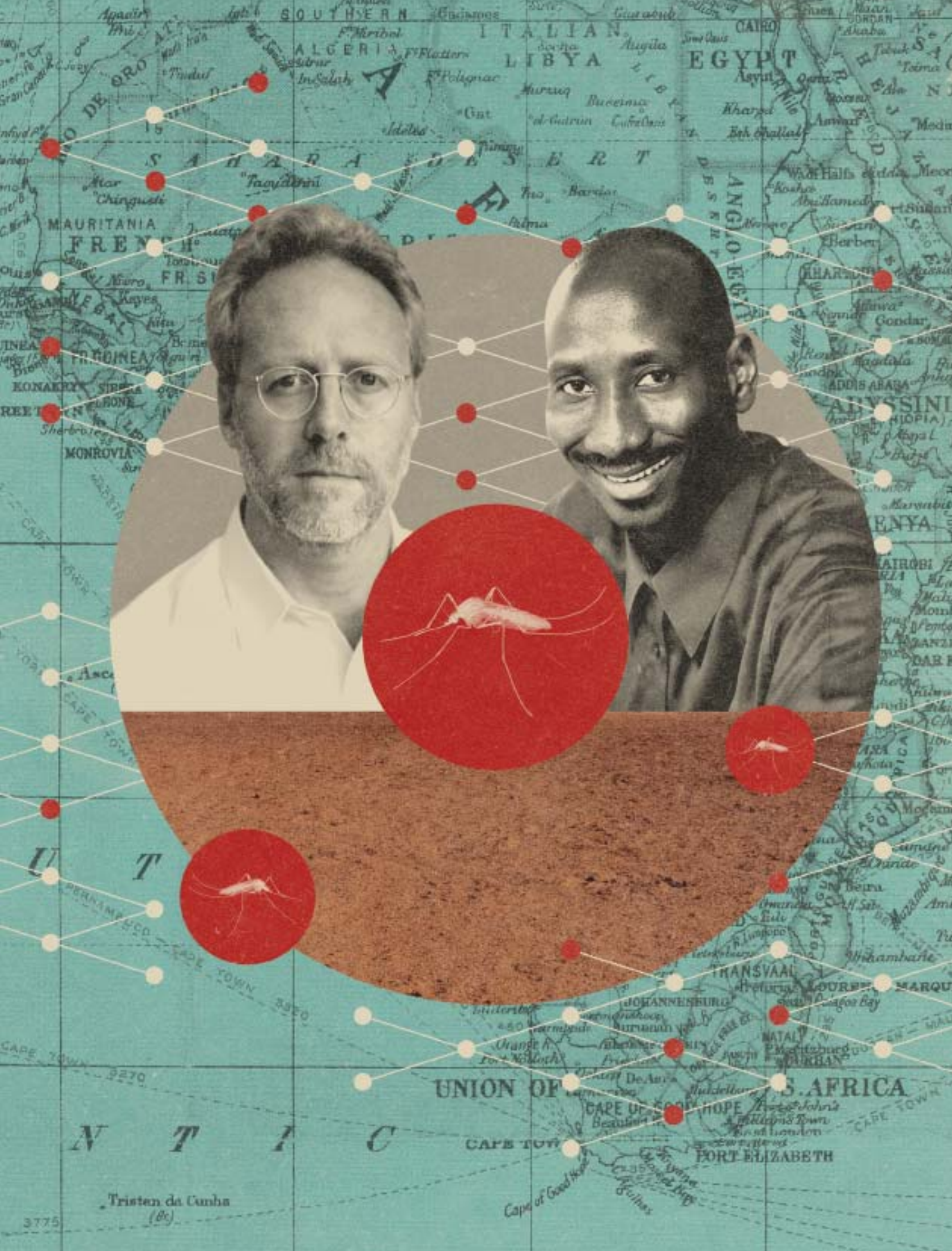


by Robert Koenig  
illustration by Mark Weaver



# TWO ROADS TO AN END

Though separated by an ocean, Christopher Plowe and Abdoulaye Djimdé are bound by their determination to stop malaria's global toll.



