Methods for Determining Vaccine Efficacy and Effectiveness and the Main Barriers to Developing a Fully Deployable Malaria Vaccine

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Abstract. The past few years have witnessed the appearance of public-private partnerships and a significant increase of resources devoted to the development of malaria vaccines. Lessons have been learned on different approaches to the clinical development plan for a vaccine candidate, including different clinical trial phases and endpoints and methods to conduct them. Improved understanding of mechanisms underlying naturally acquired immunity, definitions of surrogate markers of protection, including improved in vitro assays and animal models, and strengthened capacity in malaria-endemic countries to conduct clinical trials would accelerate the development of malaria vaccines. We are closer to having a first-generation vaccine being registered. The impact of this vaccine and subsequent products will need to be evaluated under program conditions in the context of comprehensive malaria control activities. Deployment of a safe and effective malaria vaccine administered in early childhood could become a major public health tool, particularly in sub-Saharan Africa.

INTRODUCTION

Malaria continues to exact a major toll for young children and pregnant women in disease-endemic areas, especially in sub-Saharan Africa. More than one million deaths per year remind us of how inadequate malaria control remains throughout most of the world, even though we have tools that, if better deployed, would have a significant public health impact. Improved access to prompt diagnosis and treatment with effective antimalarials needs to be combined with a multi-pronged approach to prevention that should include vector control, insecticide-treated nets (ITNs), and the use of drugs for prevention of infection in high risk groups (intermittent preventive treatment). The development of a safe and efficacious malaria vaccine has remained an elusive goal for the past 80 years. However, recent years have witnessed a new impetus and the generation of conclusive evidence that effective immunity against Plasmodium falciparum infection, mild uncomplicated malaria, and severe disease can be induced through vaccination in children living in malaria-endemic areas. It is likely that the next decade will witness the first registration of a malaria vaccine. This vaccine is unlikely to provide complete protection, but it may become a useful tool to be added to the armory in the fight against malaria.

COMPLEXITY OF DEVELOPING A MALARIA VACCINE

Developing a malaria vaccine remains an enormous scientific, technical, financial, and political challenge. The Plasmodium is a highly complex parasite, with a multi-stage life cycle, during which the parasite presents multiple antigens that show significant variability. The main hindrance to developing a vaccine is the choice of the antigen(s) to be included. This process has often relied on the use of imperfect animal models, in vitro assays with significant limitations, and seroepidemiological assessments of naturally acquired immune responses in humans and their relationship to the risk of malaria infection or disease. However, we still have no surrogate measure of protection for malaria. Numerous associations between antigen-specific immune responses and the risk of infection or disease have been described. However, an association between a given immune response and the risk of malaria does not mean that those responses play a role in protection, as previous exposure to the parasite and development of responses to multiple antigens, most of which are unlikely to be linked to protection and are simply a marker of exposure, become an important confounding factor. At present there are approximately 100 candidate malaria vaccines in development, including pre-erythrocytic vaccines, blood-stage vaccines, anti-toxic vaccines, and transmission-blocking vaccines. Nevertheless, most of them are based on a small number of P. falciparum antigens. Only a few candidates have entered clinical phase studies, and most are still in pre-clinical phases.

The malaria community has understandably placed a major effort in the antigen selection process as the key to the development of a malaria vaccine. However, the results of the RTS,S candidate vaccine have highlighted the critical role that formulation and adjuvant technology may play in the successful development of a product. This vaccine, based on the circumsporozoite antigen, had failed to provide protection in numerous trials and following commonly held views, should have been abandoned a long time ago. However, formulation of that same antigen in a new adjuvant platform radically changed the outlook for this product, which has shown safety, immunogenicity, and induction of protection in challenge models with non-immune volunteers, hyper-immune adults, and semi-immune children living in malaria-endemic areas of Africa.

CLINICAL DEVELOPMENT PLAN TO EVALUATE A P. FALCI PARUM CANDIDATE VACCINE

The different phases of a development plan for malaria vaccines have been defined. There are, however, some important differences with regards to other vaccines. First, there are potentially different types of vaccines aimed against different stages in the life cycle of the parasite, which may imply
the use of different endpoints in their evaluation. Second, there is a well established and reproducible challenge model in humans that appears to be useful in the initial screening of pre-erythrocytic vaccines. Finally, the vaccine candidate under development may be aimed at different target groups, which may determine the type of clinical development plan and particularly the age groups in which it is tested. The development pathway is not usually linear, but is an iterative process during which some trials can be repeated in different age groups or epidemiologic settings (Figure 1).

