Randomized clinical trial to assess the protective efficacy of a Plasmodium vivax CS synthetic vaccine

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Article

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Abstract

A randomized, double-blind, controlled clinical trial was conducted to assess the safety and protective efficacy of the *Plasmodium vivax* circumsporozoite (CS) protein. A total of 35 healthy adults, either malaria-naïve (n = 17) or *P.vivax* semi-immune (n = 18), were enrolled and immunized intramuscularly (i.m.) at months 0, 2, and 6, with the *Pv*CS (150 µg) formulated in Montanide ISA-51 adjuvant. Most volunteers developed *Pv*CS specific antibody and T-cell responses and were subjected to a *P. vivax* sporozoite controlled human malaria infection (CHMI) 30 days after the last immunization. Sterile protection was observed in five of 11 naïve (42%) and four of 11 semi-immune (36%) volunteers by showing no parasitemia during the 60 days follow-up, as did a semi-immune control (1/5) volunteer. All non-protected volunteers developed malaria symptoms 10–19 days after CHMI and were immediately treated with antimalarial drugs. This is the first study of a *P. vivax* sub-unit vaccine demonstrating sterile protection against *P. vivax* infection.

Introduction

An estimated ~ 229 million malaria clinical cases and > 409,000 deaths occurred worldwide in 2019, with a substantial economic impact on populations living in developing regions [1]. Epidemiological indicators have shown that *P. vivax* infections remain widely distributed worldwide, representing a significant cause of morbidity and mortality among 2.5 billion people living at risk of disease by this malaria parasite species [2]. Based on substantial evidence that supports their feasibility, vaccines are considered a critical tool to complement classical malaria control strategies that have not worked on their own [3]. *P. falciparum* RTS.S/AS01E, based on the CS protein use, is currently the most advanced malaria vaccine candidate with a mean efficacy of ~ 34% in phase III trials [3–5]. It was approved by the European Medicines Agency (EMA) [6] and is currently undergoing implementation phase in several African countries [7]. A systematic analysis of the *P. vivax* CS *(Pv*CS) antigen has been performed during the last 20 years [8]. After immunological characterization of the *Pv*CS protein and identification of multiple B- and T-cell epitopes [9], three long synthetic peptides (LSP) (N, R, and C) were designed, synthesized, and used in preclinical studies to test their immunogenicity in BALB/c mice and *Aotus* monkeys. These studies demonstrated high immunogenicity in both animal species [9, 10]. Phase I clinical study was then conducted in 69 healthy malaria-naïve volunteers to evaluate the three *Pv*CS LSP in a dose-escalating manner (10, 30, and 100µg/dose), formulated in Montanide ISA 720 [11]. This trial demonstrated good safety and tolerability, and high immunogenicity of the three individual fragments (N, R, and C-term). A second Phase I trial was further conducted in 40 volunteers who were vaccinated with mixtures of the three peptides formulated either in Montanide ISA-720 or Montanide ISA-51 at 50 and 100µg/dose [12]. Vaccine formulations were well-tolerated, and no serious adverse events (SAE) were observed with any of the vaccines. All individuals seroconverted (ELISA) and developed antibodies to all three protein regions, which also recognized the native protein on *P. vivax* sporozoites as determined by indirect immunofluorescent antibody test (IFAT). Moreover, vaccine- antibodies inhibited *P. vivax* sporozoite

invasion (ISI) to liver-cell lines [13]. Further studies allowed the standardization of a *P. vivax* sporozoite controlled human malaria infection (CHMI) in healthy and semi-immune volunteers [14–16].

Moreover, *P. vivax* CS formulations using recombinant products have been developed and tested by other groups in phase I trials, which demonstrated a good safety profile, although displayed low immunogenicity. First, the repeat region of the VK210 *P. vivax* variant was expressed in *E. coli* [17], formulated in Alum, and tested in 13 volunteers (doses of 10-1000 μg/dose); this protein displayed a good safety profile but low and no boostable antibody response detectable by ELISA. Second, a recombinant protein expressing 70% of the *Pv*CS protein sequence was produced in yeast [18], formulated in Alum and tested in doses ranging from 50 to 400 μg/dose (n = 30). Volunteers exposed to the higher doses (200–400 μg/dose) generated minimal humoral and cellular responses. More recently, a hybrid recombinant construct encompassing both VK210 and VK247 repeats alleles [19] was expressed in *E. coli* (VMP001), formulated in AS01B adjuvant, and evaluated in phase I/IIa vaccine dose escalation. Volunteers (n = 30) developed robust humoral and T-cell responses, and a slight delay in patency (1–2 days) was observed in 59% of the volunteers [20].

Herein, a phase IIa/b clinical trial to evaluate the safety and protective efficacy of *Pv*CS LSP formulated in Montanide ISA-51 adjuvant in healthy malaria-naïve, and semi-immune volunteers are described.

