Published in final edited form as:

Vaccine. 2009 September 18; 27(41): . doi:10.1016/j.vaccine.2009.07.018.

Guidance on the evaluation of *Plasmodium vivax* vaccines in populations exposed to natural infection

Ivo Mueller^{a,*}, Vasee S. Moorthy^{b,c,*},¹, Graham V. Brown^d, Peter G. Smith^e, Pedro Alonso^f, and Blaise Genton^g on behalf of the WHO Malaria Vaccine Advisory Committee (MALVAC)

^aPapua New Guinea Institute of Medical Research, P.O. Box 60, Goroka, EHP 441, Papua, New Guinea ^bInitiative for Vaccine Research, World Health Organization, 1211 Geneva 27, Switzerland ^cNuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK ^dNossal Institute for Global Health, Centre for Clinical Research Excellence in Infectious Diseases, University of Melbourne, Carlton, Victoria 3010, Australia ^eLondon School of Hygiene and Tropical Medicine, Keppel Street, WC1E 7HT, London, UK ^fBarcelona Centre for International Health Research, Barcelona, Spain ^gSwiss Tropical Institute, Socinstrasses 57, Postfach, 4002 Basel, Switzerland

Abstract

In this paper we give guidance for the design and conduct of vaccine trials against *Plasmodium vivax* malaria. The paper supplements earlier guidelines on the planning of vaccine trials against *Plasmodium falciparum* malaria [WHO. Guidelines for the evaluation of Plasmodium falciparum vaccines in populations exposed to natural infections. Geneva: World Health Organization; 1997, http://www.who.int/vaccine_research/feuille_1_4-2.pdf], with further considerations in two later documents [Moorthy VS, Reed Z, Smith PG. Measurement of malaria vaccine efficacy in phase III trials: report of a WHO consultation. Vaccine 2007 July 9;25(28):5115-23; Moorthy V, Reed Z, Smith P. MALVAC 2008: measures of efficacy of malaria vaccines in phase 2b and phase 3 trials – scientific, regulatory and public health perspectives. Vaccine 2009 January 29;27(5): 624-8]. We deal specifically with study design and methodological issues for the assessment of pre-erythrocytic and blood-stage vaccines against *P. vivax*. The role of vaccines in blocking transmission of *P. vivax* is not considered as the methodological issues are similar to those for *P. falciparum*, though longer follow-up would be required because of the potential for relapse discussed below. In this paper we discuss the rationale and background to trials of *P. vivax* vaccines, requirements for Phase IIb and Phase III field trials, implementation of clinical trials, methods of measurement and analysis, and ethical aspects.

Keywords

Plasmodium vivax malaria; Vaccine

1. Scope

In this paper we give guidance for the design and conduct of vaccine trials against *Plasmodium vivax* malaria. The paper supplements earlier guidelines on the planning of vaccine trials against *Plasmodium falciparum* malaria [1], with further considerations in two

later documents [2,3]. We deal specifically with study design and methodological issues for the assessment of pre-erythrocytic and blood-stage vaccines against *P. vivax*. The role of vaccines in blocking transmission of *P. vivax* is not considered as the methodological issues are similar to those for *P. falciparum*, though longer follow-up would be required because of the potential for relapse discussed below. In this paper we discuss the rationale and background to trials of *P. vivax* vaccines, requirements for Phase IIb and Phase III field trials, implementation of clinical trials, methods of measurement and analysis, and ethical aspects.

2. Introduction

P. vivax is estimated to cause 80–310 million cases of malaria annually, principally in the Middle East, Asia, the Western Pacific, and Central and South America [4,5]. Although generally more benign than *P. falciparum* malaria, *P. vivax* can cause repeated debilitating relapses and life-threatening complications [6–9]. The most serious manifestations include cerebral malaria, acute renal failure, circulatory collapse, severe anaemia, haemoglobinuria, jaundice, acute respiratory distress and severe thrombocytopaenia [6,10]. *P. vivax* infection during pregnancy can lead to low-birth-weight infants and hence increases the risk of death in the newborn [11]. There has been renewed interest in the control of *P. vivax* because of recent work documenting the global burden of *P. vivax* and also in the context of elimination of malaria, particularly in the Asia Pacific region and the Americas where persistent *P. vivax* presents major challenges. In areas of high transmission, a vaccine that reduced clinical malaria by, say 50%, may have high benefit to cost ratio, but such a vaccine may have only limited utility as a measure towards malaria elimination, for which vaccines that prevent infection or block transmission may have greater appeal [12].

There are several unique aspects of the biology of *P. vivax* which necessitate distinct approaches for its control and for the development and testing of *P. vivax* vaccines [13]. In *P. vivax* infections, gametocytes are made very early in the course of a blood stage infection and at very low parasite densities [14], factors enhancing the transmission to mosquito vectors [15]. In addition, *P. vivax* completes the extrinsic cycle in the mosquito faster and at a lower temperature than *P. falciparum* [16] and is thus transmissible in a wider range of climatic conditions. Relapses from persistent liverstages (hypnozoites) may lead to several waves of blood stage infections arising from a single infective mosquito bite. Hypnozoites may persist in the liver for long periods and enable the parasite to persist in the human host between mosquito breeding seasons, thus partially uncoupling the temporal distributions of blood stage infections and transmission. These aspects of its biology allow *P. vivax* to be efficiently transmitted even in areas where mosquito populations are highly seasonal. Transmission by mosquitoes feeding outdoors may also limit the effectiveness of vector control measures.

The difficulty of controlling *P. vivax* with currently available strategies is illustrated by the experiences in Thailand [17,18] and Brazil [19] where after years of relatively efficient vector control and case management, *P. vivax* is now a more common cause of illness than *P. falciparum*. Innovative control approaches specifically targeting *P. vivax*, such as drugs effective against hypnozoites or *P. vivax* vaccines, will be needed for effective control of this parasite across a large range of transmission intensities.

The ability to relapse is a phenomenon characteristic of *P. vivax* and *P. ovale* infections. It is the result of the activation of quiescent liver-stage developmental forms, known as “hypnozoites,” that remain dormant within hepatocytes for varying intervals before spontaneously dividing and developing into schizonts and subsequently releasing invasive merozoites into the bloodstream to infect red blood cells and cause a malaria episode. The

triggers for the activation of hypnozoites are not understood, but both stress and distinct genetically determined patterns of relapses linked to local mosquito seasonal abundance may play a role. In two independent studies, it was demonstrated that >60% of parasite isolates associated with relapse of infection have a genotype that is different from the parasites associated with the primary malaria episode [20,21]. This suggests that even in areas of low endemicity, individuals may be infected with a mix of genotypes. Individuals apparently aparasitaemic by microscopy or nucleic acid testing may still be infected and develop further infections and morbid episodes in the absence of new sporozoite challenge. This phenomenon of relapse has important implications for the design of *P. vivax* malaria vaccine trials and long term follow up of the trial populations particularly as there is a lack of universally effective, safe treatments for eradicating hypnozoites.

Clinical immunity to *P. vivax* is acquired more quickly than to *P. falciparum*, both under conditions of natural [22-25] and experimental [26,27] infection. Thus in areas of moderate to highly endemic transmission, *P. vivax* malaria is predominantly a paediatric illness. However, among non-immune migrants to Papua, Indonesia, suddenly exposed to heavy transmission, children and adults appeared equally susceptible to *P. vivax* malaria even after onset of age-dependent clinical immunity to *P. falciparum* malaria [28]. In low transmission areas, people of all ages may be affected. Asymptomatic infections, often of very low densities, are common at any age in both highly [22,29,30] and moderately endemic areas [31]. Difference in immune status with age and transmission levels will thus need to be taken into account in the design of *P. vivax* vaccine trials.

