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Safety and immunogenicity of GMZ2 — a MSP3–GLURP fusion protein malaria vaccine candidate

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ABSTRACT

Malaria is a major public health problem in Sub-Saharan Africa. In highly endemic regions infants, children and pregnant women are mostly affected. An effective malaria vaccine would complement existing malaria control strategies because it can be integrated in existing immunization programs easily. Here we present the results of the first phase Ia clinical trial of GMZ2 adjuvanted in aluminium hydroxide. GMZ2 is a malaria vaccine candidate, designed upon the rationale to induce immune responses against asexual blood stages of *Plasmodium falciparum* similar to those encountered in semi-immune individuals. Ten, 30 and 100 μg of GMZ2 were well tolerated in 30 healthy malaria-naïve German volunteers when given three times in monthly intervals. Antigen-specific antibodies as well as memory B-cells were induced and detectable throughout the one year follow-up of the study. We conclude that GMZ2 is a safe and immunogenic malaria vaccine candidate suitable for further clinical development.
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1. Introduction

Malaria as resulting from *Plasmodium falciparum* infection is one of the most important causes of death and morbidity in children aged one to five years and pregnant women in Sub-Saharan Africa. Resistance against chemotherapeutics and insecticides and the poor economic situation of most affected populations requires the amelioration of existing, and the invention of new strategies to control malaria through international efforts. Over the last 90 years at the least, development of an effective, safe and protective vaccine against *P. falciparum* has been one of the major goals of malaria research [1]. Besides the scientific challenge, a main motivation is the possible integration of a potential malaria vaccine into present health interventions such as the expanded program on immunization. This approach would be an extremely cost-effective tool for the control of malaria.

Presently, two main lines of clinical malaria vaccine research dominate: (i) induction of immunity against pre-erythrocytic antigens, a strategy rooted in first experiments with UV-inactivated *P. gallinaceum* sporozoites [2], and (ii) identification of antigens that induce antibodies with specificities similar to immunoglobulin preparations of semi-immune adults with a therapeutic effect in malaria patients [3,4]. Both strategies rely on the identification of plasmodial proteins that induce a protective immune response. GMZ2 belongs to the second class of malaria vaccine candidates. It is a fusion protein of parts of *P. falciparum* glutamate-rich protein (GLURP) and merozoite surface protein 3 (MSP3), two antigens that induce antibodies with activities similar to immunoglobulin preparations from semi-immune individuals. $GLURP_{27-500}$ represents the R0 non-repeat region which is a major B-cell epitope [5] and is expressed in pre-erythrocytic and erythrocytic stages of parasite development. $MSP3_{212-380}$ is a conserved part of the otherwise highly polymorphic MSP3 [6,7]. It was one of the first vaccine candidates identified by analysis of "therapeutic" immunoglobulin preparations, when sera of semi-immune and first-time *P. falciparum* infected individuals were compared in their reactivity in Western blots of crude parasite lysates and antibody (Ab) dependent cellular inhibition (ADCI) of parasite growth in the presence of monocytes [8]. The dominant ADCI effect exerted by cytophilic anti-MSP3 Abs and the association of ADCI with

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clinical protection in semi-immunes was the rational to select MSP3 as a vaccine-candidate. Besides their activity in semi-immune sera, immuno-epidemiological studies have shown an inverse association of high Ab titres against both antigens (Ag) with clinical malaria [9,10]. To date, it is not clear how Abs against GLURP and MSP3 might control parasite growth. Currently, the main hypothesis centres on the interaction between Ab, parasites and monocytes, since these three partners show an *in vitro* growth-inhibitory effect on *P. falciparum* which has been used as a surrogate marker for *in vivo* parasite growth [11–15]. Recombinant GMZ2 is produced as a secreted protein in *Lactococcus lactis*, a gram positive organism with rather low immune-stimulatory potential [16]. First studies of GMZ2 in splenectomized *Saimiri sciureus* showed good immunogenicity, safety, and partial protection against *P. falciparum* blood stage challenge [17]. These results led us to pursue the development of GMZ2 as a malaria vaccine candidate and progress towards clinical development.

Here we report the results of the first-in-man phase I clinical trial (EudraCT Number: 2005-004568-22, and ClinicalTrials.gov Identifier: NCT00397449) of GMZ2 adjuvanted in aluminium hydroxide administered in ascending doses, in thirty healthy, malaria-naïve adults from Germany.

