

Genetically attenuated malaria parasites (GAP) as a vaccine

Focusing on research performed in the Leiden Malaria Research Group, LUMC, The Netherlands (2005-2024)

What is the current status of a GAP-based vaccine (and future directions)?

Second generation GAP vaccine (GA2; 2018-2025)

The second generation genetically attenuated parasite, GA2, arrests growth late during development in the liver, compared to GA1, which arrest early during liver-stage development.

- In January 2025 the results of the second, follow up, clinical trial with GA2 were published in Nature Medicine. In this small randomized controlled clinical trial, a single immunization for malaria using GA2 parasite-infected mosquitoes showed unprecedented 90% protective efficacy.
- The results of a second, follow up, clinical trial with GA2 were presented at the meeting of the American Society for Tropical Medicine and Hygiene (ASTMH) New Orleans (USA) in November 2024. In this small randomized controlled clinical trial, a single immunization for malaria using GA2 parasite-infected mosquitoes showed unprecedented 90% protective efficacy.
- In November 2024 the results of the first (small) clinical trial comparing safety and protective immunity of GA2 and GA1 were published in N Engl J Med. GA2 was safe and had a strongly increased efficacy compared to GA1. 89% of GA2-immunized volunteers were protected compared to 13% of the GA1-immunized volunteers
- In November 2022 the results of the small clinical trial comparing safety and efficacy of GA2 with GA1 were presented at the meeting of the American Society for Tropical Medicine and Hygiene (ASTMH) in Seattle (USA). The results showed that GA2 was safe and had a strongly increased efficacy compared to GA1 after immunization of human volunteers (by delivery of GA2 and GA1 through bites of infected mosquitoes). 89% of GA2 immunized volunteers were protected compared to 13% of the GA1 immunized volunteers.
- In 2022 a paper from the Leiden group was published in [NPJ Vaccines](#) describing the generation and pre-clinical characterization of the genetically attenuated parasite PfΔmei2 (GA2) with a growth arrest late in the liver.

- Beginning of 2021 ethical and medical approval for a clinical trial using GA2 in test subjects/volunteers in the Netherlands was gained from the Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek; CCMO; NL75577.000.21).
- December 2020: approval was gained for the use of a second generation GAP, GA2 (PfΔmei2) in humans by the Dutch Ministry of Infrastructure and the Environment (GGO IM-MV 20-018). This second generation genetically attenuated parasite (GA2) arrests growth late during development in liver compared to GA1, which arrest early during liver-stage development. The late growth arrest of the parasites in the liver broadens the array of antigens displayed to the immune system and extends the duration of parasite exposure to the immune system (compared to GA1, which arrest early during liver-stage development).
- 2018-2020: Studies started to create a so-called 'second generation GAP' (GA2), a GAP with increased immunogenicity compared to the first generation GAP (PfSPZ-GA1; GA1 vaccine; see below).

First generation GAP vaccine (GA1; 2008-2020)

- In May 2020 the results of the PfSPZ-GA1 (GA1 vaccine) clinical trial were published in Science Translational Medicine. The study shows that the GA1 vaccine is safe and elicits an immune response against a malaria infection, although protection against infection was not complete. The measured immune responses and demonstrated safety were strong incentives to further develop a vaccine based on genetically attenuated malaria parasites. More information on [YouTube](#) .
- In November 2017 PfSPZ-GA1 vaccine efficacy trial (part B) was initiated in human volunteers at the LUMC (Leiden, the Netherlands) and RadboudMC (Nijmegen, the Netherlands). In a joint clinical study, volunteers in LUMC and RadboudMC received injections of the PfSPZ GA1vaccine made from genetically modified parasites/sporozoites
- In 2017 a PfSPZ-GA1 vaccine safety trial (part A; dose escalation) was successfully completed in human volunteers in the LUMC (Leiden, the Netherlands). The vaccine - a first in the world for an injectable, malaria vaccine consisting of live, genetically attenuated parasites/sporozoites - was safe and well tolerated, no volunteers developed a symptomatic/pathogenic blood stage infection. More information on the [NOS/ Nieuwsuur website](#).
- In beginning of 2017 ethical and medical approval to perform a clinical trial using PfSPZ-GA1 vaccine in test subjects/volunteers in the Netherlands was gained from the Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek; CCMO NL55657.000.16).