**Phase I studies.** Testing for the first time in humans in a phase I trial usually implies a few volunteers (less than 100) to evaluate initial safety and immunogenicity of the candidate vaccine. This first study usually takes place in the country where the vaccine was developed, usually for a combination of reasons, including logistical, ethical, and psychological comfort of the investigators, as well as for involvement of the regulatory agency. Unless the vaccine only targets non-immune adult travelers, a logical progression leads to further phase I studies in semi-immune populations because genetic differences and previous exposure to malaria may influence the safety and immunogenicity of the vaccine. There are different approaches to the age de-escalation process. Although some investigators have taken a slow and gradual process starting with adults and then moving slowly to older children, younger children, and infants, others have proposed faster processes going quickly to the final target age group. Phase I trials are also aimed at evaluating different vaccine intervals and doses (dose de-escalation) to determine the final interval and dose to be used on the basis of results of safety and immunogenicity testing.

To accelerate the development of a candidate vaccine, phase I trials are often converted to phase I/IIb trials with a sample size that enables protection to be estimated. Showing safety and efficacy in an age group provides good evidence to proceed to the following age group or phase and saves time and money.

**Phase II studies.** In malaria vaccine development, phase II trials include an initial assessment of protection. Given the development in the 1960s of a human challenge model that injected sporozoites through laboratory-reared mosquitoes, this phase is subdivided into parts “a” and “b,” which refer to whether exposure to malaria is through experimental challenge or under natural exposure in a malaria-endemic area, respectively.

Phase IIa trials have been mainly used to test pre-erythrocytic vaccines and are conducted in non-immune volunteers. However, the role of this model in assessing protection for asexual stage antigens needs to be further evaluated. The main limiting factor is the need to treat infections soon after the detection and the uncertain equivalence of experimental with natural challenge. Conversely, only a few volunteers are needed and results can shed light on the potential of the candidate vaccine. Improved experimental challenge models for pre-erythrocytic vaccines and new models for blood-stage vaccines could be a great tool for screening candidate vaccines, accelerating their development, and lowering costs. If phase IIa studies of pre-erythrocytic vaccines show protection, it is a strong evidence for proceeding to field trials. Otherwise, the candidate should be abandoned. However, negative results of phase IIa studies of blood-stage vaccines should not necessarily lead to stopping the development of that candidate because we do not know whether the results under natural exposure would be equivalent.

Phase IIb studies are proof-of-concept efficacy trials that are conducted in semi-immune volunteers in malaria-endemic areas and aim to evaluate vaccine efficacy by natural challenge. Secondary objectives always include monitoring safety and immunogenicity. The study has to be a double-blind, randomized-controlled trial and the selection of the efficacy endpoint is critical for its design. The decision of embarking on a phase IIb trial is strategic because these trials are expensive and complex. Phase IIb trials require large sample sizes (hundreds to thousands of volunteers), long follow-up periods, and complicated logistics to be deployed in malaria-endemic countries. Evidence from previous phase I studies, which show that a vaccine is safe and immunogenic, and from phase IIa studies, which provide preliminary efficacy results, sets the path to phase IIb trials, the results of which will determine whether to continue with the further development of that vaccine candidate. Prior to starting such a trial, “Go/No-go” criteria should be established, on which the decision to proceed further will be based.

**Phase III studies.** Phase III studies are efficacy pivotal trials for registration. The primary objective is the same as in phase IIb studies, but the sample size is usually larger and the vaccine is tested in the final target population, which for most vaccines is infants, in which it aims to be deployed and in the formulation that will be used for registration. Genetic features, variability of parasite strains, and intensity of malaria transmission may influence vaccine safety, efficacy, and immunogenicity. Therefore, different trials will have to be conducted in different epidemiologic settings to provide enough evidence for the regulatory authorities to license the vaccine. An issue that has not been defined and would need to be established is the number of different sites in which the vaccine has to be tested prior to licensing.

**Phase IV studies.** After a vaccine has been licensed, post-registration effectiveness studies need to be conducted in large populations. Objectives also include the assessment of duration of protection and the safety, especially the monitoring of rare adverse events. National authorities will need to

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**Figure 1.** Iterative process of the clinical development plan for a malaria vaccine.
decide whether they want to deploy a vaccine at the country level. This will depend on the cost-effectiveness of the vaccine, the availability of other control measures, and the funds to pay for the deployment of the vaccine. Once a vaccine has shown to be efficacious, it would be unethical not to administer it to the whole population at risk. Thus, phase IV trials need alternative study designs to be able to evaluate effectiveness, such as the stepped-wedge design\textsuperscript{14} or case-control studies.