2. Material and methods

2.1. Study subjects and study design

The study was performed from October 2006 until December 2007 at the Institute of Tropical Medicine of the University of Tübingen, Germany and received approval from the institutional Ethics committee (ethics committee of the medical faculty and university clinic). The study was a randomised, open-labelled, dose-escalating phase Ia clinical trial. Participants were healthy, non-pregnant, malaria-naïve, adult Europeans, negative for anti-GMZ2, -GLURP and -MSP3 antibodies. Enrolled participants were randomly assigned to one of three doses of GMZ2 (group I: 10 μg, group II: 30 μg, or group III: 100 μg), adjuvanted with aluminium hydroxide (alum). Vaccine preparation was extemporaneous and all doses were given subcutaneously. Vaccination of groups I, II, and III was done dose-staggered, with a 15 days lag until the next group's injection to ensure participant's safety and to be able to stop the trial within 14 days if unforeseen and severe reactions at one dose-level occur. Participants were observed for 30 min after each vaccination. Follow-up visits were done after 24 h and two weeks after each vaccination. In between face-to-face visits diaries were kept and regular telephone calls were made to the participants. In addition, four weeks after the last vaccination and one year after the first vaccination participants were re-examined and blood samples were taken to evaluate safety and immune responses at its peak as well as the decay phase, respectively. The clinical trial was conducted in accordance with the latest version of the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines.

2.2. GMZ2 vaccine

Recombinant GMZ2 was expressed in *L. lactis* and purified from culture supernatants following good manufacturing practice (GMP) to obtain one batch (G026/LCI/040301) for use in human subjects within this trial. Lyophilized GMZ2 (Henogen S.A., Belgium) was reconstituted into water and mixed with aluminium hydroxide (Batch 528601; Statens Serum Institut, Denmark) at a GMP facility in Tübingen before subcutaneous injection.

2.3. Objectives of the clinical trial

The primary objective was the evaluation of the safety of three doses of three different dosage of GMZ2 adjuvanted with alum and was done on intention to treat (ITT) population. The secondary objectives were (i) the assessment of humoral immune response to the vaccine before and one month after each vaccination as well as one year after the first vaccination, (ii) the assessment of the ability of antibodies to recognize the native antigen by indirect fluorescence assay (IFA), (iii) the assessment of antigen-specific memory B-cells. All analyses up to Day 84 were done on the ITT population. Exploratory analyses comprised further immunological tests and will be presented in a separate publication.

2.4. Evaluation of clinical and biological safety

Adverse events (AEs) were recorded one month after each vaccination and the serious adverse events (SAEs) throughout the study period. Participants were physically examined by the study physician on each day of vaccination and 24 h as well as 7 days thereafter. On Day 7 post-vaccination a telephone call was made to all participants. Between scheduled visits and calls a 24 hourly operated telephone line was maintained to ease reporting of adverse events. Solicited local AEs including pain, erythema, swelling, induration, pruritus, oedema and local heat occurrence were evaluated immediately (30 min) and within 14 days after each vaccination. Solicited systemic AEs such as fatigue, fever, headache, malaise, myalgia, joint pain, gastrointestinal symptoms (nausea, diarrhoea, vomiting, abdominal pain), generalized skin reactions, and contra-lateral local reaction were listed immediately (30 min) and within 14 days after each vaccination. Laboratory parameters included a full blood cell count and biochemical parameters (potassium, sodium, ASAT, ALAT, bilirubin, alkaline phosphatise, gamma GT, creatinine, glucose). Laboratory parameters were assessed before, one month after each vaccination and one year after the first vaccination. Physical examinations were performed at each visit to the study site.

2.5. Assessment of anti-GMZ2, -GLURP and -MSP3 antibodies

Determination of specific anti-GMZ2, -GLURP and -MSP3 immunoglobulin G (IgG) concentrations was done on Days 0, 28, 56, 84, and 365 by enzyme-linked immunosorbent assay (ELISA) as previously described [18]. In brief, ELISA microtitre plates (NUNC Maxisorp^{TM}, Germany) were coated with either 0.5 μg GMZ2, 0.5 μg GLURP, or 1 μg MSP3 per ml, diluted in phosphate buffered saline (PBS) overnight at +4 °C. Following four washes in PBS, 0.1% Tween 20, 0.5 M NaCl, plates were blocked in PBS, 3% non-fat milk powder, 0.1% Tween 20 for 1 h. Serum samples were diluted 1:100 in PBS, 1% non-fat milk, 0.1% Tween 20, 0.02% NaN_3. Further serial dilutions were done in the same dilution buffer without NaN_3. Ag-specific Abs were detected by a peroxidase conjugated goat anti-human IgG Ab (Caltag, USA) diluted 1:3000 in dilution buffer. As peroxidase substrate we used TMB ONE (KEM EN TEC, Belgium). Absorbance was read after addition of 0.2 M H_2SO_4 (32%, Merck, Germany) at 450 nm (reference 620 nm) on a plate reader (Asys Expert 96, Type G018065). Pooled sera from semi-immune individuals were used as positive control and sera from malaria-naïve Europeans with no cross-reaction to all three antigens served as negative controls. A reference standard curve was measured with a serially diluted, purified human polyclonal IgG (The Binding Site, UK) in PBS. Specific Ab concentrations were calculated from the standard curve and are given in μg per ml.