With few exceptions, endemic *P. vivax* malaria occurs sympatric with endemic *P. falciparum* malaria and mixed infections of the two parasites are common [30,32] with conflicting evidence on the impact of each infection on the other. There is limited evidence for cross-species interactions in asymptomatic infections but co-infection with *P. vivax* may modulate the incidence and severity of *P. falciparum* illness. In Thailand and Sri Lanka, a previous or concurrent *P. vivax* infection attenuated symptoms associated with a (subsequent) *P. falciparum* episode, whereas -thalassaemic children in Vanuatu were more prone to *P. vivax* infection in early life, and thereby appeared to gain clinical immunity to subsequent *P. falciparum* infections [33,34]. It has been argued that *P. vivax* acts as a natural 'vaccine' against *P. falciparum* morbidity and that a species-specific *P. vivax* vaccine may have a detrimental effect. However, the evidence on cross-protection is conflicting and some studies have found exacerbation of the risk of severe disease in mixed *P. vivax/P. falciparum* infections [7-9]. Of note is that in some trials of the *P. falciparum* vaccine candidate SPf66, increases were reported in the prevalence of *P. vivax* or the incidence of mixed *P. vivax - P. falciparum* infections [35,36].

Whether protective or enhancing, these interactions may have important clinical and public health implications that should be monitored in malaria vaccine trials to assess the overall impact of the intervention on malaria infection and morbidity. There are few data on potential interactions between *P. vivax* and *P. ovale* or *P. malariae* that may also be present in *P. vivax* endemic areas.

Although there is renewed awareness of the high burden of *P. vivax* disease, relatively few vaccine candidates have been evaluated. Presently, there are only two *P. vivax* sub-unit vaccine candidates undergoing clinical development, with a modest number of other candidates in preclinical trials [37]. This situation contrasts with that for *P. falciparum* for which more than 70 different vaccine formulations are available and over 20 have progressed to clinical trials [38-40]. The main focus for *P. vivax* has been on a vaccine directed against the parasite protein that binds to the putative receptor for merozoites on the

surface of the red blood cell, but the success of vaccines based on the circumsporozoite protein of *P. falciparum* has stimulated similar approaches with *P. vivax*.

3. Background

3.1. Potential use of *P. vivax* vaccines for malaria control

In areas of high endemicity, *P. vivax* malaria is usually a disease of infants and small children [22,23]. In this situation, a *P. vivax* vaccine would be best distributed with other infant vaccines through the expanded programme of immunization (EPI) infrastructure. However, in large geographical areas, *P. vivax* transmission is moderate to low and adults are also at risk of clinical disease. Consequently, the potential target groups for *P. vivax* vaccines may include special risk groups such as: (i) pregnant women, or (ii) non-immune immigrant settlers, or other non-immune visitors or temporary workers, before their arrival in an endemic area in which significant transmission is expected. In some circumstances, mass vaccination may be appropriate (iii) as part of a time-limited campaign aiming at the elimination (or near elimination) of malaria, or (iv) as part of the short-term management of a malaria epidemic. It is unlikely, therefore, that a single vaccine and dosing schedule will cover all potential applications and a *P. vivax* vaccine will require testing in a variety of target groups.

In *P. vivax* elimination campaigns, the focus will be on the efficacy of vaccines in preventing transmission of malaria, whatever stage of the parasite the vaccine targets. Though sexual stage vaccines have traditionally been thought of as transmission blocking vaccines, it is increasingly appreciated that highly efficacious pre-erythrocytic vaccines could have an important role in elimination campaigns. Furthermore if a blood stage vaccine were to be sufficiently efficacious, it could also afford substantial transmission blocking activity.

In order to work well as a vaccine in temporary migrants or other visitors, a *P. vivax* blood stage vaccine would need to provide sufficiently long protection to cover not only initial infections but also relapses from long lasting liver-stages. Similarly, vaccines for short-term management of epidemics would need to have sufficiently long protection to cover relapses, and preferably give protection after a single dose.

Given the co-endemicity of *P. vivax* with *P. falciparum* in almost all endemic areas, it is likely that the most appropriate final malaria vaccine product will be a multi-species vaccine [41]. In the context of a research agenda for elimination or eradication of malaria, *P. vivax* vaccines may have an important role [42].

3.2. Efficacy endpoints, cofactors, and the limits of extrapolation

Although the basic principles for designing trials to evaluate and compare different *P. vivax* vaccines are similar to those for *P. falciparum* vaccines, there are some differences because of the distinct biology and epidemiology of *P. vivax*. The incidence of severe manifestations and the case fatality rates associated with severe disease are estimated to be substantially lower than those associated with *P. falciparum*, though *P. vivax* infections might be a significant cofactor to mortality from other causes.

As for *P. falciparum*, the efficacy of *P. vivax* vaccines is potentially affected by variations in the exposure and susceptibility of trial participants to *P. vivax* and heterogeneity in the virulence of the parasites, but, in addition, account must be taken of the incidence and timing of relapses. Relapses would not be expected to influence efficacy measures of blood stage vaccines as their effect should not differ between primary infections and relapses. Within a single trial, bias due to such co-factors may be avoided by randomization (preceded

by stratification, if appropriate), but these factors may constrain extrapolation of findings to other geographical areas and/or age groups.

It is important to note that efficacy demonstrated in young children in highly endemic areas may not extrapolate to older age groups or individuals in low and moderately endemic areas. Adults and infants may differ in their ability to make immune responses to vaccine antigens, in their health seeking behaviour, in their general health status, and in their febrile response to infection. In low transmission areas, natural boosting is likely to occur less frequently. In addition, there may be clinically significant differences in host genetic diversity between regions and geographical differences in parasite genetic variation in vaccine targets remain poorly characterised. As a consequence it will be crucial to confirm initial efficacy results obtained in highly endemic areas with subsequent trials in other transmission settings.

For the eventual comparison of the efficacy of different vaccines, of similar or different types, eligible endpoints include all those that are not upstream from the biological targets of any of the vaccines included in the comparison. For example, a pre-erythrocytic vaccine and a blood stage vaccine could be compared in terms of incidence of disease, but not in terms of incidence of infection.

3.3. Phases, target groups and progression of clinical vaccine trials

For malaria control, in different situations, the target group for vaccination may be the total population or may be a very limited sub-group. For ethical reasons, as with other vaccines, *P. vivax* vaccines should first be tested in healthy adults, whereas those probably least tolerant of possible toxicity (young children, infants, pregnant women) may be included only later, and only if they belong to a naturally exposed population. Furthermore, before conducting an efficacy field trial in a given population, a Phase I safety and immunogenicity trial in the same population or subgroup is required.

In these guidelines Phase Ia and Ib trials refer to Phase I trials conducted in non-exposed and exposed individuals, respectively. Phase IIa trials are trials in non-exposed trial subjects who then undergo experimental infection with malaria parasites to provide preliminary efficacy data. Phase IIb refers to field efficacy trials in naturally exposed populations designed primarily to provide proof-of-concept of efficacy. Phase III refers to large field efficacy trials, usually designed to support licensure of the vaccine.

The evaluation of a *P. vivax* vaccine should involve a conditional sequence of trials, taking into account stage of product evaluation and target populations and groups. A possible sequence for a *P. vivax* vaccine eventually destined for use in infants, could be (a) Phase Ia in unexposed adults; (b) Phase IIa in the same group, particularly for pre-erythrocytic vaccines; (c) Phase Ib in adult/adolescent/school aged children in an exposed population; (d) Phase Ib in the age group and population that will be the target for initial proof-of principle and pivotal studies; (e) Phases IIb-III in the same age group; (f) and (g) Phases I and IIb-III in infants of the same population. The most appropriate sequence will vary among vaccines, target groups and epidemiological situations.

Phase IIa trials are contentious for blood stage candidates, but are generally accepted to be very helpful for screening pre-erythrocytic vaccines. There is still no agreement on the level of protection required to justify proceeding to trials in conditions of natural exposure, but some prospect of efficacy is expected before proceeding, either from *in vitro* tests, or evidence of delayed patency for pre-erythrocytic vaccines. In the absence of culture methods for *P. vivax*, artificial challenge is very difficult technically and not entirely risk free; hence further development of the phase IIa challenge model in *P. vivax* is desirable. Once a robust,

well-validated, generally accepted *P. vivax* sporozoite challenge model is available, it would be logical for all pre-erythrocytic *P. vivax* vaccines to be screened in this way.

