Leids Universitair Medisch Centrum

Genetically attenuated malaria parasites (GAP) as a vaccine

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

Second generation GAP vaccine

- December 2020: approval was gained for use of a second generation GAP, GA2, 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 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', a GAP with increased immunogenicity compared to the first generation GAP (PfSPZ-GA1; GA1 vaccine; see below).

First generation GAP vaccine

- In May 2020 the results of the PfSPZ-GA1 (GA1 vaccine) clinical trial are published in Science Translational Medicine. The study shows that the GAP vaccine is safe and elicits an immune defense response against a malaria infection, although protection was not complete. The measured immune responses and demonstrated safety are 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 volunteers at the LUMC and RadboudMC. In a joint clinical study, 67 volunteers in Leiden and Nijmegen received injections of the vaccine made from the genetically modified parasite (called PfSPZ GA1) - a first in the world for an injectable, genetically weakened malaria vaccine. A high and a low dose were administered.
- In 2017 PfSPZ-GA1 vaccine dose escalation safety trial (part A) was successfully completed in volunteers in the LUMC. The vaccine was safe and well tolerated, no volunteers developed a blood stage infection
  More information on the Nieuwsuur website
- In beginning of 2017 the ethical and medical approval to perform a clinical trial using PfSPZ-GA1 in test subjects 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 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 SANARIA has generated an aseptic, purified, vialed, cryopreserved formulation of PfΔslarpΔb9, termed PfSPZ-GA1
- 2008-2013: As part of a TI-Pharma funded project the LUMC, RadboudMC and the American 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

GAPs explained

(see also below for more detailed information and references)

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

- A GAP vaccine consists of live but attenuated parasites
- GAP attenuation 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 must ensure the complete arrest of the parasite in the liver.
- The Leiden Malaria Research Group is at the forefront of global research focused on using live-parasites as a potential malaria vaccine. Notably, the LUMC were one of the first groups to develop the concept of immunization with GAP, showing that GAP based immunity can effectively protect mice against malaria.

How would a GAP-based vaccine work?

- A GAP vaccine consists of live but attenuated parasites (so-called sporozoites). These sporozoites are able to invade the liver but are unable to produce the infectious 'merozoites' that can establish a pathogenic blood stage infection.
- Vaccination with these sporozoites induces immune responses that protect the host from re-infection. Specifically, these immune responses kill the sporozoites that are introduced by a mosquito before they get into the liver or in the liver and therefore they prevent the pathogenic blood-stage infection.

What are the benefits of vaccination with live-parasites over subunit vaccines (which consist of only specific parasite proteins)?

- Subunit vaccines are easier and cheaper to produce, and generally easier to store and administer than vaccines based on live parasites.
- However, until now no subunit vaccine has been produced that induces high-level protective immunity in humans that is comparable to that achieved through immunization with live, attenuated sporozoites.
- Immunization with live sporozoites that have either been attenuated by radiation or have been administered by mosquito bite in the presence of anti-Plasmodium chemoprophylaxis induces strong protective immunity.
- The RUNMC malaria group in Nijmegen (The Netherlands) has demonstrated that complete and long-lived immunity in humans can be achieved with only low numbers of sporozoites (i.e. limited numbers of infected mosquito bites) administered in the presence of antimalarial prophylaxis.

Why use sporozoites that have been attenuated by genetic modification and not by other means, for example by radiation?

- It has been shown in rodent models of malaria that GAP sporozoites can produce protective immune responses equal to, or even greater, than is produced by sporozoites that are attenuated by radition (RAS sporozoites).

- GAP vaccines constitute a homogeneous parasite population with a distinct genetic identity, and their attenuation is not dependent upon external factors.
- 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.

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 > 400.000 deaths per year. Years of testing a large number of (recombinant) subunit vaccines, designed to a variety of parasite antigens, have all have failed so far to induce sterile and long-lasting protective immunity in humans, consequently renewing an interest in vaccination with live-attenuated parasites.

Indeed, high-level (>90%) protection in humans has been achieved through immunization with live attenuated parasites; either based on immunisation with live irradiated sporozoites that developmentally arrest in the liver or via sporozoites administered by mosquito bite in the presence of anti-Plasmodium chemoprophylaxis. Indeed the RUNMC malaria group in Nijmegen (The Netherlands) first demonstrated that immunisation with sporozoites administered under antimalarial prophylaxis can induce sustained sterile immunity in humans.

Immunisation studies with live sporozoites attenuated by genetic modification have gathered attention as they have been shown to produce protective immune responses equal to, or even greater than, those produced by irradiated sporozoites in rodent models. These so-called **genetically attenuated parasites (GAP)** offer several advantages over radiation-based attenuation as they constitute a homogeneous population with a distinct genetic identity, and their attenuation is not dependent upon external factors (e.g. radiation, host drug metabolism).