2.6. Assessment of anti-GMZ2, -GLURP and -MSP3 antibody subclasses

To determine the concentration of IgG subclass Abs (IgG1, IgG2, IgG3, and IgG4) ELISA plates were coated as above with GMZ2. Blocking was followed by incubation with the serum samples in serum dilution buffer (1:25 to 1:100). The cytophilic Abs subclasses IgG1 and IgG3 were detected using anti-human IgG1 or IgG3 (Skybio, UK), followed by incubation with goat anti-mouse IgG (Caltag, USA). Ag-specific IgG2 and IgG4 subclass Abs were detected with a HRP conjugated monoclonal anti-human IgG2 (Sigma, Germany) and IgG4 (Caltag, USA). Purified human polyclonal IgG1–IgG4 served as the reference standard (The Binding Site, UK).

2.7. Assessment of anti-parasite immunofluorescence assay

Immunofluorescence assays (IFA) were performed according to published procedures [19]. In brief, synchronized *P. falciparum* strain NF54 were dried and frozen at −80 °C on 10 well multitest slides (Nutacon, NL) at late schizont stage. Serum samples from Days 0, 84 and 365 were tested in serial dilutions (1:40 to 1:640). Parasite-specific Abs were detected with fluorescein isothyocyanate-labelled rabbit anti-human Ig (Dako, USA). Slides were mounted using Vectashield (Vector Laboratories, USA) and read by two independent microscopists on a Leica DMLB microscope equipped with a 100 W HBO lamp (Leica Microsystems, Germany).

2.8. Enumeration of memory B-cells

Peripheral blood mononuclear cells (PBMC) were frozen at Days 0, 84, and 365 to perform a memory B-cell enzyme-linked immunospot assay (ELISPOT). PBMC were separated from heparinised full blood by gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare, USA), counted and frozen in 90% fetal calf serum (FCS Gold, PAA, Germany) and 10% DMSO (Sigma, Germany). Before ELISPOT assays, cells were thawed, counted and seeded at a density of 1×10^6 cells per ml in RPMI 1640 (Sigma, Germany), complemented with sodium pyruvate, non-essential amino acids, L-glutamine, penicillin, streptomycin, 2-mercapto-ethanol (all supplements from Life Technologies), and 10% heat inactivated FCS (FCS Gold, PAA, Germany). Maturation of circulating memory B-cells into antibody secreting cells (ASC) was done by stimulation with 2.5 μg per ml CpG-2006 (TIB-MOLBIOL, Germany) and 10 ng per ml recombinant human IL-15 (R&D Systems, USA) in 24-wells cell culture plates (Corning Costar) for 6 days at 37 °C, 5% CO_2 as described previously [20]. Following maturation, 2×10^5 cells were serially diluted on Ag-coated 96-well plates (Corning Costar). To determine the number of total ASC, anti-human IgG-gamma chain specific antibodies (Sigma, Germany) were coated instead of the Ag, and 2.5×10^4 cells were seeded. PBS-coated wells served as negative control. GMZ2 and goat anti-human IgG were coated overnight at a concentration of 5 μg per ml in PBS. Spots were detected as previously described [21] with minor modifications. Briefly, plates were washed and blocked 30 min with a blocking buffer containing PBS and 3% BSA (Serva, Germany). Meanwhile cells were washed once with HBSS, once with culture medium and counted. After seeding, cells were incubated at 37 °C, 5% CO_2 for 2.5 h to secrete Ab. Thereafter, plates were washed extensively with PBS, 3% BSA, 0.5% Tween 20 and once with PBS to remove cells and debris. Secreted Ag-specific Abs were detected using biotin labelled anti-human IgG antibody (Sigma, Germany, 1:500 in PBS, 3% BSA, 0.5% Tween 20) and streptavidin–alkaline phosphatase (Roche, Switzerland; 1:3000 in PBS). The substrate was applied as AMP-buffer (10.5 2-amino-2-methyl-1-propanol, 0.65 mM $MgCl_2$, 0.01% Triton X-405, pH 10.25), complemented with 1 mg per ml 5-bromo-4-chloro-3-indolyl-phosphate (Sigma, Germany) and 0.75% melted agarose. After 3 h incubation at 37 °C, plates were read on a stereoscopic microscope by two independent investigators.