Tristan da Cunha  
(8)

3775

# WHEN

Christopher Plowe traveled to Mali in western Africa to test a technique for detecting drug-resistant malaria, he was struck by the intelligence and skill of a local pharmacist who had volunteered to help. It didn't take long before that volunteer, Abdoulaye Djimdé, "rose to the top," Plowe recalls, and was offered a lab stint at the U.S. National Institutes of Health (NIH). Later on, Djimdé would become Plowe's first Ph.D. student.

That African encounter in 1993 led to a thriving collaboration and friendship between the two researchers—an M.D. from South Dakota and a pharmacist from malaria-ravaged Mali.

Malaria infects hundreds of millions of people worldwide and kills an estimated 900,000 a year, taking an especially high toll on children in sub-Saharan Africa. Despite more than a half century of research, no effective malaria vaccine has been approved, and the resilient parasite that causes the disease has developed resistance to numerous drugs.

Plowe and Djimdé share a passion for fighting malaria and are leaders in important research projects. Plowe—an HHMI investigator at the University of Maryland School of Medicine—focuses mainly on vaccine development. Djimdé, an HHMI international research scholar at the University of Bamako's Malaria Research and Training Center, studies antimalarial drugs and the emergence of the malaria parasite's resistance to them.

"Chris Plowe has been a major figure in the development of malaria research in Mali, especially with his work with the human populations," says Thomas E. Wellems, chief of the Malaria and Vector Research laboratory at the National Institute of Allergy and Infectious Diseases (NIAID)—and the researcher who first sent Plowe to Mali. "Both he and Djimdé perform brilliantly in

the lab as well as in the field." But the two scientists took vividly disparate routes to the field of malaria research.

## PERSONAL TRAGEDY

When Djimdé was a preteen living with his family in Koro, a Dogon village in eastern Mali, his 3-year-old brother, El-Hajj, suddenly spiked a high fever. "We took him to the hospital, but it was too late for proper treatment." The boy died from cerebral malaria a few hours later. "It was a shock for the whole family," recalls Djimdé, who was one of fifteen children. "That was the turning point when I decided to be a doctor and take care of sick kids who had malaria."

In those days, villagers relied on traditional healers to treat malaria. The common form of the malady is called the "green season disease" because it appears when mosquitoes breed during the rainy months. The more serious cerebral malaria is considered a spiritual affliction called "wabu," which healers treated with potions.

Determined to get away from tribal remedies and introduce more effective Western medications and treatment, Djimdé worked hard in school with the goal of becoming a medical doctor. The Mali educational system steered him to pharmacy school, however, and in 1989 he opened his own pharmacy. But Djimdé was still interested in malaria research, and when he heard about Plowe's project, he volunteered to help. Soon, Djimdé moved to the U.S. to work with Plowe in Wellems' NIAID lab.

## PLOWE'S PATH

Born in South Dakota, Plowe had never traveled outside North America. He studied philosophy in college and after graduating

“WE SPENT A LOT OF HOURS TOGETHER IN THE LAB AND IN THE FIELD,” RECALLS PLOWE, WHO DESCRIBES DJIMDÉ AS “AN INCREDIBLY TALENTED SCIENTIST WHO I AM PROUD TO CALL FRIEND.”

enrolled in Cornell University Medical College, planning to specialize in surgery or psychiatry. Then he spent a summer doing epidemiology research in Indonesia and two years later—while still in medical school—he “got hooked on malaria research” during a stint in western Kenya setting up a research project led by Naval Medical Research Center scientist Steve Hoffman.

“Steve convinced me that malaria was the most important disease in the world and the most challenging biological problem,” Plowe recalls. Hoffman was impressed with Plowe’s talents at working with both African villagers and Western scientists. “He played a leadership role in getting that study off the ground,” Hoffman says. Eventually published in *Science*, the study “was a testimony to the kind of groundwork Chris did, and it was predictive of his later success.”