The Leiden Malaria Research Group is at the forefront of international research on malaria vaccine development using live-parasites for immunisation. The LUMC were one of the first groups to develop the concept of immunization with GAP and showed that GAP based immunity can effectively protect mice against malaria (van Dijk et al., 2005). The malaria groups of the LUMC and RUNMC have translated these findings from murine malaria into the generation of a human, *P. falciparum*, GAP (2008-2017 PfSPZ-GA1). The LUMC together with RUNMC hold one of the first patents on the use of GAPs for vaccination. Collectively the LUMC and RUMC are experts in *Plasmodium* genetic modification, immunization and protection studies in both rodents and humans malaria and have combined to generate GAP and to develop robust pre-clinical screening protocols to evaluate their suitability for vaccination.

As part of a TI-Pharma funded project (2008-2014) RUNMC and LUMC along with their American industrial partner, SANARIA , have created a new human GAP (PfΔslarpΔb9), where 2 genes have been removed from the parasite genome in order to ensure complete liver-stage arrest. This is the first human GAP where 2 genes (slarp and b9) governing critical, but independent, cellular process have been deleted. This human GAP and the equivalent rodent GAP has been evaluated in both preclinical safety and efficacy studies. Moreover, this GAP was generated using constructs that permitted the removal of the drug selectable that not only permitted the generation of multiple gene deletion mutants but also addresses safety issues concerning the presence of heterologous DNA in genetically modified organisms used in human immunization. In November 2017 PfSPZ-GA1 vaccine efficacy trial (part B) was initiated in volunteers at the LUMC and RadboudMC. In a joint clinical study, 67 volunteers in Leiden and Nijmegen received injections of the vaccine made from the genetically modified parasite (called PfSPZ GA1) - a first in the world for an injectable, genetically weakened malaria vaccine. A high and a low dose were administered. In May 2020 the results of the PfSPZ-GA1 (GA1 vaccine) clinical trial are published in Science Translational Medicine. The study show that the GAP vaccine is safe and elicits a defense response against a malaria infection. It was observed that the vaccinated volunteers developed an immune defense against a malaria infection, although this protection was not complete. The measured immune responses and demonstrated safety are strong incentives to further develop a vaccine based on genetically attenuated malaria parasites.

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

- The Leiden Malaria group 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 mutants in rodent models of malaria, we discovered that it was possible to create attenuated parasites, which were able to invade the liver but were unable to proceed into the pathogenic blood stage infection. Importantly mice infected (immunised) with these live-attenuated parasites developed protective immunity against an infection with wild type parasites (published in PNAS in 2005).
- In 2008 we started to work on translating these findings into a human vaccine against malaria. This was performed in collaboration with the RadboudUMC (Nijmegen) and the US company Sanaria, and was supported by a grant provided by TI-Pharma. This involved testing and refining many live genetically-attenuated vaccines until we could demonstrate that one met all the necessary pre-clinical safety and protective efficacy standards; it is now ready to advance into testing in humans.
- In 2017 the first-in-human safety trials with a genetically modified malaria vaccine (i.e. PfSPZ-GA1) were performed at the LUMC. The GAP vaccine, PfSPZ-GA1, was manufactured by Sanaria and administered to volunteers intravenously by needle and syringe; the clinical trial was led by Meta Roestenberg.
- In a joint clinical study, 67 volunteers in Leiden and Nijmegen received injections of the vaccine made from the genetically modified parasite (called PfSPZ GA1) - a first in the world for an injectable, genetically weakened malaria vaccine. A high and a low dose were administered. In May 2020 The results of this clinical trial are published in Science Translational Medicine. The study show that the vaccine is safe and elicits a defense response against a malaria infection. It does not lead to infection of the blood and therefore does not cause malaria symptoms. It was also observed that the vaccinated volunteers developed an immune defense against a malaria infection, although this protection was not complete. According to the researchers, the measured immune responses and demonstrated safety are strong incentives to further develop a vaccine based on genetically attenuated malaria parasites.
- 2018-2020: Studies started to create a so-called 'second generation GAP', 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, a GAP that arrest late during development in the liver has indeed proven to induce significantly higher protective immune responses as compared to both radiation-attenuated sporozoites (RAS) and early arresting GAP (such as the first generation GAP), most likely resulting from increased antigen breadth and biomass of an LA-GAP. Our current studies are therefore focused on generating a late-arresting GAP for the human malaria parasite *P. falciparum*

More information about vaccination with live attenuated parasites (radiation and genetically attenuated parasites) can be found in the following papers:

- Review on how to perform experimental malaria infections in humans, i.e. so called Controlled Human Malaria Infections (for testing vaccine efficacy in humans).
- A N. Engl J. Med. paper showing induction of complete protective immunity in humans after only a limited number of mosquito bites.
- A review on human immunization trails 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 sterile immunity could be achieved using genetically attenuated sporozoites in rodent models of malaria.
- A Vaccine paper (from the Leiden Malaria Research Group) describing the preclinical testing of vaccines consisting of genetically attenuated parasites
- A review paper (from the Leiden Malaria Research Group) on the 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 characterisation of the human GAP vaccine PfΔslarpΔb9.
- Science Translational Medicine paper describing the results of the first-in-human safety and efficacy trial with a genetically modified malaria vaccine PfSPZ-GA1 (PfΔslarpΔb9)