Vaccine 39 (2021) 4314–4319

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Extended follow-up of children in a phase2b trial of the GMZ2 malaria vaccine

Sylvester Dassah [a,b,1], Bright Adu [c,1], Sodiomon B. Sirima [d], Benjamin Mordmüller [e], Ulysse Ateba Ngoa [e,f], Frank Atuguba [a], Fareed K.N. Arthur [b], Benedicta A. Mensah [g], Mark Kaddumukasa [h], Peter Bang [i], Peter G. Kremsner [d,e], Donnie Mategula [j], Clare Flach [j], Paul Milligan [j], Michael Theisen [k,l,*]

[a] Navrongo Health Research Centre, Navrongo, Ghana
[b] Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[c] Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana
[d] National de Recherche et de Formation sur le Paludisme, Burkina Faso
[e] Institute of Tropical Medicine, University of Tübingen, Germany
[f] Centre de Recherches Médicales de Lambaréné (CERMEL), Gabon
[g] Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana
[h] Makerere University College of Health Sciences, Uganda
[i] Department for Vaccine Development, Statens Serum Institut, Copenhagen, Denmark
[j] Faculty of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, UK
[k] Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark
[l] Centre for Medical Parasitology at Department of International Health, Immunology, and Microbiology, University of Copenhagen, and Department of Infectious Diseases, Copenhagen University Hospital, Rigshospitalet, Denmark

ARTICLE INFO

Article history:
Received 9 February 2021
Received in revised form 2 June 2021
Accepted 10 June 2021

Keywords:
GMZ2
Malaria
Vaccine
Plasmodium falciparum
Efficacy
Children
Africa

ABSTRACT

Background: The GMZ2/alum candidate malaria vaccine had an efficacy of 14% (95% confidence interval [CI]: 3.6%, 23%) against clinical malaria over 6 months of follow-up in a phase2b multicentre trial in children 1–5 years of age. Here we report the extended follow up of safety and efficacy over 2 years.
Methods: A total of 1849 (GMZ2 = 926, rabies = 923) children aged 12–60 months were randomized to receive intramuscularly, either 3 doses of 100 μg GMZ2/alum or 3 doses of rabies vaccine as control 28 days apart. The children were followed-up for 24 months for clinical malaria episodes and adverse events. The primary endpoint was documented fever with parasitaemia of at least 5000/μL.
Results: There were 2,062 malaria episodes in the GMZ2/alum group and 2,115 in the rabies vaccine group in the intention-to-treat analysis, vaccine efficacy (VE) of 6.5% (95%: CI −1.6%, 14.0%). In children aged 1–2 years at enrolment, VE was 3.6% (95 %CI: −9.1%, 14.8%) in the first year and −4.1% (95 %CI: −18.7%, 87%) in the second year. In children aged 3–5 years at enrolment VE was 19.9% (95 %CI: 7.7%, 30.4%) in the first year and 6.3% (95 %CI: −10.2%, 20.3%) in the second year (interaction by year, P = 0.025, and by age group, P = 0.085). A total of 187 (GMZ2 = 91, rabies = 96) serious adverse events were recorded in 167 individuals over the entire period of the study. There were no GMZ2 vaccine related serious adverse events.
Conclusions: GMZ2/alum was well tolerated. Follow-up over 2 years confirmed a low level of vaccine efficacy with slightly higher efficacy in older children, which suggests GMZ2 may act in concert with naturally acquired immunity.
© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Malaria is endemic in sub-Saharan Africa and contributes significantly to mortality and morbidity in children and pregnant women [1,2]. Malaria blood-stage vaccines are mainly intended to prevent clinical disease manifestation by inhibiting parasite growth and multiplication [3–5]. Such vaccine constructs are

* Corresponding author at: Department for Congenital Disorders, Statens Serum Institute, Artillerivej 5, 2300 Copenhagen S, Denmark.
E-mail address: mth@ssi.dk (M. Theisen).
[1] Contributed equally.

https://doi.org/10.1016/j.vaccine.2021.06.024
0264-410X/© 2021 The Authors. Published by Elsevier Ltd.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

designed to elicit immune responses against antigenic targets including merozoite surface proteins and invasion associated organelles [3–5]. This strategy is based on results from several sero-epidemiological studies in malaria endemic areas where higher levels of antibodies against such antigenic targets are often associated with protection against febrile malaria in semi-immune adults or children [6–10].