After he finished his residency in New York, Plowe spent three years as a postdoc in Wellem’s NIAID lab, where he studied the molecular biology of malaria parasites. He also got involved in developing a test for resistance to chloroquine—then the most widely used antimalarial drug. At that time, in the early 1990s, chloroquine resistance was spreading rapidly across the sub-Saharan region, from eastern into western Africa.

Monitoring patients for resistance was cumbersome, so it was difficult for doctors to know what antimalarial drugs to prescribe. The Wellem’s group had identified a gene associated with resistance but was searching for a practical way—rather than freezing blood samples and shipping them to labs—to use the genetic marker in remote areas. Plowe wanted to use blood spots on filter paper to extract DNA for analysis, even though previous efforts had failed.

He succeeded, and the project put Plowe on the map as an internationally known malaria researcher. “It was exciting to take a scientific advance and translate it into a public health tool that has had real impact,” Plowe says.

Adds Wellem: “It was a big challenge, but Chris made it work. Now that technique is used by researchers around the world.”

#### WORKING IN TANDEM

Plowe met Djimdé while testing his filter paper technique in the villages of Mali.

The two young researchers eventually became close friends during their time in Wellem’s lab at NIAID. “We spent a lot of hours together in the lab and in the field,” recalls Plowe, who describes Djimdé as “an incredibly talented scientist who I am proud to call friend.” Djimdé calls Plowe “a mentor who always went the extra mile to help me get established as a scientist.”

Even after Plowe left Wellem’s lab for a stint as a fellow at the Johns Hopkins University School of Medicine, he kept working with Djimdé and the NIAID group on malaria projects. “When Tom’s lab found the gene that causes chloroquine resistance, Djimdé and I developed a fairly robust test to detect mutations of the gene. Even before that was published, we shared the protocol with the World Health Organization so it could be used in the field.”

---

## POTENT BUZZ: *A Whole-Parasite Vaccine*

---

“Mosquito Crossing” warns the yellow road-caution sign. The attention-grabbing notice hangs on the door to the “challenge room” of Chris Plowe’s insectarium, at the University of Maryland, Baltimore. Inside, volunteers roll up their pant legs while researchers press a Dixie cup with four or five malaria-infected mosquitoes against their ankles. The blood-swollen insects are collected for analysis and blood samples are drawn from the volunteers. / The work is part of a “challenge study”—the first clinical trial of a promising “attenuated sporozoite” vaccine being developed by Sanaria Inc., a company headed by Plowe’s early mentor, former Navy researcher Steve Hoffman. There are dozens of other vaccine candidates in various stages of development—the farthest along being GlaxoSmithKline’s RTS,S vaccine, now in Phase III tests at 11 African sites—but Plowe says “the Sanaria vaccine is the only vaccine that uses the whole [parasite] organism.” The others, he says, which focus on one antigen variant, “are like trying to stop an elephant with a BB gun.” / The concept, Hoffman explains, is to use sporozoite-stage parasites—an early, motile stage that infects the liver—that have been weakened (attenuated) by exposure to radiation. Although the weakened parasites cannot complete their life cycle, they survive long enough to awaken the human immune system and confer protection against malaria. The overall challenge study is being led by clinical investigators Kirsten Lyke at the University of Maryland’s Center for Vaccine Development and Judith Epstein at the Navy’s research center. Right now, Plowe’s is a supporting role but eventually he will design and implement studies of the vaccine in Africa. / In a sense, the current challenge trial is part of a Maryland tradition. The first malaria vaccine trials in humans—with an attenuated sporozoite vaccine developed by researchers at New York University—were done in the early 1970s by University of Maryland professor David Clyde. (Previous malaria vaccine research had been done on ducks and rodents as far back as the 1920s.) / “The early-1970s Maryland vaccine trials proved the principle that sporozoites can give protection,” says Hoffman. “But they couldn’t produce sporozoites that met regulatory standards—that is, sterile, pure, highly attenuated, and still potent.” That is what Sanaria is now trying to do in a painstaking process that is taking years to develop and test. / Plowe says he’s “excited about taking a whole-organism vaccine to Africa” for clinical trials, which probably will begin within a few years. “With the attenuated sporozoite vaccine, even if you have diversity in one antigen, hopefully you’ll have 10 other antigens. You are taking it from the genetic level to the genomic level.” / Hoffman says Plowe is the right researcher to design and implement vaccine trials in Africa and apply genomics to understand how a whole-parasite vaccine works. “He has the capacity to work with not only his biomedical colleagues but also the people in Africa who suffer the most from this disease.” —R.K.