In order to keep sample sizes small for initial phase IIb-III trials, they will need to be conducted in highly endemic areas where incidence of infection and disease is sufficiently high. As *P. vivax* morbidity is concentrated in young children in highly endemic areas, the primary target age group for IIb-III proof-of-principle and pivotal trials of blood stage vaccines will be among children under 5 years old. Phase IIb trials of pre-erythrocytic vaccines with infection as a primary endpoint might be possible in older children (i.e. 5-10 yrs). Following proof-of-principle in highly endemic areas, it may be necessary to conduct phase IIb-III trials in additional target groups in moderate-to-low transmission areas. As sites of high transmission with *P. vivax* alone are hard to find, it will probably be necessary to conduct trials in the presence of at least some *P. falciparum* transmission, thus complicating both design and analysis.

3.4. Study design

Phases I to III trials should be based on double-blind, individually randomized, controlled comparisons, in trial participants receiving the locally used preventative tools (e.g. insecticide impregnated bed nets) and clinical care that is equivalent or superior to the national standard. In Phase I studies, more intensive care should be available in case of side-effects or increased severity of disease induced by vaccination.

The major challenge of study design is to accommodate the fact that subjects may have asymptomatic pre-existing parasitaemia, and hypnozoites in the liver and may become sick, not only from new or relapsing *P. vivax* infections, but also from *P. falciparum*. Subjects will require anti-malarial treatment for *P. falciparum* that would also treat *P. vivax*, (but usually not hypnozoites). The incidence of the endpoint(s) of interest, i.e. *P. vivax* infections or morbid episodes, will thus be reduced, to an extent that will depend of the half-life of the drug combination used and the incidence of *P. falciparum* episodes. Larger sample sizes will be needed to accommodate the loss of power on the primary outcome. At least in areas highly endemic for *P. falciparum*, it is recommended that a baseline study be conducted with comparable design, to obtain incidence rates for sample size calculations in order to have adequate power in the Phase IIb-III studies.

In natural parasite populations, many antigens are genetically diverse, and vaccines may be active against only part of the population; therefore vaccination may lead to a selective effect on the parasite population. Similarly, a reduction in incidence of *P. vivax* may lead to an increase in incidence of another human *Plasmodium* species. Therefore, in order to identify parasite genotypic selection or species interactions in vaccinees, careful assessment of all *Plasmodium* species during the surveillance period should be a part of all study designs

4. Specifications for Phase IIb and Phase III field trials

4.1. Objectives

4.1.1. Efficacy—In practical terms, regulatory considerations will have a large bearing on choice of the primary endpoint for Phase III trials, and the discussion below should be interpreted against this background.

In order to maximize the likelihood of detecting a biological effect of a vaccine, the first Phase IIb study for a given vaccine candidate should include as a key, perhaps primary, objective an assessment of the efficacy endpoint as close as possible downstream from the vaccine's molecular biological target(s). For early Phase IIb studies, incidence of infection is

an appropriate choice for pre-erythrocytic vaccines. In subsequent Phase IIb studies and in Phase III trials of pre-erythrocytic vaccines, incidence of disease should be used as a primary endpoint. Later Phase IIb studies of pre-erythrocytic vaccines could make use of two cohorts, undergoing different intensities of surveillance, designed to assess incidence of infection and clinical disease in parallel, as has occurred successfully for *P. falciparum* vaccines [43]. A clinical disease primary endpoint would allow efficacy comparisons between blood-stage and pre-erythrocytic vaccines and would be likely to satisfy regulatory requirements for an endpoint of sufficient clinically relevant benefit for licensure.

For Phase IIb and Phase III trials of blood-stage *P. vivax* vaccines, the recommended primary efficacy endpoint is the incidence of uncomplicated *P. vivax* malaria. Given the relative rarity of severe *P. vivax* disease, this is not a suitable primary endpoint for any *P. vivax* vaccine trials.

If the detection of asymptomatic infections were to result in antimalarial treatments being given, it would not be possible to measure protection against infection and protection against disease in the same cohort. Co-evaluation of incidence of infection and disease is therefore best carried out through use of separate cohorts.

Other recommended secondary efficacy endpoints include: (i) Prevalence and density of parasitaemia (including gametocytes and other *Plasmodium* species) (ii) Haemoglobin (or haematocrit), and (iii) Parasite diversity (polyclonality). When including anaemia as a secondary endpoint the potential of anaemia treatment (which includes a course of antimalarials in many malaria endemic countries) to interfere with the measurement of the primary endpoint will need to be carefully addressed. The determination of parasite diversity within isolates and genetic analysis of breakthrough parasites for polymorphisms in candidate antigens is essential to assess strain specificity of vaccine effect. Although the sample size of Phase IIb and most Phase III trials will be too small to measure efficacy against any of the severe endpoints, all severe malarial episodes, all hospitalizations, and deaths should be recorded.

4.1.2. Immunogenicity—Immune responses to the vaccine should be measured using appropriate, pre-defined tests that have been adapted to the vaccine's composition, and to field conditions. Where possible, correlations between protection and specific immune responses should be investigated, as they may assist the further development of vaccines (e.g. combination of antigens and optimization of dose and schedule), as well as their further evaluation (e.g. in control programmes). Blood samples should be taken from participants both before and after vaccination to measure immune responses and to correlate these with subsequent malaria episodes. It is also recommended for evaluation of possible boosting, through natural infection, of immune responses to the vaccine.

4.1.3. Safety—Of particular importance to vaccine safety is the monitoring of incidence and severity of *P. falciparum* episodes in areas where both parasites are present. Only in Phase III trials is it likely that sample sizes will be sufficiently large to measure a possible increase in incidence or severity of *P. falciparum* disease associated with administration of a *P. vivax* vaccine. Measuring a potential increase in *P. falciparum* risk in the *P. vivax* vaccine group is one of the major safety issues to be addressed at the phase III stage. It is thus of prime importance to monitor closely the occurrence of all *Plasmodium* parasites through both health facility-based case detection and, in some study designs, repeated cross-sectional surveys performed during the surveillance period. Ideally, parasitological assessments should be done by both microscopy and gene amplification methods in order to overcome the problems of subpatent parasitaemia.

4.2. Study design overview

The only fully adequate design requires a double-blind, randomized, controlled comparison between vaccinated and unvaccinated subjects. The appropriate unit of randomization is the individual for both pre-erythrocytic vaccine and blood-stage vaccine trials. The duration of a trial is in principle predetermined; being dependent on the calculation of the sample size, and ending with the breaking of the code. This may constrain the measurement of the duration of protection, though single-blind and even unblinded extended follow-up periods are often helpful in providing information on the long term impact of vaccination.

The need to clear long lasting *P. vivax* liver stages for the evaluation of efficacy of *P. vivax* pre-erythrocytic vaccines requires different basic study designs for pre-erythrocytic and blood stage vaccines. For pre-erythrocytic vaccines, unless it is predicted that such a vaccine may be therapeutic by acting on established hypnozoites, a treatment-reinfection design where the last vaccine dose is followed by radical treatment of liver and blood stages is most suitable if the primary endpoint is incidence of infection. This however presents a major problem as outlined below. For blood stage vaccines a traditional cohort design without radical cure is recommended.

The only drugs useful for elimination of hypnozoites belong to the eight amino-quinoline group. Recent data suggest that these drugs are more efficacious if given in association with a 4-aminoquinoline such as chloroquine or quinine, and that total dose is more important than duration of therapy [44]. This class of drug can however have serious side-effects in individuals who have deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) and this phenotype is particularly common in *P. vivax* endemic areas. The tension between the scientific benefit of radical cure to improve detection of efficacy against incidence of infection with pre-erythrocytic vaccines and the concerns stated above need to be carefully addressed when designing pre-erythrocytic vaccine trials. At the minimum, careful screening and exclusion of G6PD-deficient volunteers will be required.

4.3. Trial target groups

Given the high incidence of both *P. vivax* and *P. falciparum* disease in young children in areas highly endemic for *P. vivax*, this may be the preferred age group for both phase IIb trials and, perhaps, for an initial phase III trial in highly endemic settings. In the case of demonstrated efficacy and a lack of increase in risk of *P. falciparum* illness in trials in children less than 5 years, these should be followed by trials in infants in highly endemic areas and in school-aged children, adolescents and adults in areas of moderate and low transmission (i.e. South America and South and South East Asia). The latter is an essential part of the vaccine development plan as in low endemicity areas, where natural acquisition of immunity is slow, the vaccine target group includes all age groups.