The GMZ2 vaccine candidate is based on two blood-stage antigens, Glutamate-Rich protein (GLURP) and merozoite surface protein (MSP) 3. As GMZ2 was designed to control parasitaemia but not prevent infection, it was thought that vaccine-induced responses could be boosted by natural exposure [11]. GMZ2 absorbed on Alhydrogel® was tested in a series of Phase 1 clinical trials [12–14] and in a phase IIb multi-centre trial in malaria-exposed African children 1–5 years of age. GMZ2/Alhydrogel was administered intramuscularly three times in 4-week intervals at a dose of 100 μg [15]. Vaccine efficacy against clinical malaria, over 6 months of follow-up, was 14% (95 %CI: 3.6%, 23%) [15]. This report presents results from the extended follow up of the children in this trial for efficacy and safety, over 24 months.

2. Materials and methods

2.1. Ethics statement

The study was monitored by the GMZ2 Scientific Coordinating Committee, local safety monitors, independent clinical monitors and an independent data safety monitoring committee. Ethics Committees and regulatory authorities in each country approved the trial protocol. Written informed consent was obtained from a parent or the legal guardian of each child after explaining the aims and procedures involved. The protocol was registered with the Pan African clinical trial registry with registration number ATMR2010060002033537.

2.2. Study design, site and participants

The study was a randomized, double-blind, controlled multicentre phase IIb clinical trial of the GMZ2 malaria candidate vaccine, using human diploid cell (HDC) rabies vaccine as a control [15]. Children aged 12–60 months residing in the study areas; eligible for the trial and whose parents consented were screened for randomization into the study. A total of 1849 subjects from four countries: Gabon (n = 512), Burkina Faso (Banfora; n = 580, Sapone; n = 300), Ghana (n = 200) and Uganda (n = 257) were randomized in a 1:1 ratio to receive either three [3] doses of 100 μg GMZ2 (n = 926) or 3 doses of rabies vaccine (n = 923). Vaccine was administered on Days 0, 28 and 56. Solicited adverse events were recorded on the day of vaccination and each of the next 7 days [15]. Caregivers were asked to bring the child to the study clinician whenever they were unwell. Participants were visited 14 days after each vaccine dose and then once a month to check the health status of the child and to refer to the study clinician children who were unwell. Children were followed up for 24 months from the first vaccination.

Malaria parasitaemia was determined by microscopy as previously described [16]. Each slide was read by two experienced microscopists and discrepancies (i.e. by species or if the parasite count differed by more than 50%) were resolved by a third microscopist.

2.3. Study vaccines, randomization, and vaccination

The investigational product, GMZ2 and the control vaccine Verorab (Sanofi Pasteur, France) has been describes in detail [15]. Randomization and vaccination has been described in detail [15]. After each vaccination, participants were observed for 30 min for immediate adverse events. Participants were then followed by field workers for a six-day surveillance period after each vaccination. On Day 7 post-vaccination, participants were seen by study physician. All adverse events occurring during this period were followed until resolution or stabilization. At each visit, clinical examination was performed and information on any solicited or unsolicited signs since the last visit were recorded.

2.4. Study outcomes

The primary end point of the trial was efficacy against *P. falciparum* clinical malaria episodes (defined as fever and/or history of fever with parasitaemia of at least 5000/μL) over a six months surveillance period starting from the day of the third dose of vaccination was previously reported [15].

Secondary endpoints included fever (defined as fever and/or history of fever) and parasitaemia (using 5 threshold densities, at any density above zero, at least 2500, 5000, 10,000 and 20,000 trophozoites/μL) over 22 months, severe malaria (defined as any malaria case that results in hospital admission for more than 24 h), all cause hospitalisation and mortality at the end of follow-up.

2.5. Statistical methods

Serious adverse events were listed individually, with investigator assessment of relationship to vaccination. For any event, the relative risk (GMZ2: control) of an event during the follow up period were calculated with a 95% confidence interval and tabulated. The primary definition of a malaria episode is having a fever (tympanic temperature $\geq$ 38 °C) or history of fever with parasitaemia greater than 5000/μL. The incidence rate of malaria was calculated as the number of episodes meeting the case definition divided by the time at risk. Time at risk is the total time from start to end of the surveillance period with no deduction for periods of malaria treatment. All episodes meeting the case definition were included in the analysis of rates and vaccine efficacy, but to avoid counting the same episode twice, cases occurring within 14 days of a previous case were ignored.

Time periods: For the ATP (according to protocol) analysis, the surveillance period starts on the day of dose 3 to the end of follow-up at 24 months from the first vaccination. For the ITT (intention to treat) analysis, the surveillance period starts at the time of first vaccine administration to the end of follow-up at 24 months from the first vaccination. The incidence rate of malaria was compared between groups using Cox regression stratified by site, using time since randomization (ITT), or time since dose 3 days (ATP), as the time scale, and including all malaria episodes.