Chris Plowe and Abdoulaye Djimdé met in Mali, in the 1990s (upper left), while testing a new technique for monitoring malaria drug resistance. Once mentor and student, now they mentor each other's students and stay with each other's families while traveling.

And when Plowe joined the University of Maryland faculty in 1995, he convinced Djimdé to become his first Ph.D. student. Djimdé's thesis focused on the molecular mechanisms of malaria resistance to chloroquine.

"It was an emotional time for me. We were the first to document that resistance in the field, showing that this gene was responsible for chloroquine resistance in Mali," says Djimdé, who returned to his home country to continue his research in 2001. "When the paper came out in the *New England Journal of Medicine* I got calls and e-mails from all over the world."

Plowe says the thesis work "has been extremely influential. It laid an important foundation for the study of the molecular basis for malaria resistance."

Plowe's own interest in that area has led him to assess the new wave of resistance to artemisinin-based combination therapies, or ACTs, which replaced chloroquine over the last two decades as the most commonly prescribed antimalarial drug. Used for centuries in China, artemisinin compounds were introduced in Africa to treat malaria in the 1980s and appeared to be thwarting the typical trend of drug resistance. But researchers found signs of artemisinin resistance in western Cambodia a few years ago.

"We don't have the right tools yet to track and monitor artemisinin resistance, which is why I've shifted my attention on the drug-resistance side to Southeast Asia," says Plowe. "We're trying, in a much more accelerated fashion, what it took Tom Wellems 15 years to do in pinpointing chloroquine resistance."

In a project that involves scientists at Oxford University, Mahido University in Thailand, and the U.S. Armed Forces Institute of Medical Sciences in Bangkok, Plowe's lab is doing genomic studies to pinpoint gene loci that could be used as markers for that resistance. The goal is to give scientists around the world access to comprehensive data to help determine which antimalarial drugs should be used in which regions.

"The whole discussion of malaria eradication and elimination will come to a screeching halt if artemisinin resistance spreads throughout Asia and gets to Africa," he says. "We're trying to develop the tools to detect that resistance and head it off."

#### AN ELUSIVE PARASITE

Plowe, who became an HHMI investigator in June 2008, likes to show off his lab's new DNA extraction robot that "has vastly increased our throughput," supporting the shift in his lab's focus from drug resistance to gene resistance.

That transition is crucial to developing next-generation tools to fight malaria. Ever since British researcher Ronald Ross identified the malaria parasite in mosquitoes in 1897, thousands of scientists around the world have searched for effective treatments and vaccines. But that resilient organism—*Plasmodium falciparum* causes the deadliest form of human malaria—keeps developing resistance to drugs and presenting hurdles to vaccine makers.

At the heart of the problem is the parasite's complexity, with its life-cycle stages and uncanny ability to fool the body's immune system. Delivered by a mosquito's bite, the parasite's thread-like sporozoite stage travels through the human bloodstream and settles in the liver. There it morphs and multiplies into tear-shaped merozoites that reinvade the circulatory system and burst red blood cells, releasing toxins that sicken or kill the human host. Also in the bloodstream are the parasite's reproductive gametocytes, which biting mosquitoes suck from the human host into their salivary glands to begin a new cycle of infection.

"It has a very plastic genome," says Plowe. "Every 48 hours, the parasite multiplies roughly 10-fold," speeding the genetic recombination that strengthens resistance.

Vaccine developers target the parasite at one stage or the other, using specific parasite proteins to incite a human immune response. The challenge, Plowe explains, is to target the right combination of proteins, which can vary based on genetic code. “Part of the difficulty is the speed of mutations of the parasite and the other part is not knowing the basis of immunity in the human populations,” he says.

