Vaccine 35 (2017) 6218–6227

Contents lists available at ScienceDirect

Vaccine

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

Safety and immunogenicity of a recombinant *Plasmodium falciparum* AMA1-DiCo malaria vaccine adjuvanted with GLA-SE or Alhydrogel® in European and African adults: A phase 1a/1b, randomized, double-blind multi-centre trial

S.B. Sirima [a], C. Durier [b], L. Kara [c,d], S. Houard [i], A. Gansane [a], P. Loulergue [c,d], M. Bahuaud [f], N. Benhamouda [g,h], I. Nebié [a], B. Faber [j], E. Remarque [j,1], O. Launay [c,d,k,1,*], The AMA1-DiCo Study Group[2]

[a] *Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso*
[b] *INSERM SC10-US19, Villejuif, France*
[c] *INSERM CIC 1417, F-CRIN, I-REIVAC, Paris, France*
[d] *Assistance Publique –Hôpitaux de Paris (AP HP), Hôpital Cochin, CIC Cochin-Pasteur, Paris, France*
[i] *European Vaccine Initiative (EVI), Heidelberg, Germany*
[f] *AP HP, Hôpital Cochin, Plateforme d'immuno-monitoring vaccinal, Laboratoire d'Immunologie, Paris, France*
[g] *INSERM U970, Paris, France*
[h] *AP-HP, Hôpital Européen Georges Pompidou, Service d'Immunologie Biologique, Paris, France*
[j] *Department of Parasitology, Biomedical Primate Research Centre, Rijswijk, The Netherlands*
[k] *Université Paris Descartes; Sorbonne Paris-Cité, Paris, France*

ARTICLE INFO

Article history:
Received 9 September 2016
Received in revised form 28 July 2017
Accepted 7 September 2017
Available online 22 September 2017

Keywords:
Malaria
Plasmodium falciparum
Apical membrane antigen 1
Vaccine trial
Immunogenicity
Adjuvants
Safety

ABSTRACT

Background: *Plasmodium falciparum* Apical Membrane Antigen 1 Diversity Covering (*Pf*AMA1-DiCo) candidate vaccine is a formulation of three recombinant variants of AMA1 designed to provide broader protection against parasites with varying AMA1 sequences.
Methods: In this staggered phase Ia/Ib randomized, double blind trial, healthy French adults received AMA1-DiCo with either Alhydrogel® (n = 15) or GLA-SE (n = 15). Following a safety assessment in French volunteers, GLA-SE was chosen for the phase Ib trial where healthy Burkinabe adults received either AMA1-DiCo/GLA-SE (n = 18) or placebo (n = 18). AMA1-DiCo (50 μg) was administered intramuscularly at baseline, Week 4 and 26.
Results: AMAI-DiCo was safe, well tolerated either with Alhydrogel® or GLA-SE. In European volunteers, the ratios of IgG increase from baseline were about 100 fold in Alhydrogel® group and 200–300 fold in GLA-SE group for the three antigens. In African volunteers, immunization resulted in IgG levels exceeding those observed for the European volunteers with a 4-fold increase. DiCo-specific IgG remained higher 26 weeks after the third immunization than at baseline in both European and African volunteers. Induced antibodies were reactive against whole parasite derived from different strains.
Conclusion: AMA1-DiCo vaccine was safe and immunogenic whatever the adjuvant although GLA-SE appeared more potent than Alhydrogel® at inducing IgG responses.
Clinical Trials Registration. ClinicalTrials.gov NCT02014727; PACTR201402000719423.