Methods

Ethics statement

The study protocol was reviewed and approved by the Institutional Review Boards of the Malaria Vaccine and Drug Development Center (CECIV-MVDC) and Centro Médico Imbanaco (CMI # 0992304-493-26202) (Appendix 1). The study complied with the Declaration of Helsinki principles, International Conference on Harmonization, Good Clinical Practices guidelines, and all pertinent Colombian regulations. All participants provided written informed consent (IC) and were advised that they were free to withdraw from the study at any time. Volunteers were excluded if they had diseases or medical conditions that would alter the vaccine's assessment or any condition that could increase the risk of adverse outcomes.

Study design and participants

This was a phase IIa/b randomized, double-blind, comparative, controlled trial to evaluate the protective efficacy and safety of *P. vivax* CS protein formulated in Montanide ISA-51 adjuvant. Thirty-five healthy, Duffy positive (Fy+) men and non-pregnant women (19-44 years of age) were recruited from a larger group (n=121) and allocated into two groups: healthy malaria-naïve (n=17) and malaria semi-immune (n=18) volunteers previously exposed to *P. vivax*. Study participants were recruited based on the pre-specified (study protocol) inclusion and exclusion criteria. The eligibility criteria are provided in the Appendix 3.

Participants were recruited from 03 October 2014 (first patient in) until 22 December 2014 (Last patient in). Participants were randomly (simple) assigned in a 2:1 ratio. A blinded data manager controlled the allocation to receive the vaccine (Experimental; Exp, n=25) or placebo (Control; Ctrl, n=10) (Figure 1). Access to the randomization code was strictly controlled at the pharmacy. The naïve group was further divided into Exp (n=12) and Ctrl (n=5) and the semi-immune group into Exp (n=13) and Ctrl (n=5). Naïve volunteers were from Cali (Colombia), a non-malaria endemic city, with eligibility based on no history of malaria and negative serology against *P. vivax* blood stages. Semi-immune volunteers were recruited from Buenaventura, a low to moderate malaria-endemic area of Colombia; eligible volunteers should have had a history of malaria and antibodies against *P. vivax* blood stages with titers higher than 1:20 by IFAT or higher than 1:200 by ELISA using a recombinant *P. vivax* MSP1 (Pv200L) protein [21].

Vaccines

The three LSP (N, R, and C) synthesized under good laboratory practices (GLP) conditions at the Biochemistry Institute, University of Lausanne, Switzerland, were packaged, lyophilized, and then tested for sterility and apyrogenicity. As previously described [13], the N polypeptide corresponded to N-terminal amino acids (aa) 20-96, the C peptide to C-terminal aa 301-372. In contrast, the R peptide VK210 (type I) corresponded to a construct based on the first central non-peptide repeat (aa 96-104) in tandem three times and collinearly linked to a universal T-cell epitope (ptt-30) derived from tetanus toxin [22-24]. A 1:1:1 peptide mixture (50µg/each peptide) was emulsified in Montanide ISA-51 (Seppic Inc., Paris, France) in the same proportion according to manufacturer recommendations on the same day of subject immunizations. Saline solution (Baxter, Deerfield, IL) was emulsified with the same adjuvant and used as a placebo.

Interventions

The primary outcome measure was to assess the *P. vivax* CS LSP vaccine's protective efficacy to the *P. vivax* CHMI in malaria-naïve and semi-immune volunteers, and the secondary outcome, the immune response associated with protection. Eligible participants were enrolled to receive three doses of vaccines at months 0, 2, and 6 containing the mixture of LSP (150µg/dose) or placebo, formulated in the Montanide ISA-51 adjuvant by i.m. injection in the deltoid muscle with a volume of 0.5mL. The first immunization dose contained a mixture of peptides N and C only (50µg/peptide; total dose 100µg/dose), whereas for immunizations second and third, the doses comprised peptides N, R and C (50µg peptide/dose; a total of 150µg protein/dose). Control groups were immunized with the placebo (saline solution) formulated in the same adjuvant's total volume. Vaccines were prepared by staff researchers not involved with patient care.

Safety

During the hour following immunization, volunteers were under direct medical supervision to detect any adverse reaction to the vaccine injection, after which a physical examination was performed. Eight hours after vaccination, volunteers' physical status was assessed by a telephone call. Also, a personal follow-up

was performed one week before the following immunizations. Clinical laboratory tests were performed to evaluate vaccine tolerability and safety at months 0, 1, 2, 3, 6, 7, 9, and 10. Blood samples were collected to determine Fy blood group, G6PD deficiency test, complete blood count (CBC), prothrombin time (PT), partial thromboplastin time (PTT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, alkaline phosphatase, blood urea nitrogen (BUN), creatinine, and a pregnancy test in women.

Volunteers were also under observation for one hour after the CHMI and then by phone monitoring eight hours after and once a day until day four. Volunteers were then evaluated daily for clinical manifestations and microscopic patent parasitemia from days five to 30 after challenge and later on, every second day until day 60. Two experienced, independent microscopists evaluated parasitemia by counting the number of asexual *P. vivax* parasites per 400 white blood cells (WBC), assuming normal WBC counts (8000 cell/mL). Samples were considered negative after observing 200 microscopic fields, and qPCR was performed subsequently for retrospective analysis. Adverse Events (AE) were recorded, graded and classified according to FDA recommendations [25].