2.9. Data analysis

Data analysis was mainly descriptive because the number of enrolled participants is small. Immunological data was analysed individually and using non-parametric methods. Differences between time points were assessed using the exact Wilcoxon signed rank test. Group differences were analysed with the Kruskal Wallis Test and if this led to rejection of H_0, pairwise Mann–Whitney *U* tests were done. Correlations were analysed by Spearman's rank correlation. Randomisation of 30 screened individuals to one of the three treatment groups of 10 individuals each was done using permutated blocks with different block size from 3 to 6 using DatInf RandList v1.0.0.107. All other calculations were performed with R v2.9 (www.r-project.org). The *p*-values presented are two-sided, and a *p*-value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Clinical and biological safety

Thirty out of 57 screened volunteers were enrolled and randomised into three groups of ten. Group I, received 10, group II 30, and group III 100 μg of GMZ2 in aluminium hydroxide (Fig. 1). Mean age of the 20 female and 10 male participants was 32.1 years (range: 18.6–45.3). All but one participant in group III received all three injections. The missing injection was due to an accident immediately before the planned third vaccination and was not judged to be related to the candidate vaccine. Two participants were lost to follow-up after the third vaccination: one subject of group II left Germany and one of group III discontinued because of pregnancy. She could not be contacted for the one-year follow-up time point despite repeated attempts. During the study one SAE occurred: a fracture of one thoracic vertebrum that was not judged to be related to the intervention. The participant recovered with sequelae.

Immediate local reactions were frequent but of low intensity: all but one participant experienced at least one local reaction and all reactions were grade 1. One day after the third vaccination six subjects (1 in group I, 1 in group II, and 4 in group III) complained about grade 3 erythema and induration. The participant in group I additionally experienced an oedema at the injection site. Grade 1 erythema (1 participant in group I, following first and second vaccination) and grade 1 (5 in group I, 7 in group II, 5 in group III) as well as grade 2 (2 in group I, 1 in group II, 3 in group III) indurations were reported during the 14 days post-vaccination period. After the third vaccination 1, 4, and 5 participants experienced pruritus that lasted 1–24 h. Within two weeks after vaccination no pain, oedema, or local heat was observed. All adverse reactions resolved within 24 h and all solicited systemic reactions were of grade 1 or 2. Most common grade 2 systemic reactions were headache (2 in group II and 2 in group III), fatigue (2 in group III), and nausea (1 in group III). Seven out of 24 unsolicited adverse reactions were definitely or probably related to the intervention (3 in group I, 1 in group II, and 2 in group III). All consisted of local reactions except for one participant of group III, who experienced a rash on both legs 2 days after the first dose.

3.2. Anti-GMZ2, anti-GLURP and anti-MSP3 Ab concentrations

Ab against GMZ2 and its constituents GLURP and MSP were measured by ELISA. One volunteer of group II did not develop anti-

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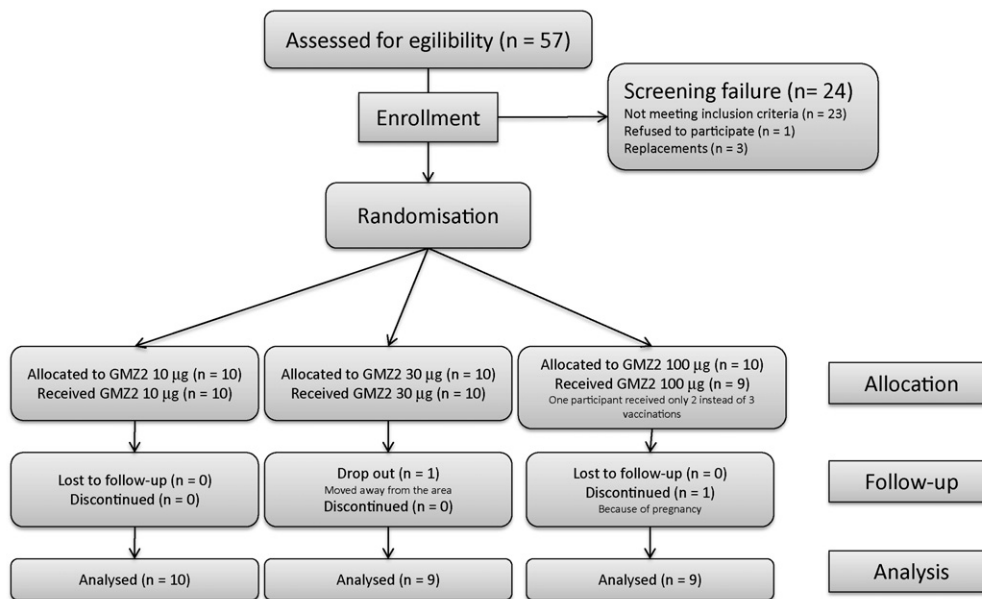

