

Frequently Asked Questions (FAQs)

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Live Attenuated Malaria Vaccine Designed to Protect through Hepatic CD8+ T Cell Immunity

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Q: What do you mean by a “whole-parasite” malaria vaccine?

A: Most malaria vaccines under development today consist of recombinant or genetically engineered proteins that represent perhaps one, two, or three of the over 5000 proteins expressed by the parasite. Instead of using a part of the parasite, the PfSPZ Vaccine is composed of the entire whole-organism. Thus, it can induce immunity against hundreds to thousands of proteins, not just the one to several proteins in a recombinant vaccine.

Q: Why are you working on the “whole-parasite” approach when it is old fashioned and difficult?

A: Our goal is to produce a vaccine that prevents infection in more than 80% of recipients for at least 6 months and preferably more than two years. The whole parasite, sporozoite approach is that only approach to malaria vaccines that has ever produced this type of potent, sustained, protective immunity. This is why we are using the whole parasite approach.

Q: What is a sporozoite?

A: In the multi-stage life cycle of the malaria parasite, the sporozoite is the form of the parasite that is concentrated in the salivary glands of an infected mosquito and that is introduced into a person's blood when the mosquito bites.

Q: How does the vaccine work?

A: The vaccine candidate uses *Plasmodium falciparum* sporozoites (PfSPZ) that are extracted from the salivary glands of irradiated, infected mosquitoes. Irradiation makes the parasite unable to multiply or colonize red blood cells and cause malaria. Following injection with the weakened sporozoites comprising the PfSPZ Vaccine, the sporozoites invade liver cells where they stimulate protective cytotoxic or killer CD8+ T cell based immunity.

Q: What evidence do you have that this vaccine will work?

A: The evidence that this approach can work is based on many human studies conducted from the 1970s through the 1990s in which irradiated, infected mosquitoes fed on volunteers (delivering live irradiated whole sporozoites). Thirteen of fourteen volunteers who had been bitten by more than a thousand irradiated, infected mosquitoes, were then protected from getting malaria when bitten by mosquitoes inoculating normal, infectious malaria parasites.

Q: Why was this approach not pursued earlier?

A: The process for the manufacture of the vaccine was not developed previously, as it was considered impossible to produce a vaccine in mosquitoes that met regulatory (FDA) standards including purity, sterility, safety and potency.

Now, after years of research and development, and with support from PATH Malaria Vaccine Initiative (from a Bill and Melinda Gates Foundation Grant), the US National Institute of Allergy and Infectious Diseases, and the U.S. Military Infectious Diseases Program, Sanaria has developed a process for manufacture that produces purified, sterile, potent, and attenuated (weakened) sporozoites.

Q: Will individuals in endemic areas such as Africa need to be vaccinated every year?

A: We don't know how long the protection will last. We only know that it lasts *at least* 10 months. It may be that natural exposure to malaria will boost the immunity without any booster doses of the vaccine.

Q: What did the clinical trial test for and in how many volunteers?

A: The trial tested the vaccine for safety, immunogenicity and protective efficacy in 80 adult US volunteers. In this trial the vaccine was administered in the skin, either intradermally or subcutaneously.

Q: Can the vaccine be used to treat malaria?

A: The vaccine is used to *prevent* malaria. Antimalarial drugs are used to treat malaria.

Q: Isn't it true that manufacturing this vaccine is impossible? Won't you need high-security insectaries to breed swarms of mosquitoes as well as hundreds of technicians to dissect each mosquito for sporozoites?

A: No, this is not true. Manufacturing of PfSPZ Vaccine that meets rigorous FDA regulatory standards has been accomplished. After years of testing, Sanaria has developed a process for manufacture that produces sterile, purified, potent, and attenuated sporozoites that are collected in large numbers from the salivary glands of laboratory-reared mosquitoes in a process that adheres to current Good Manufacturing Practices.

Manufacture of this vaccine has been demonstrated to be feasible and scalable. This type of effort is similar to many other high-quality, precision manufacturing processes. We are confident that with a highly protective vaccine in hand, it will be straightforward to manufacture vaccine to meet demand.

Q: Isn't the storage method of the vaccine, frozen in liquid nitrogen vapor phase (LNVP), a method that is difficult, impossible to handle, and unrealistic for vaccine distribution to children in Africa?

A. No, none of this is true. In reality, multiple vaccines used for veterinary purposes in Africa (and several in the US and other countries) are stored using liquid nitrogen technology (e.g. East Coast Fever, Marek's Disease) and are widely distributed in the field. In pilot studies we have already transported the PfSPZ Vaccine to clinics in several African countries and then back to Sanaria, where it was shown to have retained potency. A LNVP storage system provides a highly stable environment and is easy to use. Convenient-sized LNVP transport tanks distributed to clinics are very reliable and safe. Additionally, vaccines stored and transported this way have several advantages over conventional refrigeration methods, which are dependent on electricity: the LNVP 'cold chain' has the potential to be more reliable, require less maintenance and result in less vaccine wastage. It is also more environmentally friendly.

Q: How much will this vaccine cost?

A: At this time, we do not know how much this vaccine will cost. Sanaria is dedicated to producing a vaccine that will be affordable and accessible for geographically focused malaria parasite elimination campaigns in Africa, and is confident that the cost will allow for this indication.

Q: How can you guarantee that the sporozoites still work after they are thawed?

A: We have an assay that assesses the potency of the vaccine. This assay has repeatedly shown that thawed sporozoites are potent. We have also shown in a mouse malaria model system that immunization with cryopreserved, radiation-attenuated sporozoites generates very high levels of protective immunity.

Q: When will the vaccine be available for use?