Sporozoite production

Whole blood (15mL) was collected by venipuncture (Vacutainer tubes, Becton Dickinson, NJ, USA) from patients diagnosed with VK210 *P. vivax* in Leticia, Colombia, and used to infect colonized *Anopheles albimanus* mosquitoes. Fed mosquito batches were dissected and microscopically examined for the presence of oocysts in the midgut (day 7) and sporozoites in salivary glands (day 14) as previously described [26]. Only batches with ≥60% sporozoite infection rates were considered acceptable for CHMI to evaluate the protective efficacy. CHMI was performed three months after the last immunization by volunteer's exposure to 2-5 *P. vivax* infected-mosquito bites, as previously described [15,16]. After human-biting, each mosquito's dissection confirmed blood in midguts and sporozoites in salivary glands [27].

Humoral response

Antibody response was assessed using blood samples collected on months 0, 1, 2, 3, 6, 7 and 10 and measured by enzyme-linked immunosorbent assay (ELISA) using as antigen the N, R, or C peptides (1µg/mL), as described previously [11]. Controls were selected from a pool of sera from semi-immune blood donors (positive ctrl) and a pool of sera from malaria-naïve donors (negative ctrl). Also, peptide-specific IgG isotypes were determined using sera collected at months 0, 7, and 10 from immunized volunteers by ELISA [13]; titers <1:200 were considered negative. In addition, anti-LSP antibodies' parasite recognition was determined at 0-, 7-, and 10-months using an immunofluorescence assay (IFAT) as described before [11].

IFN-g ELIspot production

Peripheral blood mononuclear cells (PBMC) collected at months 0, 1, 2, 3, 6, 7, and 10 were separated from whole blood using Ficoll-Histopaque (Sigma-Aldrich, St. Louis, MO) density gradients and used to determine the IFN-γ-producing cells as previously described [12]. Fresh PBMCs (4×10^5 /well) were mixed

with 10 µg/mL of each LSP, and after 40 h culture, spots were counted with an ELISpot reader (AID Autoimmun Diagnostika GmbH, Germany), and the results expressed as the mean number of IFN-γ spot-forming cells (sfc) per 10^6 PBMC. Volunteers were considered responders if the number of sfc in their samples have increased from their baseline level (before immunization on day 0); any increase ≥ 5 sfc was considered positive [12].

Statistical analysis.

Data were collected and managed using REDCap (Nashville, TN, USA) electronic data capture tools, analyzed using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA), and plotted using Graph Pad Prism version 6.0 (GraphPad Software, San Diego, California, USA). The main outcome evaluated the frequency of *P. vivax* infection in volunteers vaccinated with *Pv*CS LSP formulated in Montanide ISA-51. The sample size was calculated with a confidence level of 95%, an error of 5% and an estimated prevalence of the Fy+ genotype of 30% with a population census of 5,603 in afro-descendent subjects from Buenaventura and 78% from Cali [28]. All naïve and semi-immune participants were Fy+. Immunization's protective efficacy was assessed at a 5% significance level and 80% power. Nominal variables were analyzed using descriptive statistics. Mann-Whitney U or the Kruskal-Wallis tests were used as needed. Fisher's exact test was used to compare proportions. Spearman's rank correlation was used to assess the correlation between numeric variables. Incubation and prepatent periods were determined by microscopy and qPCR and visualized using Kaplan-Meier estimator. Antibody titers and IFN-g production were compared among groups at several points in the study using Wilcoxon signed-rank and the Kruskal-Wallis test. Two-tailed, non-parametric p values ≤ 0.05 were considered significant.

Results

Volunteers enrollment and retention: Aiming to enroll 52 volunteers from malaria-endemic and non-endemic regions, a total of 121 subjects were asked to participate in the trial. After 86 were excluded, 35 volunteers were eligible and enrolled. Seventeen volunteers were allocated to the naïve group (12 Exp + 5 Ctrl) and 18 to the semi-immune groups (13 Exp + 5 Ctrl). All 35 volunteers were immunized with the *Pv*CS LSP vaccine or placebo formulated in Montanide ISA-51. After the first immunization, two volunteers withdrew (one from the naïve and one from the semi-immune groups). One more volunteer from the semi-immune group had to be dropped-out because of a diabetes mellitus diagnosis considered an SAE not-related to the immunization. Therefore, for the rest of the study (immunizations 2 and 3, and CHMI), the groups were composed of (11Exp +5 Ctrl) in each naïve and semi-immune groups (Figure 1). The mean age at enrollment was 31 years old (19-44 range). The median range was 30 years old (19-44 range) for women and 32 years old (20-43 range) for men (Table 2).

Vaccine and CHMI safety

The vaccine was safe and well-tolerated. Mild (grade 1) to moderate (grade 2) local pain was the most frequently (75%) reported AE during vaccination (24/32), followed by headache in 31.25% (10/32) and

malaise in 31.25% (10/32), which resolved in all cases the next day. Pain occurred more frequently in the Exp (16 subjects) than in the Ctrl group (8 subjects). Fever, nausea, chills, diarrhea, and abdominal pain occurred at low frequencies (1-3 subjects) during the vaccination period (Table 1).