4.4. Sample size

In any vaccine trial, it is important that the number of participants recruited should be sufficient to provide reliable answers to the main study objectives. Most trials have several objectives and, ideally, sample size computations should be carried out for each of these objectives and the size of the trial based on the largest of these. A reasonable minimum requirement, however, is that the sample size should be adequate to detect a protective effect on the primary endpoint with a power of 90% (with a two-sided significance test).

Sample size calculation should take into account the proportion of participants that will need to be censored from the analyses or excluded from time at risk for the reasons pre-specified in the protocol. Unless good quality data on both *P. vivax* and *P. falciparum* incidence rates

are available, the conduct of a baseline study with similar design and surveillance methods as the planned trial may be required to allow confidence in the sample size calculation.

5. Criteria for the selection of study areas and background information required

5.1. Epidemiological and operational criteria

Vaccine trials are best conducted in situations in which incidence is sufficient to make efficacy relatively easy to measure and in which vaccination is likely to be relevant to malaria control (the two criteria may not always coincide). The preference for locating trials in areas of intense transmission is justified on two grounds: first, protection can be assessed in a smaller number of subjects (which also limits the number of participants exposed to a new product), and, secondly, if a vaccine protects against intense transmission it is likely to protect against less intense transmission, whereas the reverse may not be true.

An endemic setting including *P. falciparum* and *P. vivax* would normally be chosen for a field efficacy trial, as no sole *P. vivax* transmission settings with sufficient *P. vivax* incidence have been described. An efficacy trial in such a dually endemic zone would have the advantage of assessing the impact of *P. vivax* vaccines on *P. falciparum* malaria. This would allow early assessment of the need to include a *P. falciparum* vaccine component to prevent potentially deleterious effects due to *Plasmodium* species replacement and interaction.

In addition, vaccine trial sites should have the following operational criteria: (i) informed and firm commitment from both study population and national and local authorities to the conduct of the trial; (ii) availability of background data on the epidemiology of malaria; (iii) low expected emigration rate for the duration of the trial (to minimize the dropout rate); (iv) reasonable expectation of social and political stability at the national and local levels for the duration of the trial and; (v) maximal involvement of national research institutions.

5.2. Background information

Background information is required for both selection of appropriate trial sites and for further specification of the study design (e.g. sample size, timing of vaccination and surveys in relation to transmission, logistics of case detection). As far as possible, the required background information should be obtained from existing data, which should be retrieved and reviewed; if important information is missing, the collection of new data may be required.

Required background information includes basic demographic characteristics of the study populations, disease burden, health services, treatment seeking behaviour and malaria incidence and prevalence rates. As the Duffy negative blood group is associated with protection from *P. vivax* infections [45], it will be essential to determine the presence of the Duffy null genotype in study populations with at least partial African ancestry.

Of key importance is the expected incidence of the primary efficacy endpoint, as this determines the required trial size. Other malariometric data (rates of infection, disease, severe disease, specific mortality; prevalence and density of parasitaemia) for all *Plasmodium* species (incl. mixed infection) will also be useful, preferably by season and age group.

In addition, data on sensitivity of malaria parasites to drugs will be required in order to select the chemotherapeutic regimens needed to clear parasitaemia (for measuring the incidence of infection) and to treat malaria in the study area. Of particular importance would

be a reliable estimate of the efficacy of primaquine (alone or in combination) for clearance of *P. vivax* liver stages, and the rate of G6PD deficiency in the trial population. However, such data are very difficult to obtain in endemic populations where re-infections are common. Background data on immunological responses induced by natural infection to the antigens present in the vaccine is desirable, as this may be related to the possibility for natural boosting after vaccination. Also valuable is local knowledge on the population field diversity of *P. vivax* antigens represented in the vaccine.

The entomological inoculation rates for both *P. vivax* and *P. falciparum* would be valuable, in particular for trials of pre-erythrocytic vaccines as they provide independent estimates of the challenge intensity and of the seasonality of transmission (which may affect timing of vaccination and surveys). However, the large temporal and spatial variability of these entomological measurements, require large samples. Unless entomological investigations are routinely undertaken in the study area, the effort required for accurate and representative measurement may not be justified.

6. Assessment of different malaria endpoints

Various aspects of measurement of malaria vaccine efficacy have recently been the subject of WHO consultations. Although these focused on *P. falciparum* vaccines, many of the recommendations are directly applicable to *P. vivax* vaccine trials [2,3]. Here, we discuss only aspects that are particular to *P. vivax* vaccine trials.

6.1. Detection of *P. vivax* parasites

The detection of *P. vivax* parasites in the blood of a trial participant is a necessary component for most relevant efficacy endpoints for the assessment of *P. vivax* vaccines. The current method of choice for detecting malaria infections of any species is microscopic examination of thick blood films. Accurate identification of infecting *Plasmodium* species in thick film microscopy is challenging, even for an experienced malaria microscopist, as in almost all areas where *P. vivax* occurs other human *Plasmodium* infections are also endemic. It is particularly difficult to detect mixed *Plasmodium* species infections, both in clinical and asymptomatic infections, using light microscopy. Even though detection of low numbers of parasites is possible, a minor population of parasites may not be detected in the presence of mixed infection dominated by another parasite. In order to determine the infecting species accurately, the inclusion of molecular diagnostic techniques, such as gene amplification (PCR), real-time PCR or post-PCR ligase detection reaction assays (LDR-FMA), should be considered. Since assays are based on DNA detection, circulating sexual forms, even at very low number will also be detected. The recommendation for the determination of parasitaemia in blood samples in *P. vivax* vaccine trials is to use light microscopy, performed by a skilled microscopist, combined with a semi-quantitative molecular assay (such as real time-PCR or LDR-FMA), recognizing that even these assays do not have ideal sensitivity and specificity.

6.2. Incidence of infection

If a study is designed primarily to determine the incidence of new *P. falciparum* infections in trial subjects, the standard recommendation is that parasitaemia should be cleared from all subjects before surveillance for new infections is started by the administration of a safe and effective schizonticidal drug after the last inoculation of vaccine or placebo. For measuring the incidence of new *P. vivax* infections, it will be necessary to add a radical cure using a drug, such as primaquine, that kills hypnozoites. Because a low dose of primaquine may not achieve a radical cure, especially with Chesson-type strains, it is important to treat with an appropriate regimen, such as an adult dose of 30 mg primaquine per day for 14 days [44,48]

(if possible, in combination with chloroquine or quinine). Even this regimen may not be completely successful. An important practical and ethical issue concerns use of drugs of this class in individuals who may be deficient in the enzyme glucose-6 phosphate dehydrogenase (G6PD) in whom the medication may trigger severe haemolysis. G6PD deficiency is common in many malaria endemic areas and one strategy would be to exclude affected individuals from *P. vivax* vaccine trials. However, this complicates trials and recently developed rapid diagnostic tests to detect G6PD deficiency need further assessment before they can be recommended for use in field studies.

For a study designed with detection of incident infections as its primary objective, the surveillance system should include repeated cross-sectional surveys by thick blood smears, supplemented by active or passive surveillance between surveys to capture incident infections in subjects with fever or other suggestive symptoms. Following initial radical cure, samples should be obtained from participants at regular intervals (weekly to monthly depending upon the level of malaria transmission), and blood films examined for *P. vivax* asexual parasites and gametocytes. During the time that individuals are under surveillance for new infections, information on anti-malarial drug usage and absence from the study site should be collected. In a vaccine trial, infection caused by any *Plasmodium* species should be recorded so that it is possible to assess whether vaccination against *P. vivax* has an effect on the incidence of parasitaemia of the other species.

7. Incidence of clinical malaria

7.1. Case definition

Case definitions developed for clinical disease due to *P. falciparum* malaria should be used for *P. vivax* and mixed infections but some modification may be required. As outlined during a previous WHO consultation on *P. falciparum* malaria [2], the clinical case definition for an uncomplicated malaria disease episode has three components: (1) the choice of morbidity criterion, (2) the parasite density threshold (which may simply be presence of any parasitaemia by microscopy, or may be restricted to parasitaemia beyond a defined level) and (3) the case detection system used. The case definition and case detection systems should ideally be defined in baseline studies conducted prior to the start of a trial. Similar considerations hold for clinical disease due to *P. vivax* malaria.