With support from the NIH, Plowe developed a vaccine test site in the same village where Djimdé did his groundbreaking study of chloroquine resistance. Plowe and his colleagues in Mali are testing vaccines developed by scientists at the Walter Reed Army Institute of Research, combined with an adjuvant (immune system booster) from vaccine manufacturer Glaxo-SmithKline Biologicals.

Among the most promising of those candidates is the AMA-1 blood-stage vaccine. Plowe’s group reported in the February 2010 issue of *PLoS One* that the vaccine candidate produced a 100-fold increase in antibodies against the AMA-1 malaria protein in 75 children in a Phase I study at the Mali test site.

Following 100 kids over time in Mali, his group sequenced the gene for AMA-1 in more than 1,300 infections. “By looking at which changes in the AMA-1 sequence in consecutive infections were more likely to make a child sick,” Plowe says, “we could pinpoint the genetic differences that seem to be important for clinical immunity.”

The new approach is aimed at a persistent problem with malaria vaccines, he says—the fact that “all the vaccines in the clinical pipeline were designed with no knowledge of whether the variant that was picked to put in the vaccine was the most common or the least common in nature.” Plowe suspects that genetic differences that matter most in natural immunity will turn out to be the same ones that drive vaccine-induced immunity. (See sidebar, “Potent Buzz.”)

In one previous trial, a malaria vaccine candidate was found ineffective, most likely because it used a protein variant that was rare in the Kenyan community where the vaccine was tested. In Mali, Plowe’s team has identified more than 200 variants of the AMA-1 protein in a single village, but the group is using genomic

analysis to narrow down the number of variations the immune system must recognize to prevent illness.

The studies have indicated that “we might be able to reduce the number of variants we would need to address [in a vaccine] from more than 200 to just 10 or so—maybe even fewer,” Plowe says. A vaccine based on just one of those variants was tested first, with the Phase I trial among children reported in the recent *PLoS One* paper and the first efficacy (Phase II) trial results expected to be reported in a paper later this year. While the results of the Phase II trial aren’t yet public, Plowe offers a hint, saying “We haven’t hit a home run, but I think we’ve finally made it to first base.

“The big question is: Can you get overall efficacy based on a single variant when there are over 200 variants in a village?” he adds. “Or, if you don’t get broad overall protection, do you at least get the information that you need to go back and develop a more broadly protective vaccine?”

#### MOSQUITOES IN MALI

Like Plowe, Djimdé divides his time between the lab and the field, where he is focusing his research on the genetics of drug resistance.

“We’ve been looking at a more holistic approach to understanding drug resistance,” says Djimdé, which would take into account “the many factors related to the response to anti-malarial drugs.” Those factors include the genetics of the human hosts—including acquired immunity and how people metabolize malaria drugs—and of the malaria parasites and the mosquitoes that spread them. “We are trying to understand the interplay among the parasite, the mosquito, and the drug,” he says. “How drug resistance is established in a community, and how it spreads across time and geography.”

Collaborating with entomologists, Djimdé’s group catches mosquitoes in the villages being studied, harvests their eggs, and develops new generations of the insects that are kept in the laboratory and used for experiments.

They also work closely with villagers. “It can take several months of preparatory work before a community agrees” to take

*(continued on page 48)*

“I DON’T THINK WE HAVE THE TOOLS TO [ERADICATE MALARIA] YET. THIS WILL REQUIRE SUSTAINED FUNDING AND DECADES OF RESEARCH.” —Abdoulaye Djimdé

part in a research project, he says, requiring approval by an ethics panel and village elders as well as cooperation with traditional healers. “You have to respect the customs and the organization of the community. You have to find a way to explain what you plan to do, what risks are involved, and the likely benefits for the community and perhaps for mankind in general.”

In one project, Djimdé’s group, in collaboration with Wellem’s NIAID lab, is examining the parasite’s resistance to quinine, the time-tested drug derived from tree bark that is often used to treat the most severe cases of malaria. (Quinine is a natural product that differs markedly from chloroquine, which was synthesized by German scientists and refined as an antimalarial drug by U.S. researchers during World War II.) He is also trying to determine the safety of, and whether resistance is emerging to, the newest generation of ACTs.

