Phylogenetic Viral Linkage
A TOOL FOR EVALUATING HIV PREVENTION EFFORTS

Major HIV prevention trials are underway in African countries, including Botswana, Kenya, South Africa, Uganda, and Zambia. These trials involve hundreds of thousands of people and cost hundreds of millions of dollars. But how will we know if they work?

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The Botswana Combination Prevention Project (BCPP) is an HIV prevention study taking place in 30 pair-matched villages in Botswana. Half of the villages receive scaled-up combination prevention measures and half of the villages act as controls. The study was designed to test whether a combination of HIV prevention measures could significantly reduce the number of new HIV infections within a community.

At the start of the BCPP, the field team rapidly scaled up HIV testing and counseling in the 15 intervention communities. Participants who tested positive were urged to begin antiretroviral treatment (ART) as soon as possible, both to improve their own health and to make them significantly less likely to infect others.

When the field work ends in 2018, the research team’s next challenge is to make sense out of the huge amount of data and thousands of blood samples collected from those enrolled during the study. Some participants were HIV+ at the start of the study. Others became infected during the course of the study, despite increased prevention efforts.

From the blood samples collected, the virus of each HIV-infected participant will be sequenced. By the end of the study, researchers expect to have up to 7000 near full-length HIV genome sequences.

### What Sequencing Reveals

When a person becomes infected with HIV, their virus is similar to the virus of the person who infected them. Because HIV mutates quickly, each infected person’s virus evolves differently over time. By sequencing the HIV genome of every infected study participant in a village, researchers can create what is known as a phylogenetic tree. HIV phylogenetics is the study of the evolutionary history and relationships among transmitted viruses. A phylogenetic tree is an illustration of those relationships and HIV transmission dynamics.

To insure every participant’s privacy, the sequenced HIV genomes are analyzed at a population (community) level. With the data, researchers can create a map that shows all sampled HIV infections in a village, as well as patterns of clustering between closely related infections. For the BCPP, a cluster is defined as two or more closely related infections.

### Targeted Response

By analyzing where clusters exist and where new infections occur, researchers can pinpoint HIV transmission chains, or distinct sub-epidemics. If performed in real time, this analysis will allow...
healthcare workers to quickly target HIV prevention efforts to high-risk groups and geographic areas.

“The goal from a public health perspective is to identify hot spots—the viral transmission chains that are feeding and fueling the epidemic—and target interventions to prevent new transmissions,” said Dr. Vlad Novitsky, the Harvard AIDS Initiative (HAI) Research Scientist leading the BCPP genetic sequencing team.

Report Card

The primary endpoint of the BCPP is the number of new HIV infections. The hope is that in the 15 villages that receive the interventions, there will be a minimal number of new infections. “But people don’t restrict interactions to their own village,” said Novitsky. “They work and socialize between communities.”

When HIV testing reveals that an individual in a village is newly infected with HIV, researchers will be able to tell if that infection is related or unrelated to viral lineages circulating within the community. If it is not related, the newly infected individual is more likely to have been infected from someone outside of the community who is not receiving the scaled-up prevention interventions.

“Basically, viral linkage tells us whether the interventions are working—whether new infections are coming from inside or outside of the community,” said Novitsky. “If there is a new infection and we have built a phylogenetic tree and see that there are 25 circulating viruses within this community, but the person does not cluster with any of those, it would be a strong confirmation that the person was infected somewhere outside of this community and interventions within this community would not prevent this infection, no matter what.”

Besides helping to gauge how well HIV prevention efforts work, phylogenetic viral linkage will also help researchers understand why different HIV lineages behave differently. HIV transmission clusters are not all the same. Some grow quickly; other are relatively stable over time. “Why are particular viral lineages still spreading despite high levels of ART? Is the answer biological or social and behavioral?” asks Novitsky.

With the powerful tool of phylogenetics, researchers are gaining a better understanding of how and where HIV spreads, allowing public health officials to rapidly target their treatment and prevention efforts to quell the epidemic.
If a boy from a Botswana village wins second place in the national science fair for a project on optimizing alcoholic brews, predictions about his future could involve his getting into trouble, or working for a large beer company, or, if you’re Dr. Simani Gaseitsiwe, becoming the director of one of Africa’s top research labs.