Mild to moderate biochemical or hematological laboratory-related AE were observed. Mild anemia (10.7-11.5 g/dL. Normal values: >12g/dL) occurred in two females (naïve group), and two (one male/one female) (semi-immune group) volunteers after the first immunization; however, all normalized before the CHMI. Transient low-level proteinuria (Grade 1-2) was observed after the second immunization in the naïve group but reached normal values in the following month. Two semi-immune group volunteers presented prolonged thromboplastin time (37.7 and 39.3 sec.; normal value: 25-35 sec.). After the third immunization, a volunteer showed glycosuria of 500 mg/dL and glycosylated hemoglobin of 8.1%; (HbA1c: normal value: 5.7%) and was diagnosed with diabetes mellitus not related to vaccination (Appendix 2). All laboratory tests for the remaining participants were within the normal range during the study period. The CHMI was well tolerated, and minor AEs were recorded. No SAEs related to malaria infection were recorded.

Vaccine Immunogenicity

Positive ELISA using the N- and C-peptides indicated seroconversion of all 22 naïve and semi-immune volunteers after the first immunization. However, lower reactivity was observed in the semi-immune than in the naïve group. The second immunization induced a moderate boosting of antibodies to the amino (N-) and carboxyl (C-) fragments, as well as priming of the response to the repeat (R) region; however, only the naïve group displayed a non-significant increase ($p < 0.093$). After the third immunization, a slight but significant boosting of antibodies to the three fragments was observed in both the naïve and semi-immune groups; however, this antibody increase was better in the naïve than in the semi-immune group (N-peptide, $p < 0.045$; R-, $p < 0.01$; C-, $p < 0.016$). All control volunteers remained seronegative during the immunization phase (Figure 3).

Single-cell IFN-g production by PBMC was similar in all volunteers from the experimental groups (naïve $p=0.66$; semi-immune $p=0.99$) with increased IFN-γ production during the immunization phase. However, after the third immunization, a significant difference was found between naïve and semi-immune groups IFN-g production in response to stimulation with the N terminal peptide ($p=0.002$) and the C terminal one ($p= 0.0194$). At this time, IFN-γ production in response to the R-peptide stimulation (post-second immunization) did not show any significant difference between naïve and semi-immune groups ($p=0.5332$). Finally, there was no boosting of the IFN-γ response upon sporozoites CHMI; instead, there was a decrease of this cytokine's levels in both groups after CHMI. This decrease was highly significant in the naïve group (N-peptide, $p=0.0001$, R-, $p=<0.0001$, and C-, $p=0.0367$) whereas it was non-significant in the semi-immune group (N-peptide, $p=0.767$, R- $p=0.189$, and C-, $p=0.293$) (Figure 4).

Vaccine efficacy

The study's primary outcome was the *P. vivax* infection's frequency as a measure of the *Pv*CS LSP vaccination's protective efficacy against *P. vivax* infection in malaria-naïve and semi-immune volunteers. From days 14 to 19 after CHMI, symptoms consistent with malaria like fever, chills, headache, and profuse sweating were shown by 6/11 and 5/5 volunteers of the naïve Exp and naïve Ctrl groups, respectively, and 9/11 and 4/5 volunteers of the semi-immune Exp and semi-immune Ctrl groups respectively, which lasted until malaria treatment. Protective efficacy determined by the presence of patent microscopic parasitemia was observed in five of 11 naïve Exp volunteers (42%) and four of 11 semi-immune Exp volunteers (36%), who did not develop parasitemia during the 60-day follow-up. One volunteer from the semi-immune Ctrl group did not develop parasitemia. In contrast, the remaining four controls were confirmed to develop blood-stage *P. vivax* parasite infections between days 15-17. All non-protected volunteers from experimental groups developed patent infection between 12-19 days after CHMI with a mean prepatent period of 15.71 days (range 14-16 days) in the naïve Exp and 15.88 days (range 12-19 days) in the semi-immune Exp group (Table 2 and Figure 2). Survival analysis showed that sterile protection was observed in four of 11 naïve volunteers (36.6%) and three of the 11 semi-immune volunteers (27.3%) who did not show parasitemia after 60 days follow-up.

Discussion

This phase IIa/b vaccine trial provides evidence that the immunization of malaria naïve and semi-immune volunteers with mixtures of LSP derived from *Pv*CS formulated in Montanide ISA-51 is safe, well-tolerated, immunogenic, and induces sterile protection. To our knowledge, this is the first report of a comparative *P. vivax* clinical trial inducing sterile protection in naïve (36.6%), and semi-immune (27.3%) volunteers against CHMI, as determined by the absence of malaria symptoms or patent parasitemia during the 60 days of close follow-up.

The safety, tolerability and immunogenicity observed are consistent with previously reported vaccination studies with similar *P. vivax* CS derived LSP formulated individually in Montanide ISA-720 [11] or as peptide mixture in Montanide ISA-720 or Montanide ISA-51 [12]. Furthermore, our results also show safety and reproducibility of the sporozoite induced CHMI [16] as well as the efficacy of the antimalarial treatment to radically cure the *P. vivax* infection as determined by the absence of clinical relapses during the close follow-up period (60 days) in any of the study participants who required anti-parasite treatment upon patent malaria confirmation. However, one of the semi-immune Ctrl group participants developed parasitemia (SAE) about two months after returning to the endemic areas, probably due to reinfection. It is striking that one of the semi-immune Ctrl group volunteers did not develop parasitemia. It might have been due to mosquito failure to infect the volunteer, as observed in a previous CHMI trial [15], or that the volunteer could have taken antimalarials drugs or beverages, which could not be documented.