- In 2016 approval was gained for use of genetically attenuated parasites, PfSPZ-GA1, in humans by the Dutch Ministry of Infrastructure and the Environment (GGO IM-MV 15-004 and GGO IM-MV 15-009).
- In 2015 the biotechnology company Sanaria generated an aseptic, purified, vialled, cryopreserved formulation of the GAP PfΔslarpΔb9, termed PfSPZ-GA1 vaccine.
- 2008-2013: As part of a TI-Pharma funded project the LUMC, RadboudMC and the American biotechnology company [Sanaria](#) , created a genetically attenuated parasite (GAP; PfΔslarpΔb9). This human GAP and the equivalent rodent GAP has been evaluated using a set of preclinical safety and efficacy studies, showing complete growth arrest of this GAP in the liver.

GAPs explained

What is a genetically attenuated parasite (GAP) malaria vaccine?

- A GAP vaccine consists of live but genetically attenuated malaria parasites (sporozoite stage).
- Attenuation of the parasites (sporozoites) is achieved through genetic modification, i.e. by deleting essential genes from the genome of the malaria parasite.
- The loss of these genes from the parasite genome should ensure complete growth arrest of the parasite in the liver. An malaria infection starts with parasites injected into the blood through the bite of an infected mosquito. The parasites injected by the mosquito, the sporozoite stage, first develop in the liver (a non-pathogenic stage) before developing into the pathogenic blood stages that multiply in the blood (inside red blood cells).
- The Leiden Malaria Research Group (LUMC, The Netherlands) is at the forefront of global research focused on using live, genetically attenuated parasites (sporozoites) as a potential malaria vaccine. Notably, the Leiden Malaria Research Group were one of the first groups to develop the concept of immunization with GAP (in 2005), showing that GAP-based immunity can effectively protect mice against an malaria infection.

How would a GAP-based vaccine work?

- A GAP vaccine consists of live but genetically attenuated parasites (so-called sporozoites). These sporozoites are able to invade the liver but are unable to produce the infectious 'liver-merozoites' that establish the pathogenic phase of infection of malaria parasites in the blood.
- Vaccination with these genetically attenuated sporozoites should induce immune responses that protect against re-infection with malaria parasites after a bite of an

infected mosquito. Specifically, these immune responses should kill the sporozoites and/or liver-stage parasites (that are introduced by a mosquito bite) before parasites can leave the liver into the blood and therefore preventing the pathogenic blood-stage infection.

What are the benefits of vaccination with live, attenuated parasites over subunit vaccines targeting only one or a few parasite proteins?

- Subunit vaccines (protein, viral-vectored, mRNA vaccines) are easier and cheaper to produce, and generally easier to store and administer than vaccines based on live, attenuated parasites. However, until now (2025) subunit vaccines do not that induce high-level (>90%) and long-lasting protective immunity in humans.
- It has been shown in multiple studies that immunization with live sporozoites, either attenuated (by radiation) or administered by mosquito bite in the presence of anti-*Plasmodium* chemoprophylaxis, can induce high-level protective immune responses (>90%) in humans.

Why use sporozoites that have been attenuated by genetic modification?

- It has been shown in rodent models of malaria that immunization of mice with genetically attenuated parasites/sporozoites (GAP) can produce protective immune responses equal to, or even greater, than is produced by sporozoites attenuated by radiation (RAS sporozoites).
- Sporozoite attenuation by genetic modification rather than by radiation, offers the advantage of a more homogeneous product, increased biosafety for sporozoite production, and potentially increased potency/immunogenicity.
- GAP can be further genetically modified to increase their immunogenicity, for example by introducing genes encoding molecules that can optimize their recognition by the immune system.

Additional (background) information

Plasmodium falciparum is the human parasite responsible for the vast majority of malaria associated morbidity and mortality; with over 200 million people infected resulting in an estimated > 500.000 deaths per year. Years of testing multiple (recombinant) subunit vaccines, designed to target a variety of parasite antigens, have failed so far to produce subunit vaccines that induce high-level (>90%) and long-lasting protective immunity in humans, consequently an interest in vaccination with live-attenuated parasites.

