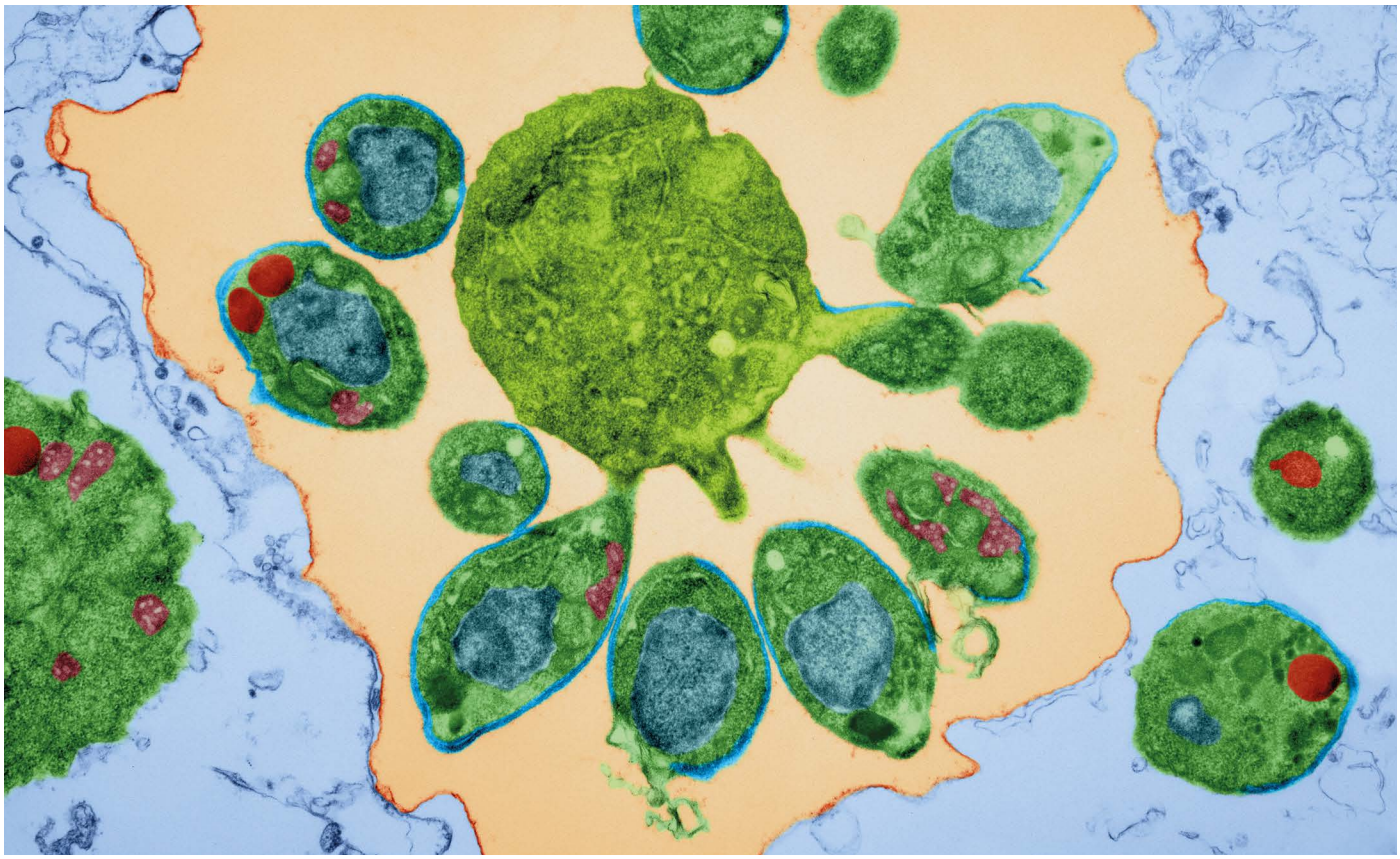


DENNIS KUNKEL MICROSCOPY/SPL

Transmission electron micrograph of merozoite-stage malarial parasites, which have caused a red blood cell to rupture.