Vaccine efficacy (VE) was defined as 100 × (1-HR), where HR is the hazard ratio from Cox regression, this is an estimate of the percentage reduction in the number of malaria episodes associated with GMZ2 vaccination [17]. A 95% confidence interval was calculated, using a robust standard error to allow for repeated episodes in the same child. The analysis was pooled across sites, adjusted for age group, with site as a stratification factor to allow for a separate seasonal pattern of incidence in the control group in each site. Analyses were repeated for case definitions defined by parasite density cut-offs as follows: >0, $\geq$2500/μL, $\geq$10000/μL, and $\geq$ 20000/μL.

3. Results

3.1. Study sites and population

The study sites and population characteristics was previously described [15]. The study took place from December 2010 to June

2013. At enrolment, the age and gender distributions were similar in the vaccine and control arms at each study site. Overall, the proportion of children who slept under bed net was different between sites but similar for both the vaccine and control arms within each site except for Lambaréné where 18% of the children who received the rabies vaccine used bed net compared to 8% in the GMZ2 group [15].

Malaria incidence was seasonal at all sites, most strongly in Burkina Faso (Banfora and Saponé) (Fig. 1). For all sites, the highest numbers of malaria episodes were recorded during or just after the peak rainfall periods. Except for Navrongo, vaccinations at all sites partially overlapped with the peak rainy season. Banfora and Saponé had the highest number of malaria episodes compared to the remaining sites.

3.2. Malaria parasite density in samples from clinical cases

Overall, 98.1% of the malaria episodes were monoinfection with *P. falciparum*. The remaining were mixed infections of *P. falciparum* and either *P. malariae, P. ovale, or P. vivax* (Table S1). Geometric mean parasitaemia in clinical cases decreased with increasing age but there was no evidence that parasite density in febrile cases with parasitaemia differed between the GMZ2 and the rabies groups (Table S2). When parasite densities were categorized based on the threshold for hyperparasitaemia (density >=200,000), the percentage of cases with hyperparasitaemia was similar in the GMZ2 and rabies vaccine groups (3.6% and 4.4% respectively) (Supplementary Table S3).

3.3. GMZ2 vaccine efficacy at different parasite thresholds

Vaccine efficacy was assessed in both according-to-protocol (ATP) analysis (i.e. data covering an average of 22 months follow up, starting from the last day of vaccination) and intention-to-treat (ITT) analysis (i.e. data covering an average of 24 months follow up, starting from the day of randomisation). The ATP analysis included a total of 1,758 malaria episodes in the GMZ2 group and 1,813 in the rabies vaccine. The overall GMZ2 vaccine efficacy (VE) using parasite density >=5,000/μL and fever/history of fever was 6.5% (95 %CI: −2.0%, 14.3%) after adjusting for age and site as strata.

In the intention-to-treat (ITT) analysis the number of malaria episodes defined by parasite density >=5,000/μL and fever/history of fever was 2,115 and 2,062 in the rabies and GMZ2 cohorts respectively and VE was 6.5% (95 %CI: −1.6, 14.0%) (Table 1). Similar estimates of VE were obtained when the ITT analysis was performed using other parasite density thresholds (greater than0/μL, >=2,500 >=10,000 or >=20,000/μL, Supplementary Table S4).

3.4. GMZ2 vaccine efficacy at different periods and in different age groups

Since both the induction and decay kinetics of GMZ2 induced antibodies have not been described, we decided to stratify the follow up time into two different intervals and assess VE within each period in the ITT analysis (with the primary endpoint parasite density >=5,000/μL and fever/history of fever) for each age group. Overall, the mean number of malaria episodes per child increased sharply in the first six months, plateaus for nine months and increases again till about the 18th month (Fig. 2A and B), mirroring the seasonality in malaria transmission patterns observed at the sites. The mean number of malaria episodes per child was slightly lower in the GMZ2 group in the older children (Fig. 2B). In the children aged 1–2 years at enrolment, there was low VE in both years (3.6% (95 %CI −9.1%, 14.8%) in year 1 and −4.1% (−18.7%, 8.7%) in year 2. On the other hand, VE was higher in children aged 3–5 years at enrolment in year 1 (19.9%, 95 %CI 7.7%, 30.4%), but there was no evidence of protection in year 2 (VE 6.3% (−10.2%, 20.3%)) (interaction by age group, P = 0.085; interaction by year, P = 0.025). Vaccine efficacy by time period is shown in Fig S1.