A previous trial using the VMP001 *Pv*CS vaccine formulation based on a recombinant *Pv*CS protein expressed in *E. coli* formulated in ASO1-B (GSK) showed it safe, well-tolerated, and immunogenic with a slight delay in the prepatent period [19]. In that dose-escalation (15, 30 and 60 µg/dose) trial, volunteers with higher immunogen doses presented a delay (two days) in parasite patency in 59 % of the vaccinees.

Here control volunteers, both naïve and semi-immune, developed a similar mean prepatent period (naïve Exp = 15.8 day) (semi-immune Exp = 16.8 days), and none of the volunteers in the experimental groups (naïve Exp and semi-immune Exp) developed significant partial delays. Another remarkable observation was the semi-immune volunteers' apparent hypo-responsiveness to the vaccine, as indicated by the significant differences observed between the naïve and semi-immune Exp groups in the specific IgG and IFN-γ responses. Recent malaria vaccine trials have also demonstrated differential immune responsiveness to pre-erythrocytic vaccine candidates (*Pf*-RTS,S; R21) in inhabitants from malaria-endemic and non-endemic areas [29], suggesting that individuals from malaria-endemic regions, either actively infected or not, display an altered basal immune status with a paucity of regulatory mechanisms and altered memory cell function leading to lower responsiveness to vaccines [30–32]. In addition, it is likely that other hosts- and environmental-factors such as sex, ethnicity, nutrition, malaria endemicity, and others influence host-immune response and immunity to malaria. Another intriguing observation was the significant decreasing trend of IFN-γ secretion displayed by most individuals exposed to sporozoite CHMI (Fig. 3). This striking result might be related to the volunteers' exposure to mosquito's saliva as some studies have shown that after biting, saliva can trigger different effects on humans' immune cells in mice grafted with human hematopoietic stem cells directly by the Th1/Th2 response [31, 33].

Importantly, AEs related to vaccination occurred with similar frequency in all groups and most volunteers; these were limited to local, transient pain at the vaccine injection site, in agreement with those reported for commercially available vaccines [38], with systemic AEs consisting of malaise and headache [34]. This mild reactogenicity has already been reported in previous studies using the same immunogen in > 100 naïve volunteers [22, 35]. In contrast, unacceptable reactogenicity previously described in HIV-derived (peptide cocktails) and *Pfs*-25 (recombinant protein) vaccines formulated in Montanide adjuvant was not observed here [36]. Only minor reactogenicity, consistent with previous phase I vaccine trials conducted with the same formulations, was observed [12]. These results agree with a significant number of vaccine trials conducted on different antigens formulated in Montanide ISA-51 with or without or with minor reactogenicity [37, 38]. The inclusion of semi-immune volunteers in this study represented an exceptional logistic challenge. Despite volunteers being from a malaria-endemic region located only 70 miles from the research center in Cali, the numerous visits during enrolment and vaccination periods and their retention post-CHMI provided essential hurdles to completing the study. Although semi-immune volunteers were requested to be outside the endemic area for the parasitemia follow-up, some decided to return to their homes about a month after CHMI, making follow-up more demanding. Therefore, one of the limitations of the study was volunteer retention. Nevertheless, it provided a unique opportunity to directly assess the vaccine candidate's protective efficacy in volunteers previously exposed to natural *P.vivax* infections, representing a proof-of-principle for further Phase IIb trials.

This study sample is not representative of the whole population of individuals living in the country's malaria-endemic areas; thus, a new trial with a larger volunteer group is being conducted in parallel in malaria-endemic and non-endemic regions (NCT 04739917). This upcoming study will also address ethnicity, the potential immunological imbalance caused by permanent exposure to malaria parasites, mosquito bites and other environmental factors in endemic areas to identify immune-signatures that may

explain and predict the hypo-responsiveness to malaria vaccination and its effect on vaccine-elicited immunity.

Declarations

Data availability

Data that support the study is into the REDCap database, available from the corresponding author upon reasonable request. This trial is registered on ClinicalTrials.gov under the identifier NCT02083068.

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Competing Interest

The authors declare no competing interest.

