuatu, with support from the United Nations Children's Fund (UNICEF), employed a drone operated by Australian company Swoop Aero to drop hepatitis and tuberculosis vaccines following a 30-mile journey over water and mountains. Last July in the Bahamas, the organization Direct Relief, with partners Merck, AT&T, Softbox, and Volans-i, tested a drone in an autonomous flight carrying a specially designed temperature-controlled pharma payload box from island to island over open water. Merck did not say if real vaccine was in the payload box but they were able to monitor the box temperature remotely at minus 70 degrees Celsius, the level required for storing and transporting many vaccines and medicines.

While these larger organizations are drawing attention with their drone experiments, efforts at drone-building using off-the-shelf, relatively inexpensive products are popular—and quite creative—in the developing world. In Tanzania, for instance, local companies are building drones with propellers and bamboo frames. While airlifting medical supplies has a history going back to the first days of flight, this kind of do-it-yourself approach, paired with a little public health expertise, may be a more accessible way to help get over that difficult last mile for delivering medicines and vaccines to the world's more difficult-to-reach populations. ■

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

Upcoming HIV-related meetings

JANUARY 2020

Tuberculosis: Immunity and Immune Evasion
January 16-20 | Santa Fe, New Mexico
www.keystonesymposia.org/KS/Online/Events/2020A2/Details.aspx?EventKey=2020A2

FEBRUARY 2020

Viruses 2020 — Novel Concepts in Virology
February 5-7 | Barcelona, Spain
viruses2020.sciforum.net

International Conference on HIV/AIDS 2020
February 10-11 | Venice, Italy
aids.gavinconferences.com

International Conference on HIV/AIDS Prevention and Control
February 27-28 | Pretoria, South Africa
hivpreventionconference.globalacademicresearchinstitute.com/main/ichiv

MARCH 2020

Conference on Retroviruses and Opportunistic Infections
March 8-11 | Boston, Massachusetts
croiconference.org

Keystone Symposia: HIV Vaccines Joint Meeting with HIV Pathogenesis and Cure
March 22-26 | Keystone, Colorado
keystonesymposia.org/KS/Online/Events/2020X5/Details.aspx?EventKey=2020X5

MAY 2020

World Congress on Control and Prevention of HIV/AIDS
May 21-22 | Osaka, Japan
conferencemind.com/conference/controlandpreventionofhivaids

JULY 2020

AIDS 2020
July 6-10 | San Francisco, California
aids2020.org

OCTOBER 2020

HIV Research for Prevention (HIVR4P)
October 11-15 | Cape Town, South Africa
hivr4p.org