The problematic parasite

This year, the first vaccine for malaria was given to children. Scientists are working on improvements, but there is little agreement on how to do this. **By Anthony King**

In 1991, when immunologist Patrick Duffy was joining the US National Institute of Allergy and Infectious Diseases, the research community was sceptical that it would be possible to vaccinate against parasites – especially the *Plasmodium* parasites that cause malaria. These organisms, each just a few micrometres across, can shape-shift and hide from the human immune system, moving from the blood into liver cells, before bursting out in a new form to take over red blood cells. They deploy similar cellular machinery to humans, and are much more complex than viral or bacterial foes – for instance, whereas the Ebola virus can encode 7 proteins, *Plasmodium* boasts genes for 5,000.

But in 2019, children in Ghana, Kenya and Malawi began receiving the RTS,S vaccine against malaria as part of a pilot programme. After 30 years in development, RTS,S is the first malaria vaccine shown to offer protection to young children in a phase III trial – malaria takes 1,200 lives each day, mostly those of children under 5. "The biggest news is that we have malaria vaccines that work," says Duffy.

RTS,S, developed by pharmaceutical firm GlaxoSmithKline, based in London, works by introducing the immune system to a fragment of a protein that is present on the surface of *Plasmodium* when the parasite enters the bloodstream through an infected mosquito. The protein stimulates the production of antibodies, and allows the body to mount a swift response to the parasite the next time it is encountered. The vaccine's pilot programme, coordinated by the World Health Organization, is expected to immunize at least 360,000 children a year until 2022. But malaria researchers are not popping the champagne corks and relaxing just yet. "RTS,S is leading the pack, but it's a suboptimal vaccine," says Michael Good, a vaccine researcher at Griffith University in Brisbane, Australia.

"Some feel it doesn't give you enough protection to be worthwhile," says Stefan Kappe, an infectious-disease scientist at Seattle Children's Hospital in Washington. Over the course of the vaccine's four-year phase III trial, it prevented around 30% of serious cases of malaria[1]. The trials also showed a rise in mortality among girls who were vaccinated. "If that is real, that is the end of that story," says Adrian Hill, a vaccine researcher at the Jenner Institute in Oxford, UK.

Malaria-vaccine researchers are working on several approaches to achieve more robust protection than that offered by RTS,S. Some

are looking to empower the immune system to go after *Plasmodium* in the liver, including using live attenuated parasites as immunizing agents. Others have their eyes on targeting the parasite inside red blood cells – if not to neutralize it entirely, then at least to prevent it spreading to other people. There are numerous strategies all jostling for the limelight, and considerable disagreement about which should be prioritized.

Following the leader

When a person is bitten by a mosquito carrying *Plasmodium falciparum*, the deadliest of the five species of malaria parasite that can infect humans, a handful of protozoa in their needle-like 'sporozoite' form enter the body (see 'Breaking the cycle'). The RTS,S vaccine stimulates the body to produce antibodies against proteins present on the surface of a sporozoite, but the parasite does not linger in this form for long – in half an hour, it can ensconce itself inside liver cells. There, it multiplies, and 7–10 days later emerges as 30,000 merozoite-stage parasites, each invisible to the RTS,S-induced antibodies.

This presents RTS,S with a daunting task. One sporozoite reaching the liver can result in full-blown malaria, so the vaccine must prime the body with enough antibodies to destroy every single sporozoite before they make it to the liver. It takes a lot, says Hill – around 500 times more antibodies than are produced by a meningitis vaccine. This requires the help of an adjuvant, which chemically stimulates the immune system. RTS,S consists of a region of repeating amino acids from the surface circumsporozoite protein and a hepatitis B surface antigen – a set-up that is the legacy of the vaccine's long development history. Back in 1987, researchers "couldn't make what they wanted, which is a virus-like protein with just malaria on the surface", says Hill. And although technology has improved, once a vaccine has entered clinical trials, you cannot simply overhaul the whole programme.