3.5. Safety

The immediate, local and systemic reactogenicity has been reported [15]. A total of 187 unsolicited serious adverse events (SAEs) were recorded in 167 individuals over the entire period of the study. Two SAEs, convulsions and bronchitis, which occurred in different children both in the rabies group a day and two days respectively after vaccination in Gabon were judged to have been related to the control rabies vaccine. Both children recovered fully without any sequelae. Apart from these, none of the SAEs recorded throughout the study were related to either the rabies or GMZ2 vaccine. In all, 91 SAEs were recorded in those who received GMZ2 vaccine and 96 in those who received the rabies vaccine. Majority (112/187) of the SAEs at all sites were malaria related. The rate of severe malaria was not different between the GMZ2

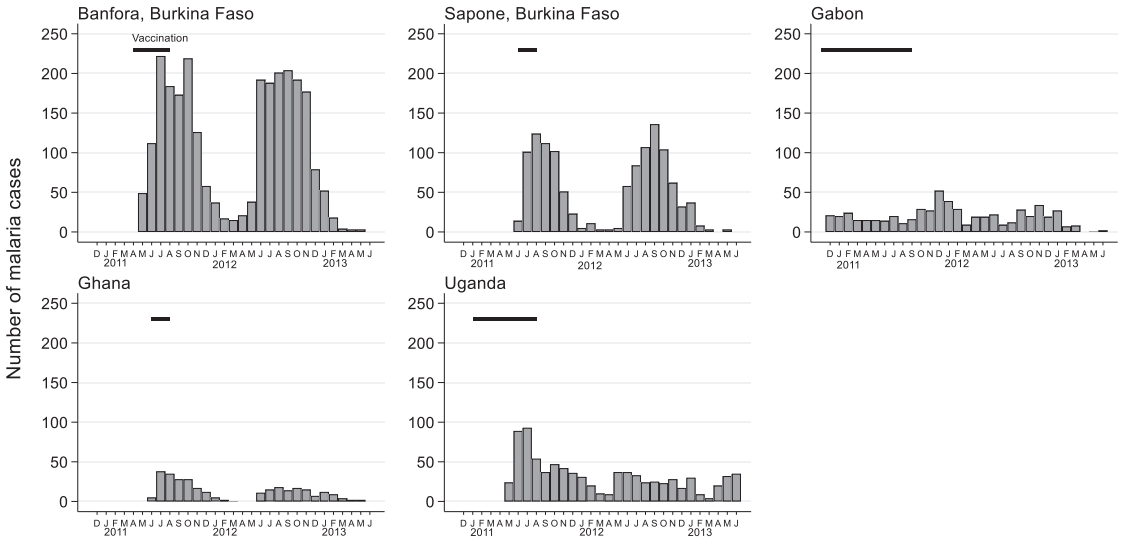

Fig. 1. Timing of vaccination and clinical malaria episodes in each site. The period of vaccine dose administration in each site is indicated by the horizontal bar. The total number of malaria episodes (at any parasite density) is shown for each month.

Table 1
Vaccine efficacy against clinical malaria (fever with parasite density >=5000/μL), in each year of the trial, ITT analysis.

	Rabies vaccine		GMZ2 vaccine		
	Events	Rate/1000	Events	Rate/1000	Efficacy
Year 1	1132	108.3	1033	96.4	11.7% (3.1%,19.6%)
Year 2	983	98.7	1029	100.1	0.6% (−10.2%,10.2%)
TOTAL	2115	103.6	2062	98.2	6.5% (−1.6%,14.0%)
Age 1–2 years at enrolment					
Year 1	562	122.7	583	117.7	3.6% (−9.1%,14.8%)
Year 2	519	119.5	607	127.8	−4.1% (−18.7%,8.7%)
TOTAL	1081	121.2	1190	122.6	−0.1% (−11.7%,10.2%)
Age 3–4 years at enrolment					
Year 1	570	97.1	450	78.0	19.9% (7.7%,30.4%)
Year 2	464	82.6	422	76.3	6.3% (−10.2%,20.3%)
TOTAL	1034	90.0	872	77.2	13.8% (2.2%,24.0%)

Interaction by age group: P = 0.085, interaction by year: P = 0.025; interaction by site: P = 0.8262.