7.1.1. Choice of morbidity criterion—The clinical presentation (types of symptoms and signs) of *P. vivax* malaria is very similar to that of uncomplicated *P. falciparum* disease, though the fever threshold (level of parasitaemia at which symptoms are likely to occur) is lower for *P. vivax*. Thus, the criterion for the clinical case definition for a primary endpoint of uncomplicated *P. vivax* disease should be *P. vivax* parasitaemia above a defined density (see below) and documented fever, defined as an axillary temperature measurement of ≥ 37.5 °C [2]. Axillary measurement of temperature can give lower readings than oral or rectal measurements, depending on the operator and method used, axillary measurement is considered to be sufficiently sensitive for a case definition where, for the purposes of measuring vaccine efficacy, specificity is the paramount concern.

Because of the highly synchronous and intermittent nature of fever associated with *P. vivax*, a significant number of patients with malaria due to *P. vivax* will not have fever at the time of presentation to health facilities. Consequently, a case definition based on measured fever may lead to a significant underestimation of incidence of *P. vivax* disease. A case definition that includes history of fever in addition to documented fever may thus be appropriate. It is recommended that prior to the conducting of a trial the performance of case definitions with and without history of fever be evaluated locally.

7.1.2. Choice of parasite density threshold—The primary case definition for clinical *P. falciparum* malaria in vaccine trials is generally based on a combination of clinical symptoms and a level of parasitaemia above some defined threshold. Because these trials are often conducted in areas with high levels of asymptomatic parasitaemia, use of a parasite density level substantially above the threshold of detection by microscopy is usually required to ensure that the specificity for disease definition is adequate. In areas of low transmission for *P. vivax*, a clinical case definition with sufficiently high specificity for clinical malaria due to *P. vivax* may use the presence of any level of *P. vivax* parasitaemia, as in such areas asymptomatic parasitaemia (and pre-existing immunity) is rare. In areas of high transmission (and hence high immunity), asymptomatic *P. vivax* infections will be more frequent and detection of parasitaemia is not a sufficient condition for the diagnosis of clinical disease. Even in areas of intermediate intensity of transmission, the incidence of asymptomatic parasitaemia may be significant, as immunity to *P. vivax* is acquired more rapidly than immunity to *P. falciparum*. For trials in such areas, a case definition that includes a parasite density cut-off may be required.

Various approaches have been proposed for determination and selection of such parasite density cut-offs that provide a high specificity for the diagnosis of clinical malaria [46,47]. In these approaches, a group of community controls and a group of clinically suspected malaria cases are used to estimate malaria attributable fractions and the sensitivity and specificity of different parasite density cut-offs. Although it would be preferable to use data from the trial itself to determine such cut-offs, it was recommended for trials of *P. falciparum* vaccines that recent historical data from the trial site, that corresponds in terms of age group, seasonality and surveillance mechanism, may be used [2]. Similar considerations will hold for *P. vivax* vaccine trials. Although optimal cut-offs have been described for *P. falciparum* for many populations and age groups, *P. vivax* specific cut-offs have rarely been investigated. Recent analyses from Papua New Guinea show that optimal cut-off densities, when determined in the same population and age-groups, are lower for *P. vivax* than those for *P. falciparum* [50].

A particular problem is raised by clinical infection caused by mixed *P. vivax* and *P. falciparum* infections. Currently there are no pyrogenic thresholds for mixed infections or standard approaches to attribute risk of illness to individual infections. For *P. vivax* vaccine trials, a conservative option is to use the same pyrogenic thresholds as in single infections, and to consider that all mixed infections exceeding the thresholds are individually assigned as clinical *P. vivax* and/or *P. falciparum* episodes. Thus a single mixed infection could be assigned as a case of *P. vivax* disease, a case of *P. falciparum* disease, or both, for the analyses.

For ethical reasons, the criteria used to implement anti-malarial therapy in such a trial will be wider than the chosen case definition (e.g. mother's history of fever in the last 24-48 h). This will have implications in terms of interference with the primary outcome measurement, and should be considered in the sample size calculations.

7.1.3. Choice of case detection system—Where the parasite cut-off level to define malaria is low, active case detection (ACD), in which an attempt is made to identify all persons with clinical symptoms of malaria and to test them for parasitaemia, facilitates detection of a high proportion of all cases of clinical malaria in the community. However, if the definition of clinical malaria used in a trial requires exceeding a defined parasite density threshold then ACD may identify many children who may qualify to be treated with antimalarials but who do not satisfy the trial case definition. This may reduce the power of the study and complicate the interpretation of trial results. For Phase III trials, passive case detection (PCD) may be preferable, in which only participants presenting to a health facility

with malaria symptoms have parasitaemia measured. With such surveillance, only disease which is severe enough to lead to presentation at a health facility is included and some true cases of malaria are likely to be missed. Thus the specificity of the case definition is increased at the expense of its sensitivity. Furthermore, the use of PCD in a trial may give the best measure of impact that the vaccine would have in reducing the burden on health facilities.

The necessity for close monitoring of the risk of *P. falciparum* infections and disease may also influence the choice of the clinical case detection system. Until it has been determined that a reduction in *P. vivax* incidence through vaccination does not lead to increased risk of *P. falciparum* disease, the incidence of *P. falciparum* disease should be closely monitored for safety reasons. The risk of severe disease associated with *P. falciparum* infection may thus require rapid access to early diagnosis and treatment for symptomatic children in the trial sites.

The choice of the appropriate case definition may thus differ between proof-of-principle Phase IIb trials and later stage field efficacy studies (large Phase IIb and Phase III). In proof-of-principle studies, the higher incidence of clinical episodes detected using ACD may allow the determination of efficacy of the vaccine in preventing clinical disease, whereas at the same time closely monitoring risk of *P. falciparum* disease. Once a sufficient body of clinical safety data indicates that a *P. vivax* vaccine does not significantly increase risk of *P. falciparum* morbidity, PCD may be preferable for Phase III trials, as it more accurately measures the clinical efficacy of the vaccine as perceived by patients or guardians and experienced by the health system. PCD also provides an efficacy measure that may have more meaning to public health planners. Of course, if PCD is used to ascertain cases in trials, it will be important to choose study settings in which there is adequate access to diagnosis and treatment.

8. Practical recommendations

In a vaccine trial in which clinical malaria is the primary endpoint, a single primary case definition for clinical malaria should be decided in advance of the start of the trial, consisting of the chosen morbidity criterion, parasite density cut-off and case detection system, so that the choice is not influenced by knowledge of vaccine effects. Applying the procedures described above to either baseline data from the study area or to data collected during the vaccine trial will allow derivation of specificity and sensitivity values for different parasite density cut-offs. Where a cut-off above zero is necessary, it should be chosen to provide a specificity in excess of 90%, if possible, with acceptable sensitivity. Low sensitivity does not bias the efficacy estimate but reduces the statistical power of a trial to detect a given efficacy, whereas poor specificity biases the efficacy estimate towards zero.

Irrespective of whether a suspected case of malaria is classified as a case for the purposes of the vaccine trial, clinically suspect cases should be treated for malaria according to locally applicable clinical guidelines (or with other therapy if local guidelines do not adequately treat locally prevalent resistant malaria).

The primary analyses of vaccine efficacy based on the incidence of clinical malaria should consider only episodes in each trial participant satisfying the primary case definition. Subsidiary analyses may consider different case definitions, determined with different morbidity criteria (for example excluding history of fever) and varying parasite density cut-offs.

In countries where national guidelines require radical cure of liver stages, treatment with primaquine following a *P. vivax* episode will reduce the risk of further episodes and thus

compromise the assessment of the efficacy of the vaccine against disease due to recurrent infections. In those circumstances, it is therefore recommended to use incidence of first-or-only episode as the primary trial endpoint. If it is required to evaluate vaccine efficacy against both primary infections and relapses, a trial design that substitutes radical cure by intensive active detection of infection may be considered.