The youngest of seven siblings, Simani was born in 1974, in Mathangwane, a small village in northeast Botswana. He attended the local primary school, then high school in Francistown, 20 miles away, where he excelled at science. After his team won second place in the national science fair for a project on the fermentation of local brews, and third place the next year for a project on how the design of airplane wings influences lift and drag, Simani was hooked on experimental science.

After high school, he taught math and science in a village junior high school to fulfill his year of National Service. “It got me interested in teaching, which is something I’m still doing,” he said. Then it was off to the capital, Gaborone, to pursue studies for a Bachelor’s of science at the University of Botswana. The first in his family to go directly to university, Simani placed in the top 5% of students, which qualified him to travel abroad for the last two years of his degree. He was accepted at the University of Pittsburgh’s program in medical laboratory technology and completed his undergraduate studies in the U.S.

He returned to Gaborone in 1998, at the peak of the AIDS epidemic. In Botswana, about a quarter of adults and over a third of pregnant women were infected with HIV. Two years earlier, the Harvard AIDS Institute, under the leadership of Prof. Max Essex, had entered into a partnership with Botswana’s Ministry of Health to combat the epidemic, establishing the Botswana Harvard Partnership (BHP).

“It was a very ambitious project,” said Dr. Monty Montano, then an Essex postdoc and now a researcher at Harvard Medical School. “Everyone was asking if you could build an infrastructure in Botswana. Max never asked if it was possible. He asked what our timeline should be.”

Essex’s team renovated a dusty warehouse on the grounds of Princess Marina Hospital, turning it into a lab for HIV testing and research. They advertised in the local newspaper for lab techs. Simani, who was teaching chemistry at the Institute of Health Sciences next door to the hospital, saw the ad and applied.

“I remember asking him why he would want to be part of the initial reference lab,” said Montano, who interviewed Simani. “He said he thought it was his obligation as a citizen of Botswana to engage in a proactive effort to combat the virus. It was a patriotic motivation on his part—a recognition of the graveness of the times.”

The first Botswana Harvard Partnership (BHP) lab assistants included Simani and three other young Batswana. It was an exciting time, as Dr. Vlad Novitisky and other scientists from the Harvard AIDS Institute taught the crew new skills and techniques. “Almost everything we were doing, it was the first time it was being done in Botswana,” remembers Simani.

After a year of performing routine HIV lab tests, Simani’s focus shifted to...
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Simani Gaseitsiwe

It was as a Fogarty Fellow that Simani began thinking about a PhD. “That was a time when I got to interact with Thumbi, who was just finishing,” he said, referring to Dr. Thumbi Ndung’u, a Kenyan-born researcher who is now Scientific Director of the HIV Pathogenesis Program at the University of KwaZulu Natal in South Africa. “Seeing a fellow African graduating with a PhD and the work that he had done, you really felt motivated. You had role models that you could look up to.”

In December 2001, Simani was back in Gaborone to see then President Festus Mogae officially open the just-constructed Botswana Harvard HIV Reference Laboratory. The three-story, 25,000-square-foot, state-of-art laboratory was located next door to the old warehouse. The new building announced Botswana’s commitment to HIV/AIDS treatment, prevention and research. It also provided BHP’s expanding staff with a headquarters.

In the brand new lab, Simani focused on HIV-1 drug resistance genotyping and other research projects. Though shy, he had a reputation for being reliable and responsible. When not at the lab, he liked to lift weights or work out at a local gym. In 2004, he married a young urban planner named Chipo.

When it came time to continue his education, Simani saw an advertisement in NatureJobs for PhD scholarships to work on HIV and TB co-infection at Sweden’s prestigious Karolinska Institute. The scholarships were funded by the European Union through the Marie Curie Fellowships and were primarily for Europeans. “The odds are stacked against me,” thought Simani, “but why not just try?”