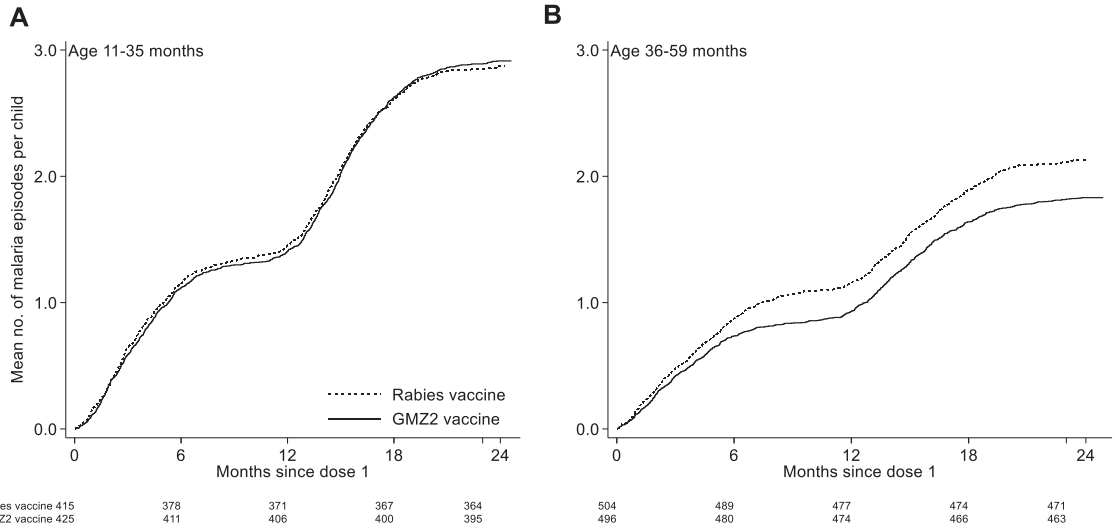

Fig. 2. Mean number of malaria episodes (fever with parasite density at least 5000/μL) per child, during 24 months of follow-up, in children aged 1–2 yrs when enrolled, and children aged 3-4yrs, by vaccine group. Nelson-Aalen cumulative hazards of malaria. The number at risk in each group is shown below the x-axis.

and rabies groups at all sites (Supplementary Table S5). When data from all sites were pooled, the Cox regression analysis adjusted for age showed that the rate of severe malaria was similar [hazard ratio (HR) = 0.93 (95% CI: 0.63, 1.38)] among the two treatment groups (Supplementary Table S5).

All cause hospitalization rate was similar in the two study groups at each site and in a pooled analysis adjusted for age [HR = 0.97 (95% CI: 0.71–1.33)] (Supplementary Table S5). A total of sixteen deaths were recorded, 9 in the rabies group and 7 in the GMZ2 group over the entire study period. The rate of death was not significantly different between the GMZ2 and rabies group either at each site or in the pooled analysis [HR = 0.76 (95% CI: 0.28–2.03)] (Supplementary Table S5). Malaria was the most common cause of death (overall = 7/16). Two meningitis related deaths were recorded, both occurring in Banfora, Burkina Faso in the GMZ2 group (Table S5). None of the deaths was judged to have been related to either the rabies or GMZ2 vaccine.

4. Discussion

This study reports the extended follow-up of the GMZ2/Alum phase IIb efficacy clinical trial. The study involved intramuscular administration of the GMZ2/Alum vaccine three times in 4-week intervals at a dose of 100 μg [15] and subsequent passive follow-up for clinical malaria episodes over 24 months. In brief: 1) GMZ2/Alum vaccine was well tolerated in these malaria-exposed children 2) Vaccine efficacy varied by age group, there was no evidence of protection in the children aged 1–2 years at enrolment, a modest efficacy in children aged 3–5 years at enrolment was observed.

The safety profile of GMZ2/Alum after 24 months of follow-up was good, consistent with previous clinical trials in which immediate common reactions were mild to moderate [13,14,18]. All adverse events were unrelated to the study vaccine. The most frequently reported adverse event at the end of the follow up period was uncomplicated malaria which was slightly lower especially for older children in the GMZ2 vaccine group although the difference was not significant. The pattern of malaria episodes observed seemed to mirror the seasonality of transmission in the study sites.

GMZ2 vaccine efficacy varied for the different age groups and was lower in younger children than those who are older. This suggests that pre-existing natural immunity in the older children worked in concert with the vaccine to protect against malaria. However, the decline in vaccine efficacy over the second year of follow up may be indicative of antibody waning as has been observed for other malaria vaccines [19,20]. Strong adaptive immune responses and long-term memory may require activation of innate immunity possibly. Such activation may occur through "danger signals" and is explained by reactions that are controlled

by specific pattern-recognition receptors [21]. Whether the inclusion of specific Toll-like receptor agonists [22] might enhance longevity, levels and functional activity of vaccine-specific antibodies in humans remains to be investigated. In malaria endemic regions, children gradually develop anti-malaria immunity as a function of exposure leading to less malaria episodes and severe disease [23]. Gradual decrease in mean parasite density with age and low malaria attack rate in older children is characteristic of development of naturally acquired immunity. The reason for the sharp increase in the mean malaria episodes per child during the first 3 months post vaccination is not known but we note that the last vaccinations were generally given during the transmission season due to late study start. The period of plateau in mean malaria episodes per child partially overlapping with the end of the transmission season suggesting that acquisition of broadly reacting malaria-specific antibodies are indeed required for protection against clinical malaria in the GMZ2/Alum group. Thus, supporting the notion that GMZ2 IgG acts in concert with antibodies against other merozoite surface antigens [24–27]. The sharp rise and plateau of malaria episodes although it cannot be explained with the present data may therefore be due to i) affinity maturation of antibodies over time to reach antigen-specific thresholds [24,26–29] ii) immune boosting by exposure to natural infection [30] and iii) antibody isotype imbalance [31–34]. Subsequent subgroup analysis of the trial cohort investigating these hypotheses may inform improved design of future GMZ2 and other blood stage malaria vaccines.