8.1. Incidence of severe malaria

Severe disease is much less frequent in *P. vivax* than in *P. falciparum* malaria and is unlikely to be a primary endpoint for evaluation of a *P. vivax* vaccine in all but the very largest studies. However, in all trials, the rate of severe malaria during the surveillance period, for all *Plasmodium* species, should be measured, both for efficacy and safety reasons. Although standard definitions for severe *P. falciparum* malaria are being finalized [2], no such definitions currently exist for severe *P. vivax* disease. However, recent studies of severe *P. vivax* malaria indicate a spectrum of severe disease that resembles that of *P. falciparum* malaria [6,10]. Until specific and standardized definitions for severe *P. vivax* are available, it is recommended that the case definitions used for severe *P. falciparum* are used as a guide for the definition of severe disease due to *P. vivax*. Severe cases are likely to be detected by PCD among patients presenting to a health facility.

8.2. Detection and investigation of deaths

Detection and investigation of all deaths is required in all trials, even though mortality is unlikely to be a primary efficacy endpoint in *P. vivax* vaccine trials, or even in *P. falciparum* vaccine trials, as the close monitoring that occurs during clinical trials is likely to reduce malaria mortality substantially. The most appropriate method for determining mortality among *P. vivax* vaccine trial subjects will be determined by local circumstances and should follow the recommendation made for *P. falciparum* vaccine trials. In areas where post-mortem examinations are not feasible, some information on cause of death may be obtained through verbal autopsy – i.e. by asking questions of the bereaved family about the final illness and the circumstances of the death. However, it is difficult to determine accurate cause of deaths where many deaths occur outside health facilities or where those in health facilities are not equipped to provide an accurate determination of the causes of death. Ascertaining the fact, rather than the cause, of death can be done more reliably and it is generally more informative to compare the overall number of deaths in vaccine and comparator groups, rather than placing too much emphasis on malaria specific mortality. An additional complicating factor may be that differentiation between deaths due to *P. falciparum* and *P. vivax* malaria will be very difficult in many trial settings. With these provisos, attempts should nevertheless be made to assign causes for all deaths occurring during the study.

8.3. Possible interference among efficacy measures

As mentioned above, the primary efficacy endpoint (i.e. *P. vivax* infection or disease) may be influenced by the level of *P. falciparum* transmission. If the risk of *P. falciparum* disease is high, participants may receive one or more anti-malarial treatments before any occurrence of *P. vivax* infection. This will reduce the effective period at risk for *P. vivax* infection, and hence the power of the study. The loss of power will be reduced if a relatively short acting anti-malarial is used to treat clinical episodes of malaria during *P. vivax* vaccine trials. It should be noted that, increasingly, combination treatments are being recommended for treatment of *P. vivax* disease and these usually include a long-acting component. Careful baseline epidemiological cohort studies will be needed to calculate the sample size required for the assessment of the primary endpoint, taking into account the interference of *P. falciparum* case management.

As discussed above, it may not be optimal to measure both infections and morbidity endpoints accurately at the same time in the same trial population. It may be preferable to measure only the efficacy endpoint most appropriate for the vaccine type being tested (i.e. infection for pre-erythrocytic vaccines and disease for blood-stage vaccines) in proof-of-principle and pivotal studies. Where both incident infection and incident disease data is required, two cohorts in different study sites with appropriately tailored surveillance systems would be necessary [43].

9. Assessment of immune responses

The methods for measuring immune responses in *P. vivax* vaccine trials are outside the scope of this document. Such measures will be chosen taking account of the vaccine's composition and are likely to be based on previous studies with the vaccine or similar vaccines. In practice, because of the difficulty of repeated bleeding of all trial participants, immune responses will usually be measured only in a sample and at selected times after vaccination, e.g. immediately before the first vaccine inoculation, shortly after the last vaccine inoculation, towards the end of the main transmission season, and/or at the end of the trial. However, ideally, vaccine-related immune responses will be measured on all participants before and after vaccination in order to assess potential immune correlates of protective efficacy. In addition, further measures will be taken throughout the trial to detect waning immunity.

10. Safety: adverse effects

The approaches for monitoring the safety of *P. vivax* vaccines are similar to those for *P. falciparum* vaccines, which have been described elsewhere [1]. However, of particular concern for *P. vivax* vaccines used in settings that are co-endemic for *P. falciparum* is the possibility that a reduction in the incidence of *P. vivax* infections and disease may be associated with an increased risk of *P. falciparum* illness. The small sample sizes in Phase I and Phase II studies preclude an accurate evaluation of this effect and monitoring of *P. falciparum* risk should thus be a primary safety endpoint for Phase IIb – III trials. It is important that all subjects be monitored for clinical illness long enough to ensure that any possible deferred increase in morbidity is observed and recorded. A previous WHO consultation[2] has recommended that there should be a minimum of 24 months post-vaccination follow-up for Phase III trials and it is difficult to conceive of a Phase IIb trial in paediatric populations which could be terminated sooner. These considerations hold equally for *P. vivax* field efficacy trials.

11. Monitoring other factors influencing malaria risk

For ethical reasons, it may be necessary to improve preventive and therapeutic interventions during vaccine trials, even though they would not exist in the population in the absence of a trial. Such measures are likely to lower the incidence of malaria in the trial population, but because they will be applied equally to the vaccine and placebo groups, they will not bias the trial results, but may reduce its statistical power. In addition, the behaviour of individual trial participants is likely to affect their risk of malaria and it is desirable, to the extent feasible, to measure these in a trial. Such factors include: use of antimalarials (including treatment-seeking behaviour and self-treatment), use of personal protection measures (e.g. mosquito nets), domestic use of insecticides, occupation and migration. Information may be obtained through interviews which may be supplemented by direct observation (e.g. of antimalarials, bed-nets or insecticides available in the home), by surveys of drug providers, or by random urine sampling for anti-malarial use.

12. Implementation of field trials of vaccines

Many of the aspects that must be considered in planning the field implementation of trials for *P. vivax* vaccines are the same as those for *P. falciparum* vaccines [1-3], but there are some special considerations.

Prior to conducting *P. vivax* vaccines trials an extended preparatory period is required to collect background information; to finalize the study design; to establish facilities; to recruit and train personnel; to test and standardize all clinical, field, laboratory and data recording methods; to test individuals for G6PD deficiency, to prepare an analytic plan; and to prepare the community for the intervention study.

The purposes and design of the trial and the procedures to be used should be discussed with representatives of the communities in which the trial is planned. Those who will be eligible for entry into the trial should be properly briefed on possible adverse effects of vaccination and on possible benefits. Of particular importance, potential participants will need to understand that the vaccine may only prevent infection and disease due to *P. vivax* but not other malarial species and, indeed, that the vaccine may have no efficacy against *P. vivax*.

Exclusion criteria in *P. vivax* blood stage vaccine trials will be similar to those in *P. falciparum* vaccine trials. However, in a pre-erythrocytic *P. vivax* vaccine trial with infection endpoints, people who are suffering from G6PD deficiency and may thus have adverse reactions to treatment with 8-aminoquinolines should be excluded.

Season of vaccination may be relevant for early field trials. The trial may be planned such that residents in areas of seasonal transmission receive their last dose of vaccine at the beginning of the rains and non-immune immigrants just before entering areas of intense transmission. No treatment with a schizonticide should be administered before vaccination. Treatment to clear parasites following vaccination should be restricted to pre-erythrocytic vaccine trials with infection endpoints and should include an efficacious anti-liver stage drug.

Once a vaccine of a certain type, e.g. a pre-erythrocytic vaccine, has demonstrated efficacy, there is likely to be an ethical requirement to test new vaccines, of a similar type, against that vaccine. This will increase the required sample size. Once registered, a highly efficacious vaccine, if available, against *P. falciparum* should be given to all trial participants.

13. Analysis of trials

Appropriate methods for the statistical analysis of randomised controlled trials of *P. vivax* vaccines are essentially the same as for *P. falciparum* vaccines [1-3]. In addition, consideration must be given to the possibility of the interference of *P. falciparum* case management with the measurement of *P. vivax* related endpoints, as discussed above, and the analytical plan should take appropriate account of this.

13.1. Disease endpoints

The preferred endpoint for proof-of-principle Phase IIb studies is the incidence of the first or only *P. vivax* clinical disease episode. For this endpoint, only the first episode of *P. vivax* disease is considered and the participant does not contribute further to either numerator or denominator following the first *P. vivax* disease episode. However, monitoring should continue with all subsequent episodes recorded and reported. If the participant experiences one or several *P. falciparum* malaria episodes prior to a *P. vivax* episode, the participant should be regarded as not at risk for *P. vivax* disease for the duration of the

chemoprophylactic effect following each treatment (the duration is drug dependent). Participants who are lost to follow-up, who withdraw, or who die, should be included up to the date of loss, withdrawal or death.