Fig. 1. Study flow of the GMZ2 phase I clinical trial.

bodies against the vaccine Ags during the study period. He was the only participant who reported no local, systemic or unsolicited reactions. The volunteer who received only two vaccinations had a weaker Ab response against all vaccine Ags compared to those who received all three injections. All other individuals had strongly increased Ag-specific Ab responses with a peak at Day 56 or 84 (Fig. 2). The median difference from baseline of anti-GMZ2 Ab concentration after the third vaccination (Day 84) was 1.20 mg per dl (95% CI: 0.83–1.81) in group I, 1.60 (0.95–2.19) in group II, and in group III 1.33 (0.90–1.90). As expected, anti-GLURP and -MSP3 antibodies were similarly increased after the third vaccination (Fig. 2b and c). After one year (Day 365) anti-GMZ2 Ab levels remained significantly elevated in all three groups (group I: $p = 0.009$, group II: $p = 0.004$, group III: $p = 0.004$) although the titre was considerably lower compared to Days 56 and 84. No difference of Ab responses between the groups was obvious, except that the concentration of anti-GMZ2 Abs after the first vaccination differed between the groups (Kruskal Wallis test: $p = 0.045$). Pairwise comparison of the groups showed that only group I and group III significantly differed from each other ($p = 0.016$), where the median for group III was highest. The main GMZ2-specific IgG subclass was IgG1 (Fig. 3), although some modest increase in IgG2 and 3 levels were observed.

3.3. Immunofluorescence analysis

Ab reactivity against *P. falciparum* NF54 parasites in a late schizont stage was determined by IFA on Days 0, 84 and 365. Titres between 1:40 and 1:640 were found. A significant increase in titres from Day 0 to Day 84 in groups II and III ($p = 0.016$ and $p = 0.008$, respectively) was present, whereas in group I no such increase was observed. At peak reactivity no significant group differences were present.

3.4. Assessment of Ab secreting cells by ELISPOT

B-cell memory was measured by ELISPOT. ASC were generated from PBMC and the total number of ASC as well as Ag-specific ASC was counted. All but three individuals developed detectable GMZ2-specific cells (Fig. 4). The participant from group II without detectable amounts of anti-GMZ2 Ab (see above) also did not develop GMZ2-specific cells. The two other non-responders were from group I. ASC number peaked at Day 84 and was still significantly elevated at the one year follow-up visit (group I: $p = 0.031$, group II: $p = 0.008$, group III: $p = 0.004$). No statistically significant difference between groups was present on Days 84 and 365. The number of Ag-specific ASC did not correlate with Ab concentration against the same Ag at Days 84, 365, and when Ab concentration on Day 84 was used to predict ASC number on Day 365.

4. Discussion

In this study we report the first-in-man clinical trial of GMZ2. It is the latest of a series of blood stage malaria vaccine candidates which have been developed starting from the idea of mimicking the naturally acquired immunity in repeatedly naturally exposed individuals. Therefore, the target population of GMZ2 are infants and children in endemic areas. In this population reduction in parasite multiplication rather than sterile immunity is the primary aim, because temporary but complete protection through immunity against the hepatic stage might reduce the encounter of blood stage parasite and the immune system to a level that leads to a rebound of malarial episodes with an increased incidence of complicated and life-threatening malaria later in life. Since healthy children are vaccinated, safety expectations are extremely high and the economic situation of most malaria-endemic countries requires that the administration of a new malaria vaccine can be embedded in existing vaccination programs. At the moment, a number of new experimental adjuvant and delivery systems are clinically tested for malaria vaccine candidates and other diseases by not-for-profit and profit-oriented companies [22]. Obviously, no long-term safety data are available for these approaches. Efficient pharmacovigilance systems are not implemented in almost all malaria-endemic countries, therefore we believe that highly experimental approaches are scientifically of enormous value but implementation of such systems in economically weak countries with only a basic health care infrastructure (the primary target of potential malaria vaccines) is problematic.