A vaccine that contains a combination of antigens, either from the same stage (multivalent) or from different stages of the life cycle (multistage), will probably be more efficacious than single antigen vaccines because of the heterogeneity of the immune response to the parasite. Nevertheless, it is not clear how evaluation of a combination vaccine would be done. There are basically two strategies: the bottom-up and the top-down.\textsuperscript{15} The bottom-up approach would first validate each antigen separately and would then build up a combination of the antigens that have been shown to be effective, which makes the development slow but avoids interference between antigens and saves money. The top-down approach starts evaluating a combination vaccine, which is much faster, but increases the cost, the possible interference between antigens, and the potential for adverse events related to the number of antigens. If the combination is efficacious, it will be difficult to know which of the antigens are the ones that confer protection and which are unnecessary. The goal for the future development of vaccines is to produce a multi-antigen vaccine, e.g., to add other antigens to the RTS,S vaccine, which is the most advanced candidate vaccine.\textsuperscript{16}

TARGET POPULATIONS

Malaria vaccines should be administered to the groups at highest risk for severe disease. There are essentially three different target groups that will require clearly distinct development plans: 1) children living in areas of stable malaria transmission, mostly in Africa; 2) pregnant women living in areas of stable transmission; and 3) non-immune adults that either travel to malaria-endemic areas or live in areas where epidemic outbreaks may take place.

**Children in malaria-endemic areas.** Ideally, children should be vaccinated during the first months of life through the Expanded Program on Immunization (EPI). Given that malaria starts early in infancy this would confer protection during the highest risk age and would enable using the delivery mechanism of the EPI, one of the few well-functioning health programs that reaches most of the childhood population in developing countries. Administering the vaccine through the EPI would ensure a high coverage and reduce the costs. Nevertheless, there is no evidence that the EPI schedule is the best suited for malaria vaccines because the immune system in early infancy is immature and does not respond to some types of vaccines. The optimal age at which to administer these vaccines to obtain a good immune response, as well as possible interactions with other EPI vaccines, will have to be studied in detail. Also, if protection of the vaccine is not sustained, the schedule will have to change according to the level of transmission, as the highest risk age varies.

**Pregnant women in malaria-endemic areas.** Protecting pregnant women from malaria complications by vaccination could have a great impact, but there are still several issues that need to be investigated further, including vaccine type, timing of vaccination, and maintenance of naturally acquired immunity that protects multiglandae.\textsuperscript{12} One option is to develop pregnancy-specific vaccines that avoid sequestration of parasites in the placenta, such as the candidate VAR2CSA.\textsuperscript{17–19} Safety and regulatory issues will have to be taken into account when testing these vaccines for efficacy if such trials were to be conducted in pregnant women. The other option is to administer general malaria vaccines (pre-erythrocytic or blood-stage) to avoid infection and disease. These vaccines would have to be administered before the first pregnancy, which could be done through vaccination of teenage girls, should the vaccine confer long-lasting protection. Development of these vaccines could be faster because they would have been tested for efficacy in non-pregnant adults and/or children. In addition, safety issues would be of a lesser concern because these vaccines would not have to be tested during pregnancy. However, teenagers and young women do not have routine contacts with the health system outside pregnancy. Therefore, a different vaccine deployment mechanism that reaches a good coverage needs to be devised.

**Non-immune adults.** Traditionally only travelers, tourists, and expatriates from countries not endemic for malaria were included in this target group. However, growing urbanization and population movements have increased the number of non-immune persons in malaria-endemic countries. Non-immune city dwellers, miners, or tourists who go to areas of malaria transmission in their own country or a neighboring country would benefit from a malaria vaccine. Likewise, such a vaccine would be useful to control malaria outbreaks in islands or areas of unstable malaria transmission.

Another issue that will have to be evaluated is the efficacy and safety of malaria vaccines in persons infected with human immunodeficiency virus. We will need to know whether the immune response is impaired, whether vaccination activates replication of human immunodeficiency virus, and the risk of increased adverse events.\textsuperscript{20}

TRIAL ENDPOINTS

Selection of trial endpoints is often a complex matter that depends on a number of factors, including the type of vaccine, the type of trial (efficacy or effectiveness), and the evidence needed for advocacy and policy decision. The endpoint will determine the sample size and will have implications in terms of cost and time. Thus, choosing an endpoint is a compromise between all these issues and finding the correct balance is challenging.