The efficacy of the GMZ2 vaccine at both the primary and secondary end points in this final report fell below the strategic target goal of the Malaria Vaccine Technology Roadmap of attaining $\geq$ 75% efficacy against clinical malaria [35]. The inability of GMZ2 and other similar blood stage candidate malaria vaccines [36–39] to demonstrate substantial efficacy against clinical malaria raise critical questions about the various models and assays used to select blood-stage antigens for vaccine development [36–38,40]. Some of the key questions that remain to be answered include whether the models and assays used in these vaccine development processes are suitable for field trials in malaria endemic populations and whether the assays are robust and useful enough to predict efficacy in field trials. A classic example is the AMA1-C1 trial where parasite inhibition in GIA was observed in malaria naïve US adults [41] but not Malian adults [42], although there was elevation in antibody titres in both populations as measured by ELISA. Despites these unanswered question, the level of protection offered by the GMZ2 candidate malaria vaccine supports the proof of concept that a blood-stage malaria vaccine is attainable [43,44]. The formulation of these vaccines, however, needs to be improved including the choice of antigens and immunostimulants. AS01 is a complex adjuvant system used with the RTS,S vaccine that appears to induce both cell and antibody-mediated immunity resulting in the current mile stone attained [45,46]. This suggests there is a potential for improving the current GMZ2 vaccine possibly with a better adjuvant system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank the children and their parents and guardians who volunteered to participate in the study, and without whose cooperation this study would have been impossible. We also thank the Data and Safety Monitoring Board (Trudie Lang (CHAIR), Brian Faragher, Blaise Genton, Angelina Kakooza, Grégoire Adzoda, John E. Williams, and Dao Fousseni) for their recommendations throughout the study.

This study was supported by grants from the European and Developing Countries Clinical Trials Partnership (grant IP.2007.31100.001), the German Federal Ministry of Education and Research (BMBF, grants 01KA0804 and 01KA1402) and Ministry of Foreign Affairs of Denmark (DFC file no.14-P01-GHA).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.06.024.