Alum, the adjuvant used in the current trial, is registered by the U.S. Food and Drug Administration (FDA) and has been used extensively in experimental and commercial vaccines since six decades [23]. Therefore its safety profile is well known. GMZ2 is expressed

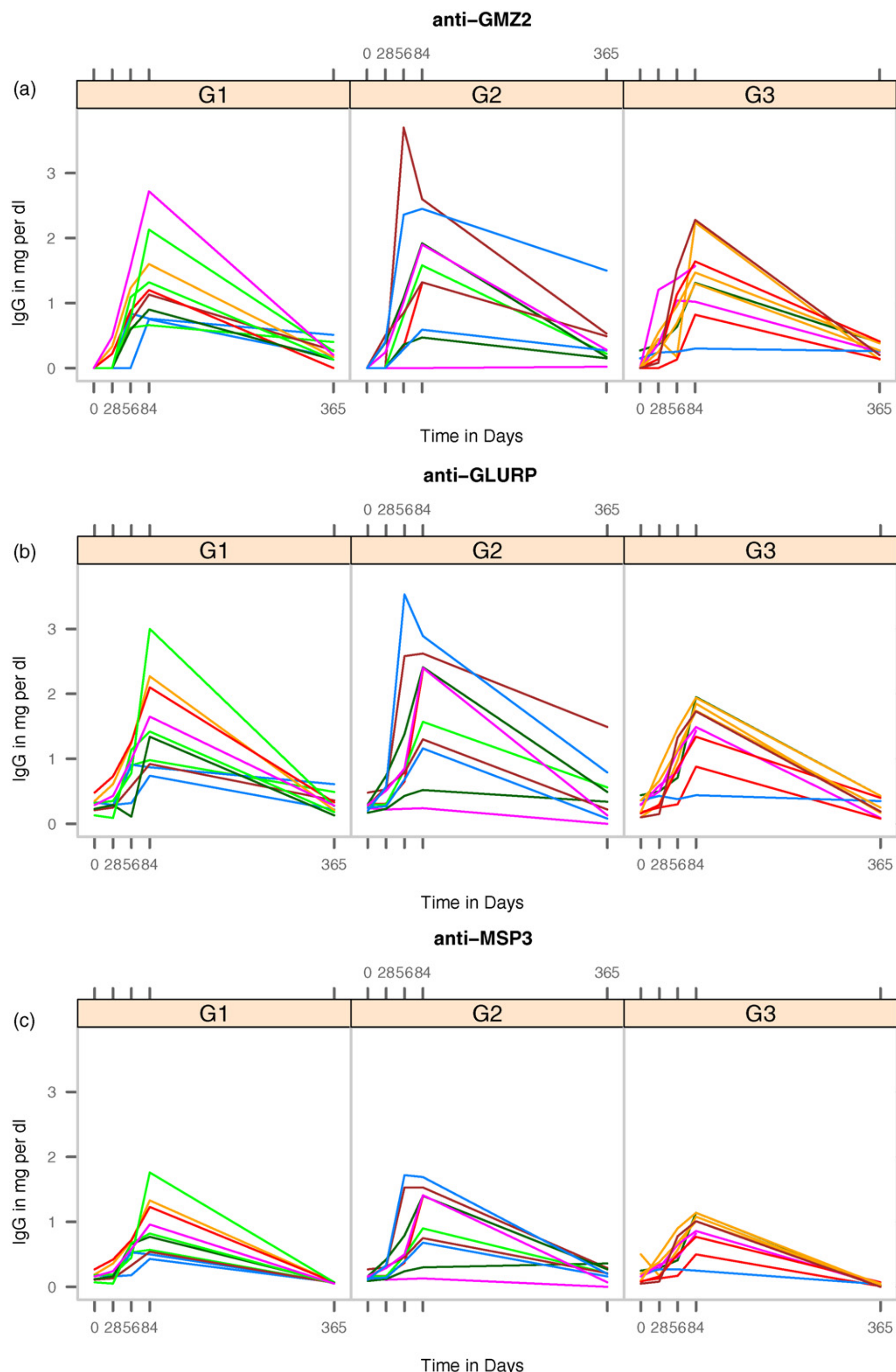

Fig. 2. Concentration of anti-GMZ2 (a), -GLURP (b) and -MSP3 (c) Abs throughout the study. Concentrations are given as mg of coated IgG control per dl for each individual in groups I (10 μg), II (30 μg) and III (100 μg). Vaccinations were given at Days 0, 28, and 56.