He didn’t have a Master’s degree, usually required for such a program. Essex, who had done his postdoc at Karolinska, sent a letter in support of his application, explaining that the papers Simani had contributed to while working at BHP were the equivalent of a Master’s.

Simani was accepted and went to Stockholm, along with Chipo and their three young children. He earned his PhD from Karolinska in 2009 and stayed for a postdoc.

At this point in his career, as a credentialed, experienced bench scientist, Simani had a number of options. He chose to return to BHP in the role of Deputy Laboratory Research Director, responsible for supervising basic research, as well as guiding and mentoring students and staff. “In terms of doing research in Botswana, there is still no place as good as BHP, especially if you’re a lab person,” said Simani.

“He’s a good mentor,” said Tapiwa Nkhisang. In 2015, while enrolled at Smith College, she spent the summer working at the BHP lab. “He has faith in people. If you need help, he’s there for you, but he gives you the freedom to try things. I like how he sort of let me fly,” she said.

“The important thing is for them to think for themselves,” said Simani about mentoring, whether it’s Harvard juniors, or Fogarty Fellows, or graduate students from the University of Botswana. “I let them develop their own ideas, get them on track, and support them. To a great extent, that has been the way I have been brought up in the science field.”

In January 2017, Simani became the BHP’s new Laboratory Director. One of his responsibilities is training young scientists to build research capacity in...
Are Migrants Driving the Epidemic?

When “Treatment as Prevention” was named *Science* magazine’s Breakthrough of the Year in 2011, there was optimism that we were closing in on AIDS. The multinational HPTN 052 study showed that providing antiretroviral therapy (ART) to people with HIV was not only good for their health, but also lowered the levels of HIV in their blood to undetectable levels, making the chance of infecting others extremely unlikely. The hope was that by testing everyone for HIV and providing ART to all HIV-positive people, the rate of new infections would steadily decline, ending the epidemic.

The Botswana Harvard Partnership (BHP) participated in that landmark study and is now a partner in the Botswana Combination Prevention Project (BCPP), a clinical trial underway in 30 villages in Botswana. The BCPP is designed to test whether ART, along with other HIV-prevention measures, can significantly reduce HIV incidence—the rate of new infections.

For its citizens, Botswana currently follows the World Health Organization (WHO) recommended policy of “universal test and treat,” but migrants are excluded from the national ART program. *(Migrant is here defined as a person who lives in Botswana but is not a citizen.)*

The number of migrants in Botswana is not well-documented and varies widely from region to region. Migrants may be seasonal workers, political or economic refugees from neighboring countries, or long-term residents living with their extended families. Migrants may have work permits or may be undocumented. In the 2011 National Census, it was estimated that 14% of the employed and 9% of the unemployed population of Francistown, the second largest city in Botswana, were migrants.

Tafireya Marukutira, who worked with the CDC on the BCPP, presented data from the on-going study at the 2017 CROI meeting in Seattle.

From October 2013 to February 2016, the BCPP field teams conducted HIV testing campaigns in 15 intervention communities. Testing was offered to anyone over 16, citizens and migrants alike.

Of the 48,640 people tested for HIV, 3% self-identified as migrants. About 20% of migrants were HIV-positive, similar to the 22% rate among citizens. But in contrast to citizens, the vast majority of HIV-positive migrants did not know their status. Only 27% of HIV-positive migrants were on ART, compared to 71% of citizens or spouses of citizens.

These results raise concerns.

“Given the high ART coverage rates in the general population in Botswana, lack of free ART coverage for non-citizens may result in a disproportionate contribution to incident HIV infections,” said Marukutira.

Dr. Vlad Novitsky, a virologist and part of Harvard’s BCPP team, agrees that migrants may contribute to new HIV infections. He would like to see more research and emphasizes that because migrants are a vulnerable population, research must meet the highest ethical standards.

“Without information, you cannot convince the government that all residents, irrespective of citizenship, should be treated because they participate in transmission,” said Novitsky.

“Determining how migrant populations are impacting HIV transmission has the potential to change government policy on access to treatment.”