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Tables

Table 1. Number of volunteers reporting vaccine-related adverse events in experimental and control groups

		Naïve	Placebo	Semi-immune	Placebo	Total
Adverse events	# doses	I II III	I II III	I II III	I II III	
	n	(11)	(5)	(11)	(5)	**64**
Local						
Injection site pain		2 1 5	2 1 1	2 3 3	2 2	24
Swelling		1 1				2
Systematic						
Headache		2 1	1 1 1	1 1 1	1	10
Malaise		1 1	1 1	1	2 2 1	10
Fever		1	1	1	1	4
Nausea/Emesis		1		1 2	1	5
Chills				2		2
Diarrhea		2	1	1		4
Abdominal pain		1			1	2

Table 2. Baseline characteristics of volunteers

	Group Code	Gender	Ethnicity	Age at enrollment	Number of immunizations	Pre-patent period (Days)	Parasite /uL (Microscopy)
NAIVE	**CS1001**	M	Mestizo	31	3	14	120
	CS1006	F	Mestizo	42	3	16	100
	CS1013	F	Mestizo	43	3	17	100
	CS1015	M	Mestizo	36	3	15	100
	CS1023	F	Mestizo	28	3	16	80
	CS1025	F	Mestizo	41	3	16	60
	CS1028	F	Mestizo	35	3	16	280
	CS1030	F	Mestizo	21	3	P	0
	CS1031	F	Mestizo	20	3	P	0
	CS1036	M	Mestizo	36	3	P	0
	CS1038	M	Mestizo	30	3	P	0
CONTROL	**CS1003**	M	Mestizo	43	3	16	400
	CS1005	F	Mestizo	32	3	16	100
	CS1012	F	Mestizo	32	3	15	220
	CS1018	M	Mestizo	21	3	16	240
	CS1037	M	Mestizo	42	3	16	100
	CS1016	F	Mestizo	23	2*	NA	NA
SEMI-IMMUNE	**CS1506**	M	Afro-descendant	30	3	12	60
	CS1511	F	Afro-descendant	27	3	P	0
	CS1535	M	Mestizo	35	3	P	0
	CS1537	F	Afro	38	3	19	20
	CS1538	F	Mestizo	38	3	15	60
	CS1547	M	Mestizo	32	3	P	0
	CS1553	M	Indigenous	20	3	19	400
	CS1565	F	Mestizo	24	3	15	160
	CS1569	F	Mestizo	44	3	16	340
	CS1575	M	Mestizo	33	3	14	128
	CS1581	F	Mestizo	26	3	17	60
	CS1584	M	Mestizo	42	1*	NA	NA
CONTROL	**CS1549**	F	Mestizo	24	3	17	50
	CS1554	F	Mestizo	28	3	16	400
	CS1570	F	Mestizo	19	3	17	70
	CS1572	M	Indigenous	20	3	P	P
	CS1574	F	Mestizo	26	3	17	220
	CS1579	M	Mestizo	19	1*	NA	NA

P: Protected; NA: Not Apply; *Withdraws

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Figures

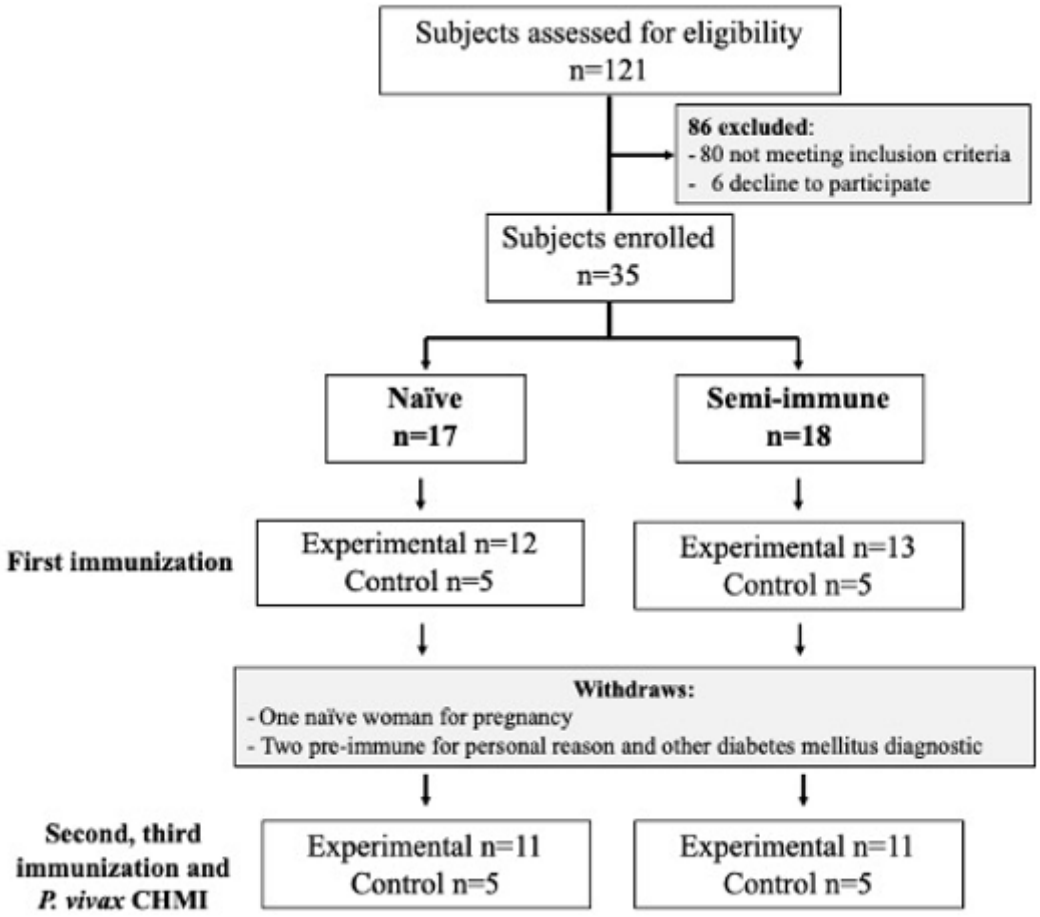

Figure 1

Trial flow diagram. The number of individuals in the screening, immunization, and CHMI steps.