Hill and his colleagues are working on an alternative vaccine, R21, that has many similarities to RTS,S. R21 fuses together a hepatitis B antigen and half the circumsporozoite protein – a larger portion than in RTS,S[2]. Hill thinks that this combination will be at least as effective as RTS,S, but it is less expensive because it can be administered in doses one-fifth the size, uses more-modern production methods and has a cheaper adjuvant. A phase II trial in Burkina Faso to test efficacy in adults and children is now under way, with results expected in 2020.

Clear the hideouts

The way in which sporozoite-targeting vaccines prepare the body to mount a defence is only good for a tight window of time after infection. The immune response, therefore, needs to be rapid and effective. An alternative strategy is to target the *Plasmodium* parasite once it is inside liver cells. With this approach, any immune response will have at least five days to eliminate infected liver cells. T cells – immune cells that react to pathogens lurking in cells – can be trained to recognize proteins on the surface of infected liver cells, and kill the cells. The symptoms associated with malaria occur only after the parasite leaves the liver and starts destroying red blood cells, so eliminating infected liver cells would prevent illness and transmission.

Denise Doolan, a vaccine scientist at the Australian Institute of Tropical Health and Medicine in Cairns, has systematically evaluated all of the parasite's proteins. She looked for proteins that would be spotted by antibodies or T cells found in the blood serum of people with a tolerance to malaria. Now, she has a promising list of proteins associated with protection against malaria that could be useful for vaccine development. "We have three antigens that we have selected from many years of screening that I would love to get to the clinic," says Doolan. She is especially keen on antigens that elicit a response against both the liver and blood stages of the parasite's life cycle, and those that show potential to stimulate an immune response in multiple *Plasmodium* species.

Wiping out all *Plasmodium*-infected liver cells requires very large numbers of T cells. One way to ramp up a T-cell response is with a one–two punch: prime the immune system by injecting a circular piece of *Plasmodium* DNA or a viral vector expressing a gene found in *Plasmodium*, and then boost it with adenoviruses engineered to express the same parasite gene. The combination triggers the immune system to muster a robust T-cell response that it will remember in future. But delivering it is logistically challenging because it requires two different vaccines to be administered sequentially. Hill points to encouraging results (67% efficacy)[3] from a small trial in Kenya, in which two viral-vector injections carry an antigen from the liver stage of the parasite. But this might not be good enough – if a single infected liver cell goes undetected, a battalion of merozoites can burst out to target red blood cells.

BREAKING THE CYCLE

Plasmodium falciparum, the parasite responsible for the most severe form of malaria in people, spends a considerable part of its life cycle inside the human body. As the parasite takes on different forms and infects different parts of the body, it presents researchers with several distinct targets for vaccines (white boxes).

Parasite life cycle

1. A mosquito carrying *P. falciparum* injects a form of the parasite called a sporozoite into the bloodstream.

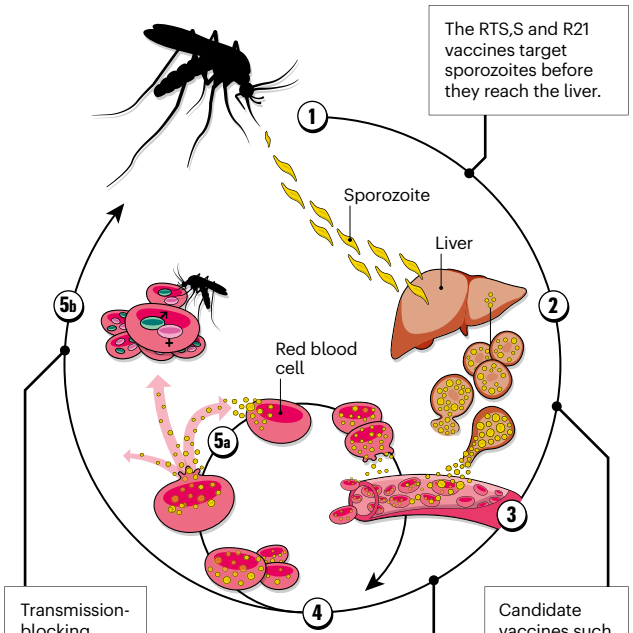
2. Sporozoites infiltrate liver cells and multiply.