The fight against malaria has continued for centuries—and intensified in recent years with the advent of the World Health Organization Global Malaria Programme and related efforts sponsored by major aid organizations. In recent years, optimistic funding agencies, such as the Bill & Melinda Gates Foundation,

have suggested that malaria can be eliminated, and more than two dozen countries have launched efforts that they hope will stamp out the disease, with some progress reported.

Plowe and Djimdé support the ambitious goal. “The malaria research world has been transformed by the call for eradication,” Plowe says. But the two say it will take years of research to stop malaria in nations where the disease is now endemic and widespread.

“I think eradication is possible, but not in the near future,” says Djimdé. “The tools we have today—notably, the artemisinin-based combination therapies and mosquito nets, with the possibility of an efficacious vaccine against malaria—can be very effective if they are available and are deployed. In specific communities, they can lessen malaria to the extent that it is no longer a serious public health problem.

“I don’t think we have the tools to [eradicate malaria] yet. This will require sustained funding and decades of research,” he adds.

Both Djimdé and Plowe will continue to lead the way. “Djimdé is now one of the best-known researchers in all of Africa,” says Plowe. Although they’re using different approaches to tackle malaria, the two scientists remain friends after nearly 20 years—sometimes mentoring the same students and discussing their separate projects. Even their families are close. “It’s been a great friendship.” ■

tug-of-war with itself, a century earlier. But Petersen and Reddien were in a better position; they knew which silenced gene had caused the oddity. It was beta-catenin, a key molecule in the Wnt signaling pathway that controls body plan polarity in many animals during embryonic development. Beta-catenin transmits the Wnt signal into the cell’s nucleus, where it directs changes to a set of other genes’ expression levels.

The two showed that, normally, beta-catenin turns on genes that suppress head formation and promote tail formation. However, when beta-catenin is absent, the default is to produce a head at any wound site, according to their 2008 *Science* paper. At the same time and also published in *Science* in 2008, Sánchez Alvarado’s group created an animal with boosted levels of beta-catenin; when its head was amputated, a tail grew back instead.

“This is the type of science you dream about as a kid. We are studying processes that are dramatic and broadly important,”

says Reddien. He says this study captivates him because it hammers home the idea that choices are being made at the sites of wounds. He wants to uncover how those decisions happen.

“We could not ask these questions in *Drosophila* or *C. elegans*. Planarians’ biology is very different and they enable a whole suite of questions that couldn’t be addressed in existing model systems that do not regenerate robustly,” notes Reddien.

Brenton Graveley, one of a handful of scientists starting to use planarians in biomedical research, gives Sánchez Alvarado, Newmark, and Reddien all the credit. “The three of them brought planarians into the molecular era,” says Graveley, a molecular biologist at University of Connecticut Health Center in Farmington.

The early genome work by Sánchez Alvarado and Newmark that showed planarian genes were closely related to human genes “propelled planarians to the forefront,” he adds. “If you are not going to work on human or mouse cells, then the leap in

translating what you find in planarians to actual human stem cell function is going to be much shorter,” he notes.

Sánchez Alvarado speaks fondly of his quirky, but potentially powerful, pet organism: “What model system isn’t funky? *C. elegans* has such a defined cell lineage, it’s uncanny. Morgan chose *Drosophila* because they were such prodigious egg layers. All model organisms got chosen because they exaggerate a particular biology.”

Sánchez Alvarado remembers the flight home after the fountain-foraging expedition in Barcelona. He and Newmark camouflaged the cooler of live worms with cardboard and duct tape. “It looked so suspicious, but they let us through.”

The journey into planarian self-renewal has been both adventurous and arduous. “It was hard,” he admits. “But when you look at the animal, and the wild type is already unbelievable—I mean, the guillotine would not work on this guy—I thought, ‘If we can actually go in and perturb these things, how amazing it would be.’” ■



This paper is certified by SmartWood for FSC standards, which promote environmentally appropriate, socially beneficial, and economically viable management of the world’s forests.