“Failure to provide ARV treatment to migrants is likely to jeopardize the Treatment-as-Prevention policy and curtail the efficacy of interventions among key populations that share sexual network with migrants,” said Novitsky. Though he supports providing ART for everyone in Botswana, regardless of citizenship, Novitsky recognizes the limits of his role as a scientist.

“It’s up to the government and the Ministry of Health to decide what to do,” he said.
Fogarty programs, including their HIV Research Training Program, launched in 1998 to strengthen research capacities in developing countries.

“The Fogarty training is unique,” said Max Essex, the Mary Woodard Lasker Professor of Health Sciences at Harvard and Chair of the BHP. “It builds expertise in exactly those places where the next round of epidemics like Ebola, Zika, AIDS, or flu are most likely to occur. This provides immediate benefits for local control of such epidemics, which in turn minimizes their impact on the U.S. and the rest of the world.”

Many of the African scientists now working at the BHP in Gaborone received training through Fogarty. Currently, in the Essex Lab at Harvard, five young women from Botswana are learning the latest laboratory techniques for genetic sequencing of HIV.

With Fogarty, training goes both ways. Their Global Health Fellowships provide U.S. scientists and clinicians with opportunities to work and learn in countries like Botswana, where HIV prevalence is high and resources are limited.

Would Eliminating Fogarty Put America at Risk?

Americans benefit from global health research. By developing scientific expertise in African countries and elsewhere, Fogarty helps to ensure that trained researchers are in place to detect and address infectious disease outbreaks at their point of origin—before they reach America.

When Ebola struck western Africa in 2014, countries with little or no scientific capacity suffered the most. When Zika spread through Brazil in 2015, Fogarty-trained Brazilian scientists used their expertise to investigate and find answers.


Harvard’s Max Essex agrees. “It’s easier and cheaper to prevent and contain a new epidemic in a small population,” he summarized.

Writing in JAMA Forum this April, Prof. Lawrence Gostin of the Institute for National and Global Health Law at Georgetown University decried the proposed elimination of the Fogarty Center. “Although we sometimes view national security in terms of military might, strengthening health systems outside the United States can do as much, or more, to keep us safe,” wrote Gostin. “Few wars would have the same cost in human lives and treasure as a pandemic . . . At about 20 cents per U.S. resident per year, why would we eliminate this national treasure?”

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Max Essex
HAI is dedicated to research and education to end the AIDS epidemic in Africa and developing countries. For over 25 years, HAI has been at the forefront of HIV/AIDS laboratory research, clinical trials, education, and leadership.

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**The Interconnected Issue**

Like it or not, we’re all connected. While officials debate whether to strengthen treaties or build walls, infectious diseases easily cross every border. This issue of Spotlight includes a story about how genetics help us understand the way HIV moves within a community, a story about how migrants may influence HIV prevention efforts, and another on how budget cuts could imperil our response to the next pandemic. Plus, a profile of Lab Director Dr. Simani Gaseitsiwe.

Botswana. In many ways, he personifies the goal himself. “Simani has the intellectual talent and the technical tools,” said Max Essex. “He’ll do great things as a scientist.”

His current research projects focus on three areas: characterizing drug resistance mutations in HIV-1C infected patients; exploring the Hepatitis B virus genotypes circulating in Botswana and their impact on response to HIV antiretroviral therapy; and investigating latent TB infection and predictors for active TB in patients on antiretroviral therapy.

Along with his work responsibilities, his family continues to grow. He and his wife now have six kids: four daughters, ages 14, 11, 3, 2, and two sons, ages 12 and 5. “It’s quite hectic, but we make it work. There’s never a dull moment,” he said, laughing. Somehow, amidst work and family, Simani recently found time to run his first marathon.

Much has happened since Simani began work at the BHP in 2000. Of the other lab assistants who started with him, sadly one died a few years later, the other two, like Simani, earned advanced degrees and continue to work in medical science.

“Life, at the end of the day, is finite, so everyday counts,” said Simani. “If you can come up with something that could add one more day to someone’s life, that’s so important. Biomedical research is one of those areas that can really help in terms of adding one more day.”