3. Tens of thousands of merozoite-stage parasites burst from liver cells and enter the bloodstream.

4. Merozoites hijack red blood cells and multiply.

5a. Merozoites burst out and infect more red blood cells, releasing toxic substances that cause many of the clinical symptoms of malaria.

5b. Some merozoites mature into a sexual form. These are taken up by mosquitoes, and sexual reproduction occurs and new sporozoites are generated.



Stephen Hoffman, a malaria researcher and chief executive of biotechnology company Sanaria in Rockville, Maryland, has an idea about how to elicit a T-cell response without relying on segments of protein. In the early 1970s, research suggested that people gained immunity to *P. falciparum* if they were bitten by irradiated mosquitoes carrying sporozoites. Hoffman founded Sanaria in the 1990s to continue this work and develop a vaccine containing live, but radiation-weakened, parasites. Sanaria injected volunteers with weakened *P. falciparum* parasites that travelled to and developed inside liver cells for a few days, but then stopped replicating and did not leave the cells to cause disease. Because the parasite remained in the liver stage, immune cells learnt how to identify it in future infections.

Sanaria says that its vaccine, PfSPZ, has shown 100% protection in studies[4,5] in which adults who have not been previously exposed to malaria, mostly in the United States, are infected with *P. falciparum*. However, in endemic regions, people can be infected by other species, such as *Plasmodium vivax*. In February, Hoffman says, a trial will begin on the island of Bioko, 32 kilometres off the coast of West Africa, where malaria is endemic. The trial will involve 2,100 people between the ages of 2 and 50, and gather safety and efficacy data for regulatory approval. "I'm pretty confident that the vaccine also works," says Duffy. Duffy is involved in another PfSPZ study, to be based in Mali, which is now recruiting 900 women to assess the protection the vaccine offers during pregnancy. Hoffman is also planning a trial in Indonesia to see whether PfSPZ protects against *P. vivax*, and is trying to produce *P. vivax* sporozoites for testing in controlled human studies.

"Whole-parasite vaccines have shown significant promise, and we can make them even better," says Kappe. Rather than training the immune system to recognize and respond to one parasite protein, the live parasite teaches it about lots of antigens at once, and should, therefore, generate a considerable immune response. Kappe is also working on a live attenuated malaria vaccine. Instead of hobbling the parasite with radiation, Kappe uses gene editing. A crucial DNA-packaging protein is sabotaged using the gene-editing tool CRISPR–Cas9, creating a parasite that can enter liver cells but never leave them. "Our new parasite can infect the liver, replicate and become an antigen-making machine," says Kappe.

Kappe has shown that this strategy prevents parasites from coming out of the liver and that it triggers a potent immune response, but, so far, only for mice hosting human liver and blood cells. He and Hoffman might collaborate to insert *P. vivax* genes into attenuated *P. falciparum* sporozoites, with the aim of providing protection against both. Together, this pair of parasites accounts for 95% of malaria.

Despite their promise, live attenuated vaccines have engendered some scepticism in the malaria-vaccine community. Some suggest that their efficacy might not be as impressive in the real world as it seems in studies that deliberately expose volunteers to malaria. In these, a defined parasite strain is introduced, says Doolan, "whereas in the field you typically have hundreds of thousands of different strains of malaria and multiple species". Doolan also warns that gene-edited vaccines could "revert to virulence", shaking off the shackles of attenuation and causing malaria rather than preventing it.

Whether Sanaria can make its vaccine affordable is also a subject of debate. The manufacturing process involves infecting sterile mosquitoes with the parasite, incubating them for several weeks, and then removing their salivary glands by hand. The parasites in these tiny glands are then purified and packaged. "It's obviously a tedious process, and will make the vaccine more expensive," says Good.

"Many people say it is crazy to make a malaria vaccine in mosquitoes, but we have made vaccines in pus and poo."