It has been shown that high-level (>90%) protection in humans can be achieved through immunization with live sporozoites; either by immunization with sporozoites attenuated by radiation that developmentally arrest in the liver or by sporozoites administered by mosquito bite in the presence of anti-*Plasmodium* chemoprophylaxis.

Immunization studies with live sporozoites attenuated by genetic modification (so-called **genetically attenuated parasites, GAP**) have gathered attention as they have been shown to produce protective immune responses in rodent models equal to, or even greater than, those produced by immunization with sporozoites attenuated by radiation. Sporozoite attenuation by genetic modification rather than by radiation, offers the advantage of a more homogeneous product, increased biosafety for sporozoite production, and potentially increased potency/immunogenicity. In addition, GAP can be further genetically modified to increase their immunogenicity, for example by introducing genes that encode molecules that can optimize their recognition by the immune system.

The Leiden Malaria Research Group (LUMC, The Netherlands) is at the forefront of international research on malaria vaccine development using live, genetically attenuated sporozoites for immunization and was one of the first groups to develop the concept of immunization with GAP and showed that GAP-based immunity can effectively protect mice against an malaria infection ([van Dijk et al., 2005](#)). The malaria group of the LUMC have translated these findings from murine/rodent models of malaria into the generation of human malaria *Plasmodium falciparum* GAPs (GA1 and GA2). The LUMC hold one of the first patents on the use of GAP for vaccination. Collectively, the Leiden Malaria group are experts in *Plasmodium* genetic modification, immunization and vaccination studies in both rodent malaria models and human malaria and have used their expertise to generate GAP and to develop robust pre-clinical and clinical screening protocols to evaluate their suitability for vaccination.

The development of GAP vaccines in the Leiden Malaria research group is the culmination of studies that were first initiated here in the mid-90s:

- The Leiden Malaria research group (LUMC, The Netherlands) was the first group to develop genetic modification in malaria parasites (2 papers published in Science in [1995](#) and [1996](#)).
- Through analysis of gene-deletion parasite mutants in rodent malaria models, it was discovered that it was possible to create genetically attenuated parasites (GAP), which were able to invade the liver but were unable to proceed into the pathogenic blood stage infection. Importantly mice infected (immunized) with these live-attenuated parasites developed protective immunity against an infection with wild type parasites (published in PNAS; [van Dijk et al., 2005](#)).
- In 2008 the Leiden group started to work on translating these findings into a human GAP vaccine against malaria. This was performed in collaboration with the RadboudMC (Nijmegen, The Netherlands) and the US biotechnology company [Sanaria](#), and was supported by a grant provided by Top Institute Pharma (TI-Pharma). This involved testing and refining different live genetically-attenuated parasites and demonstration of necessary pre-clinical safety and protective efficacy

standards for use of GAP in humans, resulting in the generation of the GAP Pf Δ slarp Δ b9.