1. Introduction

Apical Membrane Antigen 1 (AMA1) is a protein of apicomplexan parasites with an essential role in host cell invasion [1]. The AMA1 ectodomain is an important *Plasmodium falciparum* blood-stage vaccine target and antibodies against the ectodomain have been shown to interfere with AMA1 processing and prevent red cell invasion *in vitro* [2–6]. This effect requires immunization with correctly folded AMA1 [7,8]. The challenge however is the extreme sequence diversity of AMA1. In a single trial site in Mali,

* Corresponding author at: CIC Cochin Pasteur, Bâtiment Lavoisier, Hôpital Cochin, 27 rue du Faubourg St Jacques, 75679 Paris Cedex 14, France.
E-mail address: odile.launay@aphp.fr (O. Launay).
[1] These two authors contributed equally to the manuscript.
[2] See complete list of the AMA1-DiCo Group co-authors in the online version.

https://doi.org/10.1016/j.vaccine.2017.09.027
0264-410X/

214 AMA1 variants were identified among 506 subjects [9]. With a database currently at 2372 entries, we have identified 841 unique AMA1 variants and 140 polymorphic amino acid positions in the ectodomain [E. Remarque; unpublished]. The importance of this polymorphism is best illustrated by the outcome of a vaccine trial with a single allele vaccine; overall vaccine efficacy was only 17% whereas efficacy against the homologous (3D7) allele was 64% [10].

The AMA1 Diversity Covering (DiCo) proteins were designed to overcome AMA1 polymorphism. To this end, three artificial protein sequences were constructed incorporating a high degree of variation based on 355 sequences available at the time of design [11]. Immunization with a mixture of three DiCo proteins yielded antibodies capable of inhibiting *in vitro* growth of a panel of AMA1 variants in rabbits and non-human primates [11–13]. Studies in monkeys were done to compare the potency of different adjuvants available at the time (CoVaccine HT). It was subsequently found that continuous access to the patented CoVaccine HT adjuvant was not guaranteed, so we opted for another adjuvant with guaranteed access (GLA-SE). Rabbit studies were therefore performed with GLA-SE and Alhydrogel, but studies were not repeated in monkeys with the adjuvants selected for clinical trials.

For comparative reasons, Alhydrogel® is used as reference adjuvant in European Vaccine Initiative (EVI) malaria phase Ia clinical trials. However, *P. falciparum* AMA1 adjuvanted with Alhydrogel® induced modest (inhibitory) antibody responses in European adults [14–16] and failed to protect Malian children from natural exposure [17]. One possibility was to use the adjuvant AS02 which has an acceptable safety profile and yielded about 4-fold higher antibody levels than Alhydrogel [15]. AS02 contains a squalene emulsion, a TLR4 agonist (MPL), saponin (QS21) and tocopherol. Because AS02 was not available for the AMA1-DiCo trial, it was decided to use another adjuvant GLA-SE which contains a squalene emulsion and a TLR4 agonist (GLA). In addition GLA-SE has a guaranteed continuous access. This proprietary adjuvant (Infectious Disease Research Institute Seattle, IDRI, USA) is a stable oil-in-water emulsion containing glucopyranosyl lipid adjuvant (GLA), a synthetic monophosphorylated lipid, which is a Toll-like receptor 4 agonist.

We conducted a phase Ia trial in French volunteers to evaluate the safety and immunogenicity of three doses of 50 μg AMA1-DiCo formulated with either GLA-SE or Alhydrogel® adjuvant. Following a positive safety assessment of the two first doses in all French volunteers, GLA-SE was chosen for the phase Ib trial. Subsequently we conducted a phase Ib in Burkinabe volunteers to evaluate the safety and immunogenicity of three doses of 50 μg AMA1-DiCo formulated with GLA-SE compared to a saline control. Malaria transmission is seasonal, being low during the dry season (November to May) and high during the rainy season from June to October. During the rainy season, the clinical malaria incidence rate in children 2.4 per child-year at risk in children under five years of age with *P. falciparum* accounting for more than 95% of infections [18]. The main vectors are *An. gambiae* and *An. funestus*. From February to May, the number of bites per person per night (Entomological Inoculation Rate; EIR) due to *An. Gambiae s.l.* was negligible. However, the EIR increased from June to September, decreased from September to November and remained low until the next rainy season. Findings from the same area highlighted that targeting domain I of AMA1 revealed the presence of AMA1 alleles without a statistical overexpression of a particular allele [19].