But improvements in production methodology are possible. "If the vaccine works, there will be research to speed up the process," Good says. Hill takes the opposing view, saying that it is "non-manufacturable to even a few thousand people without huge expense". Storage and administration are problematic: in its current form, the vaccine must be transported in dry ice and delivered intravenously.

Hoffman brushes off cost criticisms, saying that vaccines such as Prevnar, used to protect against pneumococcal bacteria, take longer to make and require more arduous quality control. He also says that it might be possible to reduce the number of parasites required for a single vaccination. Each dose of standard PfSPZ harbours 900,000 sporozoites. But when volunteers also took antimalarial medication, a vaccine containing only 50,000 sporozoites gave superior results – all of the nine participants exposed to the parasite were protected[5].

Kappe is also confident about the prospects of live attenuated vaccines. "Many people say it is crazy to make a malaria vaccine in mosquitoes," he says, "but we have made vaccines in pus and poo."

During the life cycle of malaria parasites, the microorganisms exit the liver and hijack red blood cells. Once inside, merozoites multiply inside the cells and burst out. That can lead to seizures, severe anaemia and coma. Researchers have long targeted this part of the malarial life cycle with vaccines, but have had little success.

In the 1960s, hopes were raised when it was shown[6] that parasite load could be dampened by giving someone blood serum from an adult living in a malaria-endemic region. But trials focusing on the blood phase of the life cycle fell flat – researchers targeted vaccines at a small number of surface antigens within which merozoites have evolved extreme variability to outwit their host's immune system. "The targets of some of the earlier vaccines had a lot of genetic diversity, meaning they looked different in every parasite," says Simon Draper, an immunologist at the University of Oxford, UK.

To avoid this problem, Draper is looking at a protein that *P. falciparum* uses to harpoon red blood cells, called RH5. "We know RH5 is highly conserved. It looks nearly identical in every parasite," says Draper. His group currently targets the entire protein, but it is redesigning the molecule to elicit a stronger immune response and putting RH5 on a virus-like particle to ramp up the immune response. A trial to study safety and efficacy in UK volunteers showed highly promising RH5 antibody responses. A trial in Tanzania, where malaria is endemic, finished in July, and a second is on the cards for 2020.

In Australia, Good led the first clinical evaluation[7] of a whole-parasite blood-stage vaccine in 2018. Merozoites were grown in red blood cells, disabled with an antimalarial drug, and then injected into eight volunteers. "The volunteers all developed a good T-cell response," says Good. His group is now infecting vaccinated volunteers with malaria to see whether this approach limits the number of parasites in their blood. Again, Hill sees manufacturing problems ahead – growing parasites in blood cells will be just as difficult as carving them out of mosquitoes' salivary glands, he says.

Stopping the spread

Vaccinating against the malaria parasite is "an incredibly complex problem, like sending someone to the Moon", says Kappe. He thinks that greater investment is required, but this is not likely to be forthcoming. Parasite-targeting vaccines commonly fail to excite pharmaceutical companies – few have invested substantially in malaria-vaccine research. And there is a feeling among the researchers *Nature* spoke to that philanthropic funding organizations have begun to place

more attention on tackling the mosquitoes that carry the disease and preventing transmission, rather than priming people to fight off the parasite. Hill thinks eradication of malaria is not possible with the tools currently available, and that new vaccines are ultimately necessary. Duffy agrees: "An effort like elimination means you have to bring lots of tools together," he says. "A vaccine can be an important addition."

Vaccines could be useful in the battle to prevent transmission, too. One way to stop the parasite jumping between human and mosquito is to sabotage the parasite's sexual stage. Duffy's team has developed a transmission-blocking vaccine that targets the sexual forms that are taken up by mosquitoes when they ingest human blood cells. In an initial field trial of this type of vaccine, conducted in Mali in 2018, it proved safe[8]. Next, Duffy is planning to report the results of a trial of a vaccine that combines Pfs230, a protein on the outside of the parasite gamete, with GlaxoSmithKline's adjuvant ASO1, which is included in the RTS,S vaccine to boost T-cell response. A major challenge with these transmission-blocking vaccines, however, will be developing the methods to measure any reduction in malaria transmission in the field, and to prove that the vaccines work.