- In 2015 the biotechnology company Sanaria generated an aseptic, purified, vialled, cryopreserved formulation of the GAP Pf Δ slarp Δ b9, termed PfSPZ-GA1.
- In 2017 (in a joint clinical study) volunteers in LUMC (Leiden) and RadboudMC (Nijmegen) received injections of the GAP vaccine, PfSPZ GA1, - a first in the world for an injectable, malaria vaccine, consisting of live, genetically attenuated parasites/sporozoites.
- In 2020 the results of this first clinical trial were published in [Science Translational Medicine](#). The study showed that the vaccine was safe and elicited strong immune response against a malaria infection. Immunization with the GAP PfSPZ-GA1 did not lead to blood infection (and therefore does not cause malaria symptoms). Although PfSPZ-GA1 immunization induced strong immune responses, it did not result in complete protection against a malaria infection. According to the researchers, the measured immune responses and demonstrated safety were strong incentives to further develop a vaccine based on genetically attenuated malaria parasites.
- 2018-2020: Studies started in the Leiden group to create a so-called 'second generation GAP' (GA2), a GAP with increased immunogenicity compared to the first generation GAP (PfSPZ-GA1; GA1 vaccine). One of the likely ways to improve the immunogenicity of GAP vaccines is to broaden the array of antigens displayed to the immune system and by extending the duration of parasite exposure to the immune system. In mouse models of malaria, GAP that arrest growth late during development in the liver (late-arresting GAP; LA-GAP) have indeed proven to induce significantly higher protective immune responses as compared to early growth-arresting GAP (EA-GAP; such as the first generation GAP) or to radiation-attenuated sporozoites, most likely resulting from increased antigen breadth and biomass of an LA-GAP. The studies in the Leiden group therefore focused on generating a late-arresting GAP for the human malaria parasite *P. falciparum*.
- In November 2021 a small safety and efficacy trial was initiated with the second generation GAP, GA2 (a GAP with a late growth-arrest in the liver) in volunteers at the LUMC (Leiden). Volunteers were infected and immunized with GA2 via mosquito bite delivery of attenuated sporozoites. For comparison, a group of volunteers was immunized with GA1 via mosquito bite delivery of attenuated sporozoites.
- In 2022 a paper from the Leiden group was published in [NPJ Vaccines](#) describing the generation and pre-clinical characterization of the genetically attenuated parasite Pf Δ mei2 (GA2) with a growth arrest late in the liver.
- In November 2022 the results of the small clinical trial comparing safety and efficacy of GA2 and GA1 were presented at the American Society for Tropical Medicine and Hygiene (ASTMH) meeting in Seattle (USA). GA2 was safe and had a

strongly increased efficacy compared to GA1. 89% of GA2-immunized volunteers were protected compared to 13% of the GA1-immunized volunteers.

- In November 2024 the results of the first (small) clinical trial comparing safety and protective immunity of GA2 and GA1 were published in N Engl J Med. GA2 was safe and had a strongly increased efficacy compared to GA1. 89% of GA2-immunized volunteers were protected compared to 13% of the GA1-immunized volunteers.
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More information about vaccination with live attenuated parasites (radiation and genetically attenuated parasites) can be found in the following papers:

- A [review](#) in Nature Review Immunology on how to perform experimental malaria infections in humans, i.e. so called Controlled Human Malaria Infections (for testing malaria vaccine efficacy in humans).
- A New England Journal of Medicine [paper](#) showing induction of complete protective immunity in humans by sporozoite inoculation after only a limited number of mosquito bites.
- A [review](#) on human immunization trials performed using radiation-attenuated sporozoites administered by mosquito bite.
- A Science paper demonstrating protective immunity in humans after immunization with radiation-attenuated and cryopreserved sporozoites administered by intravenous injection.
- A PNAS [paper](#) (from the Leiden Malaria Research Group) was one of the first proof-of-concept studies demonstrating that sterile immunity could be achieved using genetically attenuated sporozoites in rodent models of malaria.
- A [paper](#) (from the Leiden Malaria Research Group) in Vaccine describing the preclinical testing of vaccine candidates consisting of genetically attenuated parasites.
- A review [paper](#) (from the Leiden Malaria Research Group) on considerations behind and the progress in developing genetically attenuated sporozoites suitable for vaccination in humans.
- [FASEB J](#) and [eLIFE](#) papers describing the generation and pre-clinical characterization of the human GAP vaccine candidate PfΔslarpΔb9.
- A Science Translational Medicine [paper](#) describing the results of the first-in-human safety and efficacy trial with a genetically attenuated malaria vaccine PfSPZ-GA1 (PfΔslarpΔb9)
- An NPJ Vaccine [paper](#) (from the Leiden Malaria Research Group) describing the generation and pre-clinical characterization of the genetically attenuated parasite PfΔmei2 (GA2) with a growth arrest late in the liver
- A [review](#) in Expert Rev Vaccines on the use of attenuated sporozoites as a vaccine against malaria
- A N Engl J Med [paper](#) describing the results of the first-in-human safety and efficacy trial with a genetically attenuated malaria vaccine GA2, a second generation GAP (PfΔmei2)
- A Nature Medicine [paper](#) describing results of the second, follow up, clinical trial with GA2 showing in a small clinical trial that a single immunization with GA2 results in an unprecedented 90% protective efficacy.