2. Materials and methods

2.1. Vaccine formulations

The investigational vaccine is AMA1-DiCo formulated with the adjuvant GLA-SE (IDRI, USA) or Alhydrogel® manufactured by Brenntag (Denmark), aseptically diluted at Nova Laboratories Ltd (United Kingdom).

AMA1-DiCo vaccine consists of three highly purified recombinant *P. falciparum* AMA1 Diversity Covering proteins (*Pf*AMA1-DiCo 1, 2 and 3). The sequences of the artificial proteins *Pf*AMA1-DiCo 1–3 are derived from the naturally occurring variants of the AMA1-protein in different parasite strains.

AMA1-DiCo is artificial and the sequences of proteins do not match with any variants from AMA1 in natural parasite population; however, when used as a mixture, the AMA1-DiCo proteins induce antibodies that are broadly reactive with many AMA1 variants [11,20]. Similar observations were made for mixtures of natural alleles [12,21]. We hypothesize that this is due to the fact that strain (DiCo)-specific epitopes are diluted out relative to conserved epitopes [20,21]. No interactions between the three DiCo proteins in rabbit immunogenicity studies other than the relative dilution of strain specific epitopes relative to conserved epitopes have been observed.

Each of the three *Pf*AMA1-DiCo proteins was expressed individually in *Pichia pastoris*, purified, mixed in a 1:1:1 mass ratio and freeze-dried to obtain the AMA1-DiCo lyophilized malaria vaccine [22].

The content of one vial of lyophilized AMA1-DiCo was reconstituted at each trial site pharmacy with 0.3 mL saline for injection. For the Alhydrogel® formulation, 0.3 mL Alhydrogel® was added to one vial of reconstituted AMA1-DiCo. Each 0.5 mL dose contained 50 μg AMA1-DiCo and 0.85 mg aluminum. For the GLA-SE formulation, 0.3 mL of diluted GLA-SE was added to one vial of reconstituted AMA1-DiCo. Each 0.5 mL dose contained 50 μg AMA1-DiCo and 2.5 μg GLA in 2% oil.

2.2. Study design

2.2.1. Fast-track clinical trial strategy

To accelerate early stage vaccine development, EVI has designed a fast-track strategy where the first-in-human evaluation is done through a staggered multicenter phase Ia/Ib clinical trial. This allows proceeding quickly with the immunization of the African volunteers of the phase Ib trial after a review of the safety data by an independent data safety monitoring board of the phase Ia first vaccination dose in European adults. It was planned to test both GLA-SE and Alhydrogel adjuvants in the phase Ia and, in view of the low immunogenicity results observed with AMA1 vaccine adjuvanted with Alhydrogel in previous trials [14–16], to drop the Alhydrogel adjuvant in the phase Ib provided safety of the GLA-SE adjuvant was confirmed in the European volunteers. The control arm of phase Ib has received saline.

This staggered phase Ia/Ib, randomized, double-blind, multicenter trial was designed to assess the safety and immunogenicity of three intramuscular injections of 50 μg AMA1-DiCo administered in the deltoid muscle at Day 0, Week 4 and 26 with either Alhydrogel® or GLA-SE adjuvants. Healthy volunteers were included in the following two cohorts: non-exposed European volunteers in France (Centre Clinique d'Investigation Cochin Pasteur, Hôpital Cochin, Paris, Cohort A, n = 30) and malaria-exposed African volunteers in Burkina Faso (Centre National de Recherche et de Formation sur le Paludisme, CNRFP; Ouagadougou, Cohort B, n = 36).

2.2.2. Participants

Participants were healthy males and non-pregnant females aged 20–45 years. Exclusion criteria for all subjects included: symptoms, physical signs or laboratory values suggestive of systemic disorders; positive HIV, HBV and HCV tests. For European volunteers, additional exclusion criteria were: a history of malaria or travel in malaria endemic areas within the past six months; pos-