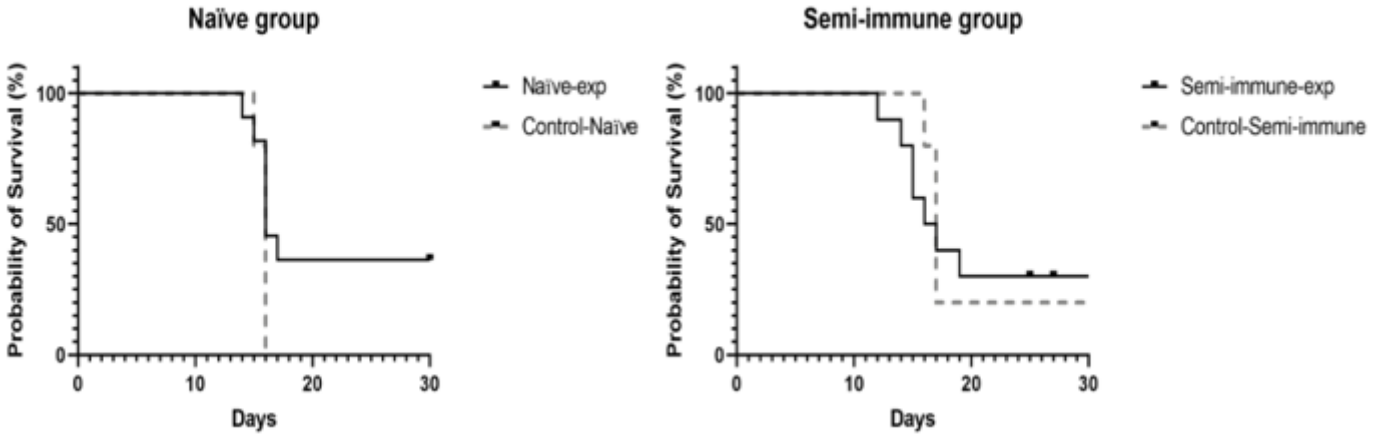

Figure 2

Survival Curve for Naïve and Semi-immune groups. Protective efficacy Kaplan-Meier curves for Naïve and Semi-immune group. One volunteer of the semi-immune Ctrl group did not develop parasitemia.