References

[1] World Health Organization. Fact sheet about Malaria [Internet]. 2019 [cited 2020 Jul 2]. Available from: https://www.who.int/news-room/fact-sheets/detail/malaria.
[2] Aguzie ION. Pregnancy-associated Malaria, Challenges and Prospects in Sub-Saharan Africa. Clinics Mother Child Health 2018;15(1):1–18.
[3] Waters A. Malaria: new vaccines for old? Cell 2006 Feb 24;124(4):689–93.
[4] Genton B. Malaria vaccines: a toy for travelers or a tool for eradication? Expert Rev Vaccines 2008 Jul;7(5):597–611.
[5] Vekemans J, Ballou WR. Plasmodium falciparum malaria vaccines in development. Expert Rev Vaccines 2008 Mar;7(2):223–40.
[6] Richards JS, Stanisic DI, Fowkes FJI, Tavul L, Dabod E, Thompson JK, et al. Association between naturally acquired antibodies to erythrocyte-binding antigens of Plasmodium falciparum and protection from malaria and high-density parasitemia. Clin Infect Dis 2010 Oct 15;51(8):e50–60.
[7] Soe S, Theisen M, Roussilhon C, Aye KS, Druilhe P. Association between Protection against Clinical Malaria and Antibodies to Merozoite Surface Antigens in an Area of Hyperendemicity in Myanmar: Complementarity between Responses to Merozoite Surface Protein 3 and the 220-Kilodalton Glutamate-Rich Protein. Infect Immun 2004;72(1):247–52.
[8] Tran TM, Ongoiba A, Coursen J, Crosnier C, Diouf A, Huang C-Y, et al. Naturally acquired antibodies specific for Plasmodium falciparum reticulocyte-binding protein homologue 5 inhibit parasite growth and predict protection from malaria. J Infect Dis 2014 Mar 1;209(5):789–98.
[9] Dodoo D, Theisen M, Kurtzhals JAL, Akanmori BD, Koram KA, Jepsen S, et al. Naturally Acquired Antibodies to the Glutamate-Rich Protein Are Associated with Protection against Plasmodium falciparum Malaria. J Infect Dis 2000 Mar 1;181(3):1202–5.
[10] Theisen M, Dodoo D, Toure-Balde A, Soe S, Corradin G, Koram KK, et al. Selection of glutamate-rich protein long synthetic peptides for vaccine development: antigenicity and relationship with clinical protection and immunogenicity. Infect Immun 2001 Sep;69(9):5223–9.
[11] Theisen M, Soe S, Brunstedt K, Follmann F, Bredmose L, Israelsen H, et al. A Plasmodium falciparum GLURP-MSP3 chimeric protein; expression in Lactococcus lactis, immunogenicity and induction of biologically active antibodies. Vaccine 2004 Mar 12;22(9–10):1188–98.
[12] Esen M, Kremsner PG, Schleucher R, Gässler M, Imoukhuede EB, Imbault N, et al. Safety and immunogenicity of GMZ2 - a MSP3-GLURP fusion protein malaria vaccine candidate. Vaccine 2009 Nov 16;27(49):6862–8.
[13] Mordmüller B, Szywon K, Greutelaers B, Esen M, Mewono L, Treut C, et al. Safety and immunogenicity of the malaria vaccine candidate GMZ2 in malaria-exposed, adult individuals from Lambaréné. Gabon Vaccine 2010 Sep 24;28(41):6698–703.
[14] Bélard S, Issifou S, Hounkpatin AB, Schaumburg F, Ngoa UA, Esen M, et al. A randomized controlled phase Ib trial of the malaria vaccine candidate GMZ2 in African children. PLoS ONE 2011;6(7):e22525.
[15] Sirima SB, Mordmüller B, Milligan P, Ngoa UA, Kironde F, Atuguba F, et al. A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children. Vaccine 2016;34(38):4536–42.
[16] World Health Organization, editor. Basic malaria microscopy. 2nd ed. Geneva: WHO; 2010. 2 p.
[17] Cheung YB, Xu Y, Tan SH, Cutts F, Milligan P. Estimation of intervention effects using first or multiple episodes in clinical trials: The Andersen-Gill model re-examined. Stat Med 2010 Feb 10;29(3):328–36.
[18] Esen M, Kremsner PG, Schleucher R, Gässler M, Imoukhuede EB, Imbault N, et al. Safety and immunogenicity of GMZ2 — a MSP3–GLURP fusion protein malaria vaccine candidate. Vaccine 2009 Nov;27(49):6862–8.
[19] Olotu A, Fegan G, Wambua J, Nyangweso G, Leach A, Lievens M, et al. Seven-Year Efficacy of RTS, S/AS01 Malaria Vaccine among Young African Children. N Engl J Med 2016 Jun 30;374(26):2519–29.
[20] Datoo MS, Natama MH, Somé A, Traoré O, Rouamba T, Bellamy D, et al. Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial. Lancet 2021 May 15;397(10287):1809–18.
[21] Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335–76.