Figure 2 shows the different factors that play a role in endpoint selection. As we move from an efficacy trial to an effectiveness trial, the endpoints used are further downstream and the distance between the biologic target of the vaccine and the trial endpoint increases. This decreases the generalizability of results because the number of local cofactors modifying the risk of malaria is larger, and increases the sample size needed. Conversely, it increases the public health importance of the vaccine.

In early phase IIb stages, it is critical to obtain early evidence of protection. This may imply working closer to the biologic target of the vaccine, i.e., incidence of infection for
pre-erythrocytic vaccines and transmission-blocking vaccines and incidence of clinical malaria for blood-stage and anti-toxic vaccines.

We agree that it may be difficult to predict how the effect on one endpoint will impact on endpoints that are further downstream, i.e., what will be the reduction in clinical malaria given a reduction in new infections. However, everyone will agree that if a vaccine prevents infections, there will be an impact downstream in the natural history of the disease and the vaccine will therefore prevent disease and death. Also, severe disease can be included as a secondary endpoint in phase IIb trials because although the trial will not be powered to estimate efficacy against severe disease, results can be significant and precise if there are enough severe malaria cases, and a positive result may be important in the decision making on whether to continue with the clinical development plan of that candidate. The RTS,S/AS02A phase IIb trial conducted in Mozambican children was designed with two cohorts. Cohort 1 had clinical malaria as the primary endpoint and severe malaria as an exploratory endpoint, and cohort 2 had time to new infection as the primary endpoint. The results of the two cohorts were correlated and the vaccine showed efficacy against all three endpoints.

In such a scenario, and once a reproducible correlation between infection and disease is demonstrated for a pre-erythrocytic vaccine, it could be argued that the product is registered using data on protection against infection, which would accelerate time to registration and reduce the costs.

For further proof-of-principle trials, endpoints that are further downstream are more suitable to provide evidence on the potential public health role of the vaccine. Although efficacy against severe malaria or mortality would be powerful in advocating use of the vaccine, trials using these endpoints require large sample sizes, are difficult to conduct because of the difficulty of defining and diagnosing severe malaria in many malaria-endemic areas, and are expensive. We believe that using clinical malaria also provides strong evidence for advocacy because following the reasoning above, protection against uncomplicated malaria has to necessarily decrease severe disease and mortality, and would reduce the complexity and costs because sample sizes are smaller and case definitions are easier to establish.

Establishing case definitions for the endpoints to be measured is crucial. This is especially important for clinical malaria because there is no standard definition. The malaria-attributable fraction of fever and the sensitivity and specificity of different malaria case definitions have to be calculated for each age group. In a vaccine trial, the case definition with the highest specificity and sensitivity for that age group and study area should be used. A low specificity of the case definition would bias the vaccine efficacy towards zero, and a low sensitivity would reduce the power of the study. There is also no established definition for severe malaria. Definitions used have a low specificity and severe malaria cases are often misclassified. In a study conducted in Malawi, in which autopsies were performed on 31 patients who were examined by expert clinicians and classified as having clinically defined cerebral malaria (Blantyre coma score ≥ 2), 23% had actually died of other causes.

Defining malaria-specific mortality is also difficult, given that a large percentage of malaria deaths occur at home and that the verbal autopsies used to define them have a low specificity. Thus, using overall mortality, which also includes indirect malaria mortality, as a trial endpoint is better than using malaria-specific mortality.

Guidelines will be developed to specify which endpoints should be used in trials of vaccines to prevent malaria complications in pregnant women. It will have to be decided whether endpoints of the mother (clinical malaria, anemia), the pregnancy (placental malaria, abortion, still birth), or the child (low birth weight, prematurity, overall neonatal mortality) will be included as trial primary and secondary endpoints.

\textbf{PLASMODIUM VIVAX}

The burden of \textit{P. vivax} malaria has been underestimated and more detailed epidemiologic, clinical, and immunologic research on \textit{P. vivax} is needed. Far less effort has been devoted to development of \textit{P. vivax} vaccines than to \textit{P. falciparum}.