A: We don't know exactly. This will depend on the results of subsequent trials and discussions with regulatory authorities. However, planning has started to use the vaccine to assess the capacity to eliminate *Plasmodium falciparum* malaria from geographically focused areas in 2-3 years.

Q: Is there a risk of contracting malaria from this approach?

A. All current, attenuated, whole infectious agent vaccines, such as the Sabin oral polio vaccine, are associated with a very small risk of infection. During the past 20 years, no volunteer immunized with attenuated sporozoites by the bite of infected mosquitoes has developed malaria. We have developed laboratory tests that indicate that the vaccine is safe. None of the immunized volunteers in the first clinical trial with the vaccine trial developed malaria. Based on these prior data we are confident that the vaccine will not cause malaria.

Q: How will you keep the vaccine pure and sterile?

A: One of Sanaria's major accomplishments has been to produce a sterile, purified vaccine in accordance with current Good Manufacturing Practices. Once the vaccine is vialled in the bottle, it is kept sterile with the appropriate closure and stored at a low temperature using the vapor phase of liquid nitrogen.

Q: How do you know that all of the sporozoites are adequately de-activated? Specifically, how do you know whether each of the thousands of mosquitoes has received the planned dose of radiation?

A: We have worked hard to determine very accurately the highest and lowest doses of irradiation that any mosquito could possibly have received. We worked with consultant radiation physicists and the staff of the National Institute of Standards to identify and design an irradiator and the containers in which the mosquitoes are irradiated. Every time the mosquitoes are irradiated the National Institute of Standards actually measures the doses of irradiation to which the mosquitoes have been exposed. Thus, we do know the highest and lowest amount of irradiation that any one mosquito could have received. In the trial, we injected millions of attenuated sporozoites into the volunteers, and saw no “breakthrough” malaria, even though a single inadequately weakened parasite could cause disease.

Q: Is the radiation safe for humans?

A: Only the mosquitoes are irradiated. There is no evidence that after the mosquitoes have been exposed to radiation, there are residual, radioactive molecules passed through during the production and final bottling of the vaccine. Therefore, we do not believe that there is any radiation present in the vaccine. Irradiation is used as a safe and effective technology for preventing food-borne diseases. International health and safety authorities have endorsed the safety of irradiation for all foods up to a dose level of 10,000 Gy. A much lower dose of irradiation, only 150 Gy, is used to irradiate the mosquitoes carrying PfSPZ.

Q: Do other commonly used vaccines use radiation?

A: To our knowledge, no other commonly used vaccines use irradiation.

Q: Is there a risk that other, unidentified pathogens might be delivered with the vaccine since you are using parasites that have been grown in human erythrocytes (i.e. in cultures of infected human, red blood cells)?

A: The human red blood cells are rigorously tested before use. They meet the same standards as the blood used for transfusion in hospitals. The vaccine is tested after bottling and before use to determine if it contains any contaminating microorganisms or pathogens. Any vaccine lot that would contain any contaminants or pathogens would be discarded. Thus, it is extremely unlikely that the vaccine could contain unidentified pathogens. Nonetheless, this possibility cannot be excluded, just as it cannot be totally excluded for other drugs and vaccines. However, the US Food and Drug Administration’s regulations and review process dramatically reduce the chance of using a contaminated vaccine.

Q: Why is a malaria vaccine so valuable for the U.S. military?

A: Developing a malaria vaccine to protect troops deployed to malaria endemic areas remains a top priority for the Department of Defense. During the past 150 years in every campaign in which the U.S. military was involved where malaria was intensely transmitted there have been more casualties (person days lost) from malaria than from hostile fire.

Q: Why is Sanaria’s approach to vaccine development of such importance for the military?

A: A vaccine for the military must be highly effective, effective enough so that a vaccination regimen can replace continuous chemoprophylaxis in the field, which is often difficult to maintain due to compliance in taking a drug consistently for a prolonged period of time. The approach being pursued by Sanaria capitalizes on prior clinical studies using radiation-attenuated sporozoites delivered by mosquito bites that achieved sustained sterile protection exceeding 90%.

Q: What role has the Department of Defense played in the development of this vaccine?

A: The Naval Medical Research Institute, the predecessor of Naval Medical Research Center (NMRC), was involved in the 1970s in the original studies in which irradiated sporozoites were introduced by mosquito bite. Then, in the 1980s and 1990s NMRC and the Walter Reed Army Institute of Research (WRAIR) conducted numerous studies that helped to establish the model of protection by attenuated sporozoites. In the current trial, NMRC served as one of the two clinical testing sites and NMRC investigators played an important role in the immunological studies reported in the *Science* paper.

Q: What role has the University of Maryland Center for Vaccine Development (UMD-CVD) played in the development of this vaccine?

A: UMD-CVD was involved in the 1970s in the original studies in which irradiated sporozoites were introduced by mosquito bite. Then, in the 1980s and 1990s UMD-CVD conducted studies that helped to establish the model of protection by attenuated sporozoites. In the current trial, UMD-CVD served as one of the two clinical testing sites and UMD-CVD investigators conducted the parasite PCR studies reported in the *Science* paper.

Q: What role has the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Disease (NIAID) played in the development of this vaccine?

A: The mission of the Vaccine Research Center (VRC) is to conduct research that facilitates the development of effective vaccines for human disease. The VRC uses basic research to establish mechanisms of inducing long-lasting protective immunity against HIV and other pathogens that present special challenges to vaccine development, which includes laboratory analysis, animal testing, and clinical trials of such candidates. The VRC conducted the non-human primate studies reported in the *Science* paper as well as T cells studies during the clinical trial.

Q: What is Sanaria?

A: Sanaria is a biotechnology company dedicated entirely to malaria vaccine development. Sanaria was founded in 2003 with a primary mission to develop, license, and commercialize a whole-parasite (sporozoite stage) vaccine to prevent malaria.