in *L. lactis*, a non-pathogenic gram positive bacterium widely used by the dairy industry, where extensive experience with this organism is present. Its immune-stimulatory potential is low, although some adjuvantive effect was shown [24]. Currently, it is explored as a whole cell vaccine delivery system. In general GMZ2 vaccination was well tolerated and produced very few adverse reactions. Most were local and since we administered GMZ2 subcutaneously, the rate was expected to be higher than after intra-muscular injection, which would be used in the final product. If GMZ2 in its current formulation proves to be efficacious, it will be very interesting to assess the additive effect of new and well tested adjuvants on GMZ2 efficacy and associate those findings to immunological markers, particularly Ab subclass distribution, growth inhibition, and memory B-cell responses.

The use of GLURP- as well as MSP3-sequences contained in GMZ2 has been reported in previous phase I clinical trials [19,25]).

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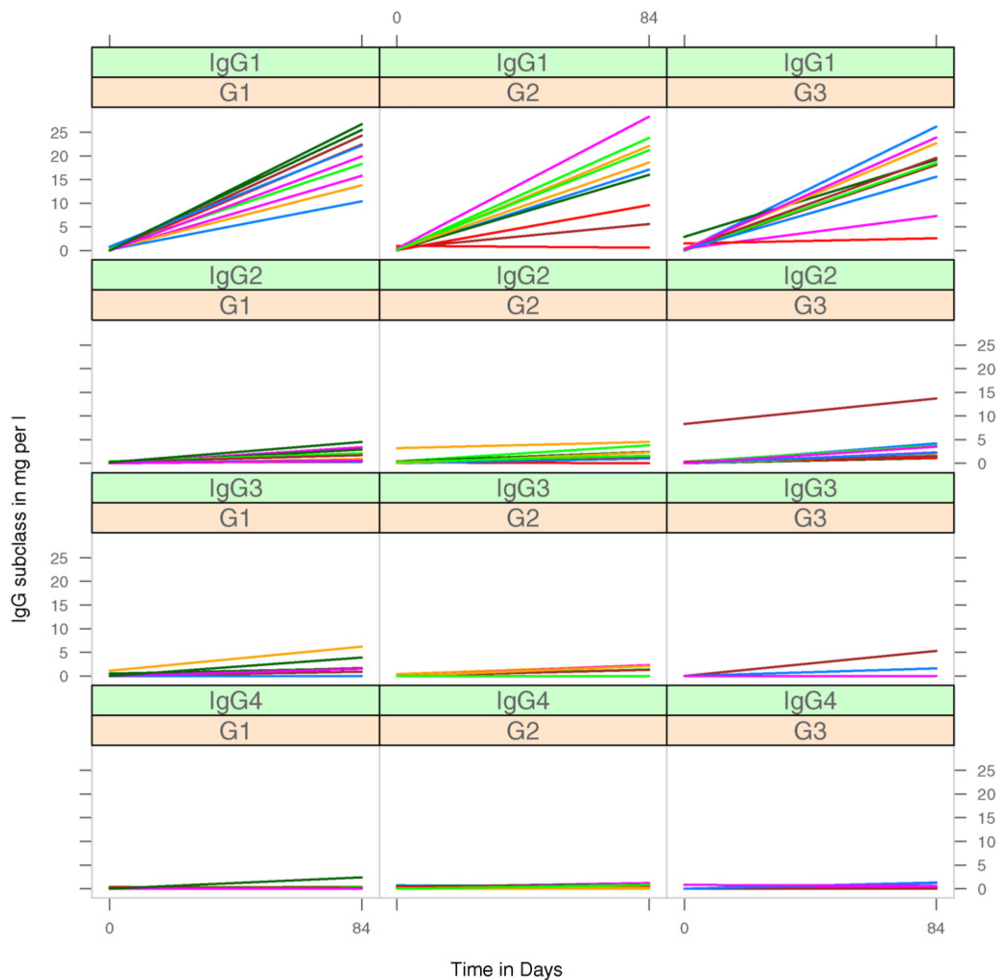

Fig. 3. Concentration of anti-GMZ2, -GLURP and -MSP3 subclass Abs on Days 0 and 84 (one month after the third vaccination). Concentrations are given as μg of coated IgG subclass control per ml for each individual in groups I (10 μg), II (30 μg) and III (100 μg). Vaccinations were given at Days 0, 28, and 56.