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\caption{Choice of endpoints in development of a malaria vaccine.}
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parum vaccines, with only one candidate having entered clinical trials. Development of P. vivax vaccines should be included in malaria vaccine programs and guidelines for the evaluation of these vaccines are being established to address the differential characteristics of the P. vivax life cycle and epidemiology.

However, if a single-species vaccine is used in areas with both P. vivax and P. falciparum transmission, the effect of the vaccine on the incidence of the other species is unknown. Ultimately, the aim is to obtain a combined P. vivax-P. falciparum vaccine to be used in areas of co-endemicity. Given that P. vivax coexists almost everywhere with P. falciparum, a combination vaccine will probably be the only malaria vaccine that can be used outside sub-Saharan Africa.

DEPLOYMENT

First- and second-generation vaccines will probably be only partially effective. The desired performance a candidate vaccine needs to attain to justify licensing and deployment has been outlined. However, it warrants further consideration, taking into account the evaluation of the potential impact of a partially effective vaccine deployed by itself and deployed in the context of other control tools such as ITNs or intermittent preventive treatment in infants (IPTi). Malaria control needs to be tackled using multiple approaches, and unless a scale up of a combination of control tools is achieved, there will be no impact on the burden of malaria. A vaccine will have to be used in combination with other control tools. Therefore, assessment of vaccine efficacy when deployed together with ITNs or IPTi is required to determine whether vaccines add protection to these already available tools.

Licensing an efficacious malaria vaccine for children or pregnant women will be the first step in the pursuit of improving malaria control through vaccination. Nevertheless, having a malaria vaccine is no guarantee of achieving an impact, the final goal being to reach high-risk populations. Deployment and funding mechanisms have to be foreseen so that once the vaccine is ready for wide-scale use, it can be rapidly and efficiently deployed. Only recently have hepatitis B and Haemophilus influenzae type b vaccines been introduced in some developing countries, after having been used for many years in western countries. We cannot accept such delay in the implementation of future malaria vaccines. Cooperation among the whole international public health community is needed to work together with governments in developing countries, pharmaceutical companies, and funding sources to achieve a good uptake. The Program for Appropriate Technology in Health Malaria Vaccine Initiative is working in this direction by not only accelerating the development of malaria vaccine candidates but also by preparing the introduction and accessibility of licensed vaccines.

To help in national decision-making about introducing a malaria vaccine, policymakers will need epidemiologic and economic data, including the burden of malaria; the cost-effectiveness of the new vaccine, of other control tools, and of the combination of both; the health system capability to deliver the vaccines; and the available financing mechanisms. These data would need to be produced in advance to generate strong advocacy for vaccine introduction. Malaria vaccines will probably be much more expensive than currently available EPI vaccines. Thus, new financing mechanisms must be established to make them accessible to governments of developing countries. Commitment from international organizations such as the Global Alliance for Vaccines and Immunization, together with The Vaccine Fund, its financial partner, and contributions from governments of industrialized countries will be needed. Pharmaceutical companies have to be encouraged to participate in the development and scaling-up of malaria vaccines because their role is critical, but mechanisms have to be put in place to ensure the return of the investment. The cost of the research is enormous; conducting the whole development plan takes several years and the licensure process may be complex. Strategies such as the Advance Market Commitments, which aim at pre-purchasing vaccines when they are being developed, should be encouraged to make the malaria vaccine market attractive for pharmaceutical companies.

CONCLUSIONS

Malaria vaccine development has greatly advanced during the past decade. Although the process from pre-clinical research to licensing requires several years, we hope that as a result of the increased international commitment, it can be accelerated and a malaria vaccine can be licensed in the near future. Some obstacles still have to be overcome. These obstacles include the lack of a surrogate of protection and the lack of sufficient capacity in malaria-endemic countries to conduct clinical trials. The Malaria Clinical Trials Alliance, an African-led institution funded by the Bill & Melinda Gates Foundation, has been recently founded with the aim of strengthening the capacity of conducting antimalarial drugs and vaccines clinical trials. Developing adequate and validated experimental challenge models for asexual blood-stage vaccines and improving those for pre-erythrocytic vaccines would facilitate screening of candidate vaccines. This development should be complemented by a better model for the prediction of the performance of a candidate vaccine under natural exposure according to its performance in previous phase IIa studies. Finally, evaluating the impact of vaccines when used in combination with other malaria control tools (ITNs or IPTi) will be required. We trust that the increase in the institutions and funding devoted to combat malaria will have in the coming years a significant impact on the burden of disease.

Received August 21, 2006. Accepted for publication September 22, 2007.

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