Safety and preliminary protective efficacy of immunisation with genetically attenuated *PfΔmei2* (GA2) malaria parasites in healthy Dutch volunteers

O.A.C. Lamers[1], J.P.R. Koopman[1], G.V.T. Roozen[1], J.J. Janse[1], S.C. Chevalley-Maurel[2], F.J.A. Geurten[2], H.M. de Bes-Roeleveld[1], E.D. Colstrup[2], E. Wessels[3], G.J. van Gemert[4], M. van de Vegte-Bolmer[4], W. Graumans[4], T.R. Stoter[4], E.L. Houlder[1], R.A. Murugan[2], C.J. Janse[2], B.M.D. Franke-Fayard[2], M.B.B. McCall[4], M. Roestenberg[1].

[1]Department of Parasitology, Leiden University Center for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

Malaria Group

[2]Leiden Malaria Research Group, Leiden University Center for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

[3]Department of Medical Microbiology, Leiden University Center for Infectious Diseases, Leiden University Medical Centre, Leiden, Netherlands

[4]Department of Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands

Introduction: Given the recent resurgence of malaria, an effective vaccine is needed now more than ever. The only vaccine candidates that have achieved >90% protection in clinical trials consist of live attenuated sporozoites. Attenuation can be achieved by radiation, chemoprophylaxis or genetic modification. Irradiated parasites cause few side effects and abort development 1-2 days after hepatocyte invasion, yet have a lower potency than chemo-attenuated parasites. Depending on the pharmacological intervention, chemo-attenuated parasites can persist longer in the host, which may improve immunogenicity. The advantage of genetic attenuation is that parasites can be engineered to arrest at a desired timepoint, combining biosafety with increased potency.

We previously tested an early-arresting (EA) genetically attenuated *Plasmodium falciparum (Pf)* parasite (GAP) that interrupts development 2-3 days into the liver stage. While the EA-GAP GA1 (*Pf*NF54Δ*slarp*Δ*b9*) was safe and well-tolerated, its protective efficacy in malaria-naïve Dutch participants was lower than desired[1].

We therefore created GA2 (*Pf*NF54Δ*mei2*), a late-arresting (LA) GAP that aborts development just before the end of the liver stage (after 6-7 days)[2]. We hypothesized that by prolonging exposure and broadening the variety of antigens presented to the immune system, immunogenicity and therefore protective efficacy would be increased.

Methods: We conducted a phase 1/2a partially double-blind placebo-controlled clinical trial, assessing the safety, tolerability and preliminary protective efficacy of GA2. Forty-three healthy male and female, non-pregnant, malaria-naïve volunteers, aged 18-35, participated in the study. The primary endpoints were the frequency and severity of adverse events (AE) and parasitaemia, assessed by 18s qPCR.

Stage A was a dose-escalation study: participants were exposed once to 15 or 50 GA2-infected mosquito bites (MB). In stage B, GA2 was compared to its predecessor GA1 and a placebo. Participants were exposed to 50 GA2-, GA1-infected or uninfected MB, three times at 28-day intervals. Three weeks later, all participants underwent controlled human malaria infection (CHMI) by means of 5 MB infected with the unattenuated homologous *Pf* isolate (3D7).

Results: The GA2 parasite was found to be safe and well-tolerated and the frequency and severity of AEs was low and comparable across groups. No breakthrough infections occurred following GA2-administration at any dose.
Immunisation with GA2 resulted in 89% protection against homologous CHMI: 8 out of 9 participants remained qPCR negative until the end of the trial. GA1 immunisation achieved a protective efficacy of 13%: 7 out of 8 participants developed parasitaemia following CHMI. Immunisation with uninfected MB offered no protection.
GA2- and GA1-immunised participants exhibited a significant increase in anti-*Pf* circumsporozoite protein (CSP) antibodies compared to baseline (paired samples t-test: $p<0.001$), yet titres did not differ between experimental groups and between protected and non-protected individuals. Immunisation did not induce significant antibody responses against apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP1), two antigens expressed predominantly during blood-stage of malaria.

Conclusion: The novel *Pf* LA-GAP GA2 has a favourable safety profile, causes no breakthrough infections and induces high protective efficacy against homologous CHMI. Our results provide the pivotal proof-of-concept that longer liver stage development of attenuated whole sporozoites results in greater vaccine efficacy. We anticipate that cellular immunity against late liver stage antigens may play a dominant role in mediating sterile protection. Our findings warrant further investigation and evaluation of GA2 as a promising *Pf* vaccine candidate.

[1]Roestenberg, M. *et al. Science translational medicine* (2020).
[2]Franke-Fayard, B.M.D et al. *NPJ Vaccines* (2022).