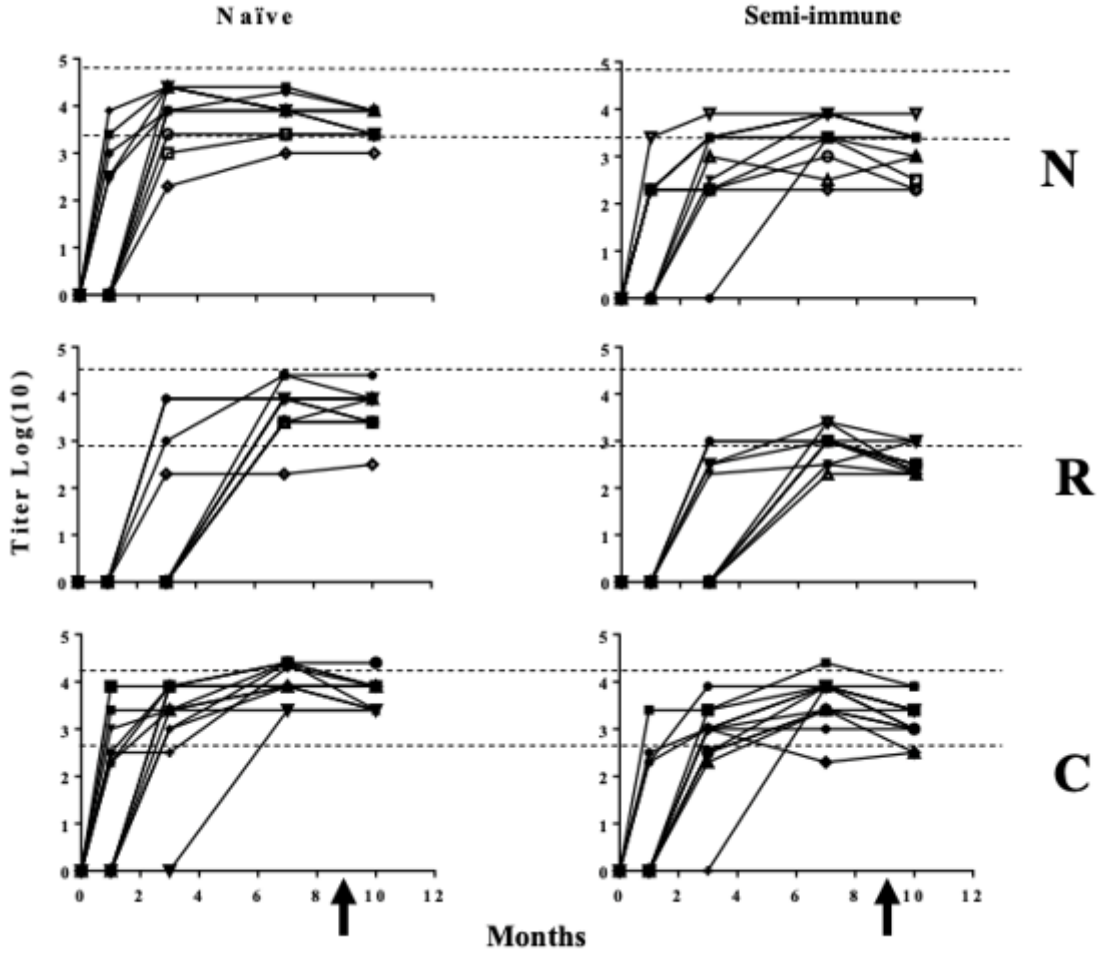

Figure 3

Anti-LSP antibody response in Naïve and Semi-immune volunteers. IgG antibody response of volunteers vaccinated with a mixture of PvCS N, R and C peptides, 150□g/mL/dose formulated in Montanide ISA-51. Values are expressed as reactivity index (RI) defined as sample OD at 1:200 serum dilutions divided by the cut-off value. All participants seroconverted after the first vaccination, and none had a boosting effect observed after the second and third vaccination or CHMI (arrows)

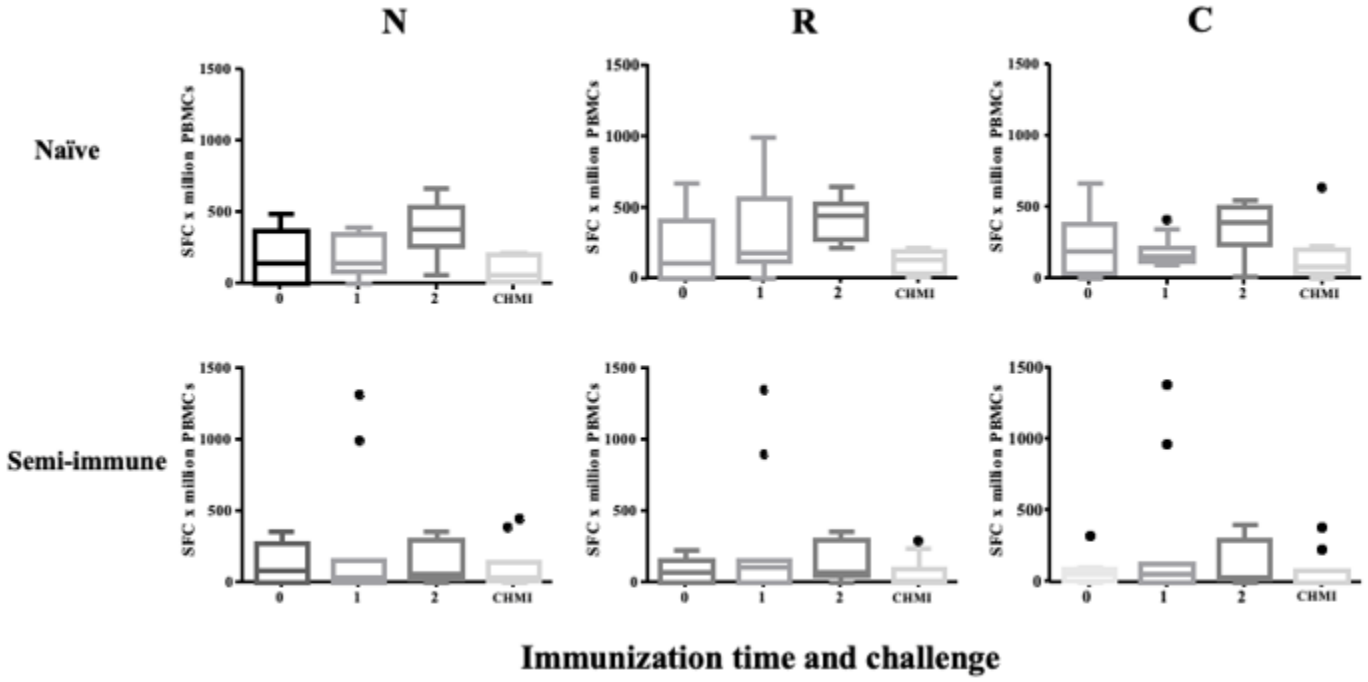

Figure 4

LSP specific induction of IFN-γ in Naïve and Semi-immune groups. IFN-γ production by PBMC stimulated with N, R and C peptides. The number of IFN-γ-sfc/106 cells was evaluated by ELIspot using fresh PBMC from volunteers after each immunization cultures for 36 h in the absence or presence of individuals peptides. The results are expressed as the number of IFN-γ-sfc/106 cells PBMC of immunized volunteers.

Supplementary Files

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