An additional endpoint that will usually be considered as a secondary endpoint is the incidence rate of all *P. vivax* disease episodes. In these analyses a standard rule must be used to ensure that treatment failures are not enumerated as separate episodes. By convention, events during the 4 week period after an episode are not included, and these 4 weeks do not contribute to the time at risk. This rule applies to subsequent episodes with the same but not with different malaria species. An episode with a different species that follows an earlier episode can, by definition, not be a treatment failure and can thus be enumerated.

Testing for statistical significance and deriving confidence intervals on the estimates of vaccine efficacy against all *P. vivax* episodes is not straightforward if different episodes within an individual are not independent, as is likely to be the case. Although, there is currently no consensus on the most appropriate method to analyse such an endpoint, it should nevertheless be considered for analyses of *P. vivax* vaccine efficacy as it is less affected by concurrent *P. falciparum* episodes. As a minimum, all *P. vivax* episodes in all subjects should be recorded.

Methods which require censoring at time of treatment for first *P. falciparum* episode are not recommended, as these may require an unacceptably large increase in sample size and may result in biased efficacy estimates.

Measurement of incidence of *P. falciparum* disease, using both first episode and all episodes endpoints, should also occur as part of the safety assessment of *P. vivax* vaccines.

13.2. Infection endpoints

In trials of pre-erythrocytic vaccines, the primary outcome measure will usually be the time to first infection, which will be calculated as the period between the last dose of radical treatment following last vaccine dose and the first incident infection detected during the efficacy follow-up period. If a drug with a longer half life is used together with primaquine, time at risk should start when the chemoprophylactic effect of the drug has passed. Again, following a *P. vivax* infection, the individual should not contribute further to either numerator or denominator. Cox proportional hazards regression analysis is the preferred analysis method.

The impact of *P. falciparum* case management on this outcome will depend crucially on whether there is an ethical requirement to diagnose and treat asymptomatic infections. If this is the case, participants would be excluded from further analysis when any malaria infection is diagnosed. Depending on the relative transmission levels of *P. falciparum* and *P. vivax* this may require a substantial increase in trial sample size for the evaluation of the efficacy of the vaccine against *P. vivax* infection. If only symptomatic *P. falciparum* infections are treated, the loss of power will be significantly less.

Alternatively, incidence of first or only infection may be used as an endpoint. For this endpoint, the participants who receive treatment for a *P. falciparum* infection prior to experiencing a *P. vivax* infection will need to be excluded from the at risk period for the duration of the chemoprophylactic effect of the drug used to treat the *P. falciparum* infection.

13.3. Analytical plan

An analytical plan for the trial, specifying the planned trial analyses, should be prepared and finalised prior to the breaking of the vaccine codes. It is best practice to finalize the analysis plan before, or close to, the start of an efficacy trial. The analysis plan should address the issue of multiple comparisons by denoting one primary endpoint only, in such a way that it is clear that the results themselves have not influenced the choice of the primary endpoint. The analytical plan should also detail the inclusion and exclusion criteria, the primary case definition to be used, and the methods of data analysis, including how interference by *P. falciparum* case management will be addressed in the analyses. Alternate case definitions and secondary analyses should be specified in the analysis plan. Any “post hoc” analyses not specified in the analysis plan, will be interpreted with caution.

The first analysis should be the comparison of vaccine and control group with respect to their baseline characteristics. Variables included in the analysis should include: age, sex, initial parasitological status, area of residence and bed-net use.

14. Ethical considerations

Similar ethical considerations apply to *P. vivax* vaccine trials as apply to *P. falciparum* vaccine trials [1,49]. However, the conduct of *P. vivax* vaccine trials in areas where *P. falciparum* is the leading cause of malaria morbidity and mortality raises additional ethical questions. Indeed, the impact of such a *P. vivax* vaccine on the health of the population may be smaller compared to a vaccine that reduces *P. falciparum* disease. In addition, there is a possibility, but probably a small one, that an effective *P. vivax* vaccine may lead to species replacement and increase of *P. falciparum* morbidity and mortality. It can be argued that proof of principle Phase IIb trials using single species *P. vivax* candidates are justified in such settings for accurate assessment of the effect of the vaccine on *P. vivax* infections and morbidity, as well as potential effects on *P. falciparum* infections and morbidity. In the absence of any data confirming or refuting a possible increase in *P. falciparum* risk it may be necessary to conduct the trial using an intensive active case detection system to minimize the risk to participants. The findings in such Phase IIb trials will influence decisions whether or not to proceed further with single species *P. vivax* candidates or to include one or several *P. falciparum* component(s).

Acknowledgments

The authors gratefully acknowledge the contribution of Dr Zarifah Reed and Dr John Aponte for the writing process of this document and for comments on the manuscript. We acknowledge the key role of the WHO MALVAC Advisory Committee for highlighting the need for this document, thus initiating the writing process. Funding support to WHO Initiative for Vaccine Research from Fondazione Monte dei Paschi di Siena is gratefully acknowledged. Authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions or the stated policy of the World Health Organization.

References

- [1]. WHO. Guidelines for the evaluation of *Plasmodium falciparum* vaccines in populations exposed to natural infections. World Health Organization; Geneva: 1997. http://www.who.int/vaccine_research/feuille_1_4-2.pdf
- [2]. Moorthy VS, Reed Z, Smith PG. Measurement of malaria vaccine efficacy in phase III trials: report of a WHO consultation. *Vaccine*. Jul; 2007 25(28):5115–23. [PubMed: 17577487]
- [3]. Moorthy V, Reed Z, Smith P. MALVAC 2008: measures of efficacy of malaria vaccines in phase 2b and phase 3 trials – scientific, regulatory and public health perspectives. *Vaccine*. Jan; 2009 27(5):624–8. [PubMed: 19061928]