[22] Lousada-Dietrich S, Jogdand PS, Jepsen S, Pinto VV, Ditlev SB, Christiansen M, et al. A synthetic TLR4 agonist formulated in an emulsion enhances humoral and Type 1 cellular immune responses against GMZ2–a GLURP-MSP3 fusion protein malaria vaccine candidate. Vaccine 2011 Apr 12;29(17):3284–92.
[23] Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. Proc Biol Sci 2015;282(1801):20142657.
[24] Murungi LM, Kamuyu G, Lowe B, Bejon P, Theisen M, Kinyanjui SM, et al. A threshold concentration of anti-merozoite antibodies is required for protection from clinical episodes of malaria. Vaccine 2013 Aug 20;31(37):3936–42.
[25] Theisen M, Adu B, Mordmüller B, Singh S. The GMZ2 malaria vaccine: from concept to efficacy in humans. Expert Rev Vaccines 2017;16(9):907–17.
[26] Garcia-Senosiain A, Kana IH, Singh SK, Chourasia BK, Das MK, Dodoo D, et al. Peripheral merozoite surface proteins are targets of naturally acquired immunity against malaria both in India and in Ghana. Infection and Immunity [Internet]. 2020 Jan 21 [cited 2020 Mar 10]; Available from: https://iai.asm.org/content/early/2020/01/15/IAI.00778-19.
[27] Kana IH, Singh SK, Garcia-Senosiain A, Dodoo D, Singh S, Adu B, et al. Breadth of Functional Antibodies Is Associated With Plasmodium falciparum Merozoite Phagocytosis and Protection Against Febrile Malaria. J Infect Dis 2019;220(2):275–84.
[28] Tijani MK, Reddy SB, Langer C, Beeson JG, Wahlgren M, Nwuba RI, et al. Factors influencing the induction of high affinity antibodies to Plasmodium falciparum merozoite antigens and how affinity changes over time. Scientific Reports [Internet]. 2018 [cited 2019 May 28];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5998021/.
[29] Reddy SB, Anders RF, Beeson JG, Färnert A, Kironde F, Berenzon SK, et al. High affinity antibodies to Plasmodium falciparum merozoite antigens are associated with protection from malaria. PLoS ONE 2012;7(2):e32242.
[30] Palacpac NMQ, Ntege E, Yeka A, Balikagala B, Suzuki N, Shirai H, et al. Phase 1b Randomized Trial and Follow-Up Study in Uganda of the Blood-Stage Malaria Vaccine Candidate BK-SE36. PLoS ONE 2013 May 28;8(5):e64073.
[31] Bouharoun-Tayoun H, Druilhe P. Plasmodium falciparum malaria: evidence for an isotype imbalance which may be responsible for delayed acquisition of protective immunity. Infect Immun 1992 Apr;60(4):1473–81.
[32] Wahlgren M, Berzins K, Perlmann P, Persson M. Characterization of the humoral immune response in Plasmodium falciparum malaria. II. IgG subclass levels of anti-P. falciparum antibodies in different sera. Clin Exp Immunol 1983 Oct;54(1):135–42.
[33] White WI, Evans CB, Taylor DW. Antimalarial antibodies of the immunoglobulin G2a isotype modulate parasitemias in mice infected with Plasmodium yoelii. Infect Immun 1991 Oct;59(10):3547–54.
[34] Falanga PB, D'Imperio Lima MR, Coutinho A, Pereira da Silva L. Isotypic pattern of the polyclonal B cell response during primary infection by Plasmodium chabaudi and in immune-protected mice. Eur J Immunol 1987 May;17(5):599–603.
[35] World Health Organization (WHO). WHO | Malaria vaccine technology roadmap [Internet]. WHO. 2018 [cited 2018 Aug 13]. Available from: http://www.who.int/immunization/topics/malaria/vaccine_roadmap/en/.
[36] Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, et al. A Field Trial to Assess a Blood-Stage Malaria Vaccine. N Engl J Med 2011 Sep 15;365(11):1004–13.
[37] Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, et al. A Recombinant Blood-Stage Malaria Vaccine Reduces Plasmodium falciparum Density and Exerts Selective Pressure on Parasite Populations in a Phase 1–2b Trial in Papua New Guinea. J Infect Dis 2002 Mar 15;185(6):820–7.
[38] Spring MD, Cummings JF, Ockenhouse CF, Dutta S, Reidler R, Angov E, et al. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. PLoS ONE 2009;4(4):e5254.
[39] Sheehy SH, Duncan CJA, Elias SC, Choudhary P, Biswas S, Halstead FD, et al. ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: assessment of efficacy against mosquito bite challenge in humans. Mol Ther 2012 Dec;20(12):2355–68.
[40] Laurens MB, Thera MA, Coulibaly D, Ouattara A, Kone AK, Guindo AB, et al. Extended safety, immunogenicity and efficacy of a blood-stage malaria vaccine in malian children: 24-month follow-up of a randomized, double-blinded phase 2 trial. PLoS ONE 2013;8(11):e79323.
[41] Malkin EM, Diemert DJ, McArthur JH, Perreault JR, Miles AP, Giersing BK, et al. Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for Plasmodium falciparum malaria. Infect Immun 2005 Jun;73(6):3677–85.
[42] Dicko A, Diemert DJ, Sagara I, Sogoba M, Niambele MB, Assadou MH, et al. Impact of a Plasmodium falciparum AMA1 vaccine on antibody responses in adult Malians. PLoS ONE 2007 Oct 17;2(10):e1045.
[43] Cohen S, McGREGOR IA, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature 1961 Nov;25(192):733–7.
[44] Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P, et al. Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg 1991 Sep;45(3):297–308.
[45] Didierlaurent AM, Berger A, Heineman TC, Henderickx V, Tavares Da Silva F, Vekemans J, et al. Chapter 14 - The Development of the Adjuvant System AS01: A Combination of Two Immunostimulants MPL and QS-21 in Liposomes. In: Schijns VEJC, O'Hagan DT, editors. Immunopotentiators in Modern Vaccines (Second Edition) [Internet]. Academic Press; 2017 [cited 2018 Oct 8]. p. 265–85. Available from: http://www.sciencedirect.com/science/article/pii/B9780128040195000141.
[46] Baldridge JR, Crane RT. Monophosphoryl lipid A (MPL) formulations for the next generation of vaccines. Methods 1999 Sep;19(1):103–7.