In both cases long synthetic peptides were given together with either alum or Montanide ISA 720 as adjuvants. As in the current study, alum was well tolerated but Montanide ISA 720 was not so. Comparison of some of the samples from those studies showed that Ab levels against the respective Ag were similar to those achieved with GMZ2 (data not shown). Nevertheless, the main disadvantage of long synthetic peptides is their difficult and expensive synthesis. In addition, size restrictions and non-controlled folding of the peptides can be problematic and led to the discontinuation of this approach.

Since GMZ2 is a synthetic fusion of parts of two proteins, expressed in a heterologous organism it is of particular interest if antigenic properties are similar to those of their *P. falciparum* counterparts. Pre-clinical testing in splenectomized *S. sciureus* showed very good immunogenicity and partial protection when Freund's adjuvant was used [17]. The GLURP–MSP3 fusion showed better

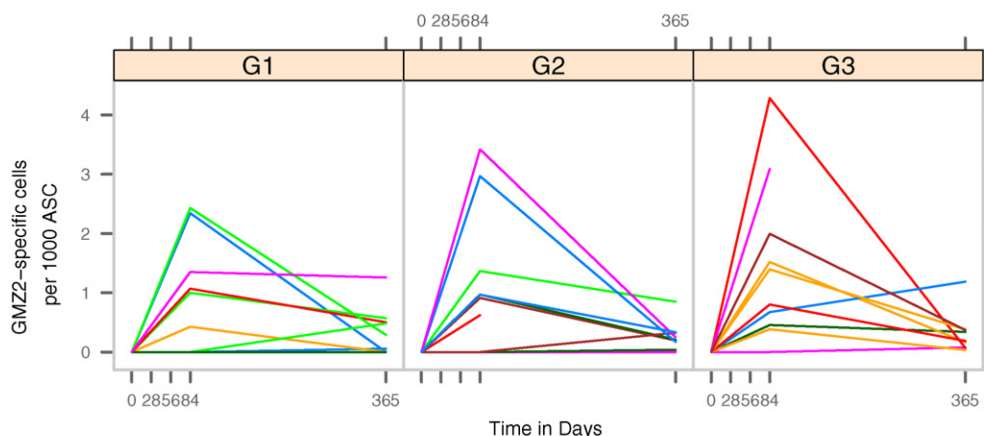

Fig. 4. Memory B-cell ELISPOT on Days 0, 84, and 365. Results are depicted as Ag-specific memory B-cells per 1000 antibody secreting cells.

immunogenicity compared to the administration of the two subunits either at the same time or one after the other, although growth-inhibitory activity was similar [16]. In the current clinical trial we observed an increase in anti-schizont immune-reactivity of sera similar to the pre-clinical studies and, in addition, measured good Ab titres against GLURP and MSP3 expressed in a different expression system (*Escherichia coli*). Ab concentrations against all vaccine Ags after the three vaccinations were similar to the level seen in a random sample of semi-immune adults from Lambaréné, Gabon, an area with high malaria endemicity [26,27].

Unfortunately, it is not known if and how GMZ2 induced immune responses reduce parasite multiplication in naturally exposed individuals. The lack of surrogate markers extents to the whole field of malaria research and should re-appear on the agenda of current and future efficacy trials on malaria vaccine clinical trials. Most evidence comes from notoriously artificial animal models and associative studies that do not allow deducing causative relationships. We extended the immunological test set-up in malaria vaccine clinical trials by implementation of an Ag-specific memory B-cell ELISPOT. Since mature ASC are difficult to access we generated them from circulating memory B-cells by maturation in the presence of IL15 and CpG [20]. The assay proved to be very robust and clearly showed the vaccine response in all but three participants: one individual that did not respond at all and two individuals from group I (the low dosage group). A response is still seen one year after the first vaccination and indicates that memory B-cells may contribute to long-term memory against the candidate vaccine. If and how Ab-affinity and antiparasitic action is different after memory B-cell maturation after booster vaccinations or natural exposure should be investigated in future studies. Interestingly, no significant correlation between Ab concentrations and the amount of Ag-specific ASC was seen after vaccination. On first sight this seems paradox but in a previous study a similar lack of association was seen with other vaccines [28]. We believe that this and additional experiments should be implemented, validated and miniaturized to be tested in efficacy clinical trials for their capacity to serve as a surrogate or at least a predictive marker of vaccine efficacy.

In conclusion, the GMZ2 malaria vaccine candidate was safe and immunogenic and the clinical development program is ongoing. Next steps would be safety and subsequent efficacy clinical trials in the target population in malaria-endemic countries.

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