- [4]. Mendis K, Sina BJ, Mechesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg.* 2001; 64(1-2 Suppl.):97–106. [PubMed: 11425182]
- [5]. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. *Am J Trop Med Hyg.* Dec; 2007 77(6 Suppl.):79–87. [PubMed: 18165478]
- [6]. Kochar DK, Saxena V, Singh N, Kochar SK, Kumar SV, Das A. Plasmodium vivax malaria. *Emerg Infect Dis.* Jan; 2005 11(1):132–4. [PubMed: 15705338]
- [7]. Barcus MJ, Basri H, Picarima H, Manyakori C, Sekartuti, Elyazar I, et al. Demographic risk factors for severe and fatal vivax and falciparum malaria among hospital admissions in northeastern Indonesian Papua. *Am J Trop Med Hyg.* Nov; 2007 77(5):984–91. [PubMed: 17984364]
- [8]. Genton B, D'Acremont V, Rare L, Baea K, Reeder JC, Alpers MP, et al. Plasmodium vivax and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Med.* Jun.2008 5(6):e127. [PubMed: 18563961]
- [9]. Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, et al. Multidrug-resistant Plasmodium vivax associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Med.* Jun.2008 5(17):e128. [PubMed: 18563962]
- [10]. Baird JK. Neglect of *Plasmodium vivax* malaria. *Trends Parasitol.* Nov; 2007 23(11):533–9. [PubMed: 17933585]
- [11]. Nosten F, McGready R, Simpson JA, Thwai KL, Balkan S, Cho T, et al. Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet.* Aug; 1999 354(9178):546–9. [PubMed: 10470698]
- [12]. Greenwood BM. Control to elimination: implications for malaria research. *Trends Parasitol.* Oct; 2008 24(10):449–54. [PubMed: 18760671]
- [13]. Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar D, Alonso PL, del Portillo HA. Key gaps in knowledge of Plasmodium vivax, a neglected human malaria parasite. *Lancet Infect Dis.* 2009; (9) in press.
- [14]. McKenzie FE, Jeffery GM, Collins WE. Plasmodium vivax blood-stage dynamics. *J Parasitol.* Jun; 2002 88(3):521–35. [PubMed: 12099421]
- [15]. Collins WE, Jeffery GM, Roberts JM. A retrospective examination of the effect of fever and microgametocyte count on mosquito infection on humans infected with Plasmodium vivax. *Am J Trop Med Hyg.* Jun; 2004 70(6):638–41. [PubMed: 15211005]
- [16]. Gilles, HM.; Warrell, D. Essential malariology. Arnold; London: 2002.
- [17]. Chareonviriyaphap T, Bangs MJ, Ratanatham S. Status of malaria in Thailand. *Southeast Asian J Trop Med Public Health.* 2000; 31:225–37. [PubMed: 11127318]
- [18]. Luxemburger C, Thwai KL, White NJ, Webster HK, Kyle DE, Maelankirri L, et al. The epidemiology of malaria in a Karen population on the western border of Thailand. *Trans R Soc Trop Med Hyg.* Mar-Apr;1996 90(2):105–11. [PubMed: 8761562]
- [19]. Chaves SS, Rodrigues LC. An initial examination of the epidemiology of malaria in the state of Roraima, in the Brazilian Amazon basin. *Rev Inst Med Trop Sao Paulo.* 2000; 42:269–75. [PubMed: 11058937]
- [20]. Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of Plasmodium vivax infection result from clonal hypnozoites activated at predetermined intervals. *J Infect Dis.* Apr; 2007 195(7):934–41. [PubMed: 17330782]
- [21]. Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, et al. Relapses of Plasmodium vivax infection usually result from activation of heterologous hypnozoites. *J Infect Dis.* Apr; 2007 195(7):927–33. [PubMed: 17330781]
- [22]. Michon P, Cole-Tobian JL, Dabod E, Schoepflin S, Igu J, Susapu M, et al. The risk of malarial infections and disease in Papua New Guinean children. *Am J Trop Med Hyg.* Jun; 2007 76(6): 997–1008. [PubMed: 17556601]
- [23]. Maitland K, Williams AI, Bennett NM, Newbold C, Peto TE, Viji J, et al. The interaction between *Plasmodium falciparum* and *P. vivax* in children on Espiritu Santo island, Vanuatu. *Trans R Soc Trop Med Hyg.* 1996; 90:614–20. [PubMed: 9015495]
- [24]. Balfour MC. Malaria studies in Greece. *Am J Trop Med Hyg.* 1935; 15:301–29.
- [25]. Earle WC. Epidemiology of malaria in Puerto Rico. *Puerto Rico J Pub Health Trop Med.* 1939; 15:3–27.

- [26]. Collins WE, Jeffery GM, Roberts JM. A retrospective examination of reinfection of humans with *Plasmodium vivax*. *Am J Trop Med Hyg.* Jun; 2004 70(6):642–4. [PubMed: 15211006]
- [27]. Collins WE, Jeffery GM. A retrospective examination of secondary sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity following secondary infection. *Am J Trop Med Hyg.* Jul; 1999 61(Suppl.)(1):20–35. [PubMed: 10432042]
- [28]. Baird JK. Host age as a determinant of naturally acquired immunity to *Plasmodium falciparum*. *Parasitol Today.* Mar; 1995 11(3):105–11. [PubMed: 15275362]
- [29]. Genton B, Al Yaman F, Beck HP, Hii J, Mellor S, Narara A, et al. The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trials. I. Malariometric indices and immunity. *Ann Trop Med Parasitol.* 1995; 89(4):359–76. 1995. [PubMed: 7487223]
- [30]. Kasehagen LJ, Mueller I, McNamara DT, Bockarie MJ, Kiniboro B, Rare L, et al. Changing patterns of *Plasmodium* blood-stage infections in the Wosera region of Papua New Guinea monitored by light microscopy and high throughput PCR diagnosis. *Am J Trop Med Hyg.* Oct; 2006 75(4):588–96. [PubMed: 17038678]
- [31]. Alves FP, Durlacher RR, Menezes MJ, Krieger H, Silva LH, Camargo EP. High prevalence of asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* infections in native Amazonian populations. *Am J Trop Med Hyg.* 2002; 66(6):641–8. [PubMed: 12224567]
- [32]. Snounou G, White NJ. The co-existence of *Plasmodium*: sidelights from *falciparum* and *vivax* malaria in Thailand. *Trends Parasitol.* Jul; 2004 20(7):333–9. [PubMed: 15193565]
- [33]. Williams TN, Maitland K, Bennett S, Ganczakowski M, Peto TE, Newbold CI, et al. High incidence of malaria in alpha-thalassaemic children. *Nature.* 1996; 383:522–5. [PubMed: 8849722]
- [34]. Maitland K, Williams TN, Peto TE, Day KP, Clegg JB, Weatherall DJ, et al. Absence of malaria-specific mortality in children in an area of hyperendemic malaria. *Trans R Soc Trop Med Hyg.* 1997; 91:562–6. [PubMed: 9463668]
- [35]. Nosten F, Luxemburger C, Kyle DE, Gordon DM, Ballou WR, Sadoff JC, et al. Phase I trial of the SPf66 malaria vaccine in a malaria-experienced population in Southeast Asia. *Am J Trop Med Hyg.* 1997; 56(5):526–32. [PubMed: 9180603]
- [36]. Noya O, Gabaldon Berti Y, Alarcon de Noya B, Borges R, Zerpa N, Urbaz JD, et al. A population-based clinical trial with the SPf66 synthetic *Plasmodium falciparum* malaria vaccine in Venezuela. *J Infect Dis.* Aug; 1994 170(2):396–402. [PubMed: 8035026]
- [37]. Herrera S, Corradin G, Arevalo-Herrera M. An update on the search for a *Plasmodium vivax* vaccine. *Trends Parasitol.* Mar; 2007 23(3):122–8. [PubMed: 17258937]
- [38]. Malkin E, Dubovsky F, Moree M. Progress towards the development of malaria vaccines. *Trends Parasitol.* Jul; 2006 22(7):292–5. [PubMed: 16707275]
- [39]. WHO Initiative for Vaccine Research. Candidate malaria vaccines in clinical development; October 2008. Available from: www.who.int/vaccine_research/documents/en/ under “Malaria Vaccines”
- [40]. WHO Initiative for Vaccine Research. Candidate malaria vaccines in preclinical development; October 2008. Available from: www.who.int/vaccine_research/documents/en/ under “Malaria Vaccines”
- [41]. Higgs ES, Sina B. *Plasmodium vivax* vaccine research: steps in the right direction. *Am J Trop Med Hyg.* Nov; 2005 73(Suppl.)(5):1–2. [PubMed: 16291759]
- [42]. Lancet. Is malaria eradication possible? *Lancet.* 2007; 370:1459. [PubMed: 17964329]
- [43]. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the RTS, S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. *Lancet.* 2004; 364:1411–20. [PubMed: 15488216]
- [44]. Baird JK, Hoffman SL. Primaquine therapy for malaria. *Clin Infect Dis.* Nov; 2004 39(9):1336–45. [PubMed: 15494911]
- [45]. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. *N Engl J Med.* 1976; 295:302–4. [PubMed: 778616]

- [46]. Smith T, Genton B, Baea K, Gibson N, Taime J, Narara A, et al. Relationships between *Plasmodium falciparum* infection and morbidity in a highly endemic area. *Parasitology*. Dec; 1994 109(Pt 5):539–49. [PubMed: 7831089]
- [47]. Vounatsou P, Smith T, Kitua AY, Alonso PL, Tanner M. Apparent tolerance of *Plasmodium falciparum* in infants in a highly endemic area. *Parasitology*. Jan; 2000 120(Pt 1):1–9. [PubMed: 10726260]
- [48]. Hill DR, Baird JK, Parise ME, Lewis LS, Ryan ET, Magill AJ. Primaquine: report from CDC expert meeting on malaria chemoprophylaxis I. *Am J Trop Med Hyg*. Sep; 2006 75(3):402–15. [PubMed: 16968913]
- [49]. WHO. Ethical considerations arising from vaccine trials conducted in paediatric populations with high disease burden in developing countries. 2002. Available from: www.who.int/vaccine_research/documents/ethics/en/index.html
- [50]. Müller I, Genton B, Rare L, Kiniboro B, Kastens W, Zimmerman P, Kazura J, Alpers M, Smith TA. Three different *Plasmodium* species show similar patterns of clinical tolerance of malaria infection. *Malaria J*. 2009; 8:158.