Hill doubts that a stand-alone transmission-blocking vaccine will ever emerge, but can see it being part of a multi-component vaccine. A combination vaccine that includes a transmission blocker could be more effective and practical.

The need for a malaria vaccine is not diminishing, and researchers are optimistic. "We can see the top of the mountain now," says Kappe of his team's work. Although scientists might not agree on the best approach to take, they can see progress being made – not least the wide distribution of the RTS,S vaccine. "Our best products will be combined in different ways," says Duffy. "RTS,S has found a very specific role to reduce clinical malaria in children." It might not be perfect, Hill says, but malaria is such a horrendous problem that even a partially effective vaccine could make a big difference.

Anthony King is a freelance science writer based in Dublin.

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Q&A

Jeffrey Bethony: Taking on worms

OLIVIA ANDERSON/GW SCHOOL OF MEDICINE AND HEALTH SCIENCES

About two billion of the world's poorest people are infected with parasitic worms. Treatments are available, but Jeffrey Bethony, a microbiologist at George Washington University in Washington DC, explains why only vaccines can eradicate infection.

Why is it more difficult to develop a vaccine for parasites than for many viruses?
Parasites go through a series of life stages and occupy several different niches in the body. They've also developed clever mechanisms to evade the immune system. So parasitic infections are the ultimate challenge.

Which diseases caused by parasitic worms do we most need a vaccine for?
Schistosomiasis results in the greatest disease burden, especially in sub-Saharan Africa and Brazil. There is an effective treatment, praziquantel, but without a vaccine we can't prevent reinfection, so it has proved impossible to eliminate the parasite in low-income countries. And more than 500 million people are infected with hookworms, which can impair physical and mental development.

What progress are you making on a schistosomiasis vaccine?
We are developing a candidate vaccine based on proteins found on the outer surface of the worms. We're currently running a phase II trial in Uganda, funded by the US Department of Defense, that targets a fragment of such a protein on the worm *Schistosoma mansoni*. A group at Leiden University Medical Centre in the Netherlands is working on a controlled human infection model, or CHIM, for schistosomiasis. This approach allows researchers to give people an experimental vaccine and then challenge them with a dose of pathogen. This would allow us to use fewer volunteers and reduce the costs of trials.

What vaccine strategies are you developing against hookworms?
We have developed two subunit vaccines, each containing a protein given with an immune stimulant. One protein degrades haem, a component of the blood protein haemoglobin. Haem is potentially toxic, and antibodies against the degradation protein reduce the worm's ability to eliminate haem from its blood meals. The other protein prevents hookworms from breaking down haemoglobin for consumption. We have done separate phase I trials of our two vaccines in the United States, Brazil and Gabon, and have just received funding to test both proteins using a controlled human infection model in endemic areas of Brazil. We then plan to test the simultaneous delivery of both proteins in a single vaccine.

So a CHIM study involves injecting healthy people with parasites?
Yes. We borrowed the idea from malaria researchers. I immunize people against hookworms by administering the proteins in the subunit vaccines, and then challenge the volunteers with hookworms. If the vaccines don't work, we can get rid of the infection with drugs. If we had to wait for people to get infected, studies would take longer and cost more; CHIM studies accelerate vaccine development.

Isn't it tricky to get people to volunteer to be infected with a parasitic worm?
We have no problem getting volunteers. There are lots of people who think that hookworms, because they can modulate the immune system, can be therapeutic for coeliac disease, Crohn's disease or irritable bowel syndrome. That strategy is being trialled by other researchers now. We suspect that's why some people volunteer.

Do you have any problems getting funding?
People who need a vaccine against parasitic worms can't afford to pay hundreds of dollars for it. So there's not lots of money spilling around. Malaria is better funded than disease caused by parasitic worms – it's considered more important. Malaria researchers are usually one or two steps ahead of us. But success with a malaria vaccine would help all of us. If they can do it, so can we.

By Anthony King
This interview has been edited for length and clarity.