Malaria vaccines: current situation, challenges and strategy for the future; a developer's perspective from the PATH Malaria Vaccine Initiative

The new paradigm of elimination and eradication and constrained economic environment – combined with a first decade of experience – have lead the PATH Malaria Vaccine Initiative (MVI) to redefine its vaccine development strategy. This strategy has two targets: the pre-erythrocytic and sexual stages of the parasite, the latter including the parasite's evolution inside the mosquito. It encompasses four components: the selection of new antigens, identification of presenting platforms, adjuvant development and vaccine formulation and evaluation technologies. However, the development of a malaria vaccine cannot occur in isolation from other interventions; rather, it should be carried out in coordination with them. RTS,S is now in a Phase III trial, after demonstrating levels of efficacy from 30 to 50% against clinical malaria. Bringing a partially effective vaccine to licensure and use is a long and complex process that will require efficacy as well as public health impact data. Prime-boost and attenuated sporozoite approaches are, or will be, part of the portfolio required to build on these results, the former including use of adenovirus-based platforms. Current investments in blood-stage approaches are limited to attempting to resolve the challenge of *AMA1* polymorphism and to an approach aimed at blocking re-entry of merozoites into red blood cells. MVI has prioritized the development of transmission-blocking vaccines and has identified some promising projects, while also researching their regulatory pathway. Two projects targeting *Plasmodium vivax* are supported. As part of its strategy, MVI has adopted a new classification of projects into small-scale preclinical feasibility studies, larger translational projects and, finally, vaccine candidates, the latter requiring proof-of-concept to be established in endemic countries.

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The last 10 years have seen important developments take place in the field of malaria. Major players began investing in expanding coverage of existing tools and in the development of new ones. Three events of the last 3 years stand out. First, in October 2007, Bill & Melinda Gates challenged the malaria community to embrace eradication of malaria as a long-term goal. Then, in October 2008, the global economy went into crisis, with subsequent negative impacts on public and private donors' capacity to give. In addition, in May 2009, RTS,S entered a large-scale Phase III trial, moving the field an important step closer to the eventual introduction of the first licensed malaria vaccine.

As a public–private partnership standing at the intersection of the nonprofit/public and for-profit sectors, the PATH Malaria Vaccine Initiative (MVI) has had to revisit its strategy, taking into account the above events and the results of 10 years of investments in malaria vaccine development.

Positive results have been observed with a vaccine candidate based on one antigen only, the circumsporozoite protein (CSP). The development of RTS,S offers a unique opportunity to further our understanding of the complexity of the technical, clinical and regulatory aspects of malaria vaccine development.

Our approach to malaria vaccine development is now based on several major axes: the successful completion of the RTS,S Phase III trial and introduction; the development of a more effective, next-generation vaccine candidate; the definition of the vaccine that will be needed to complement other malaria interventions in 10–15 years; and the development of new transmission-blocking research projects. Progress on all these fronts will necessitate addressing challenging technical and scientific issues, and overcoming any policy and regulatory challenges in order to make this intervention available more quickly. In addition, vaccine development efforts to date have primarily targeted *Plasmodium falciparum;* a vaccine targeting *Plasmodium vivax* now becomes more important. Finally, one has to consider the level of effort that should be dedicated to the development of blood-stage vaccines.

A vaccine developer also has to take into account criteria that go beyond finding the answers to scientific questions. These include manufacturability, time to licensure, cost of

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goods, development costs and the potential impact of this intervention alongside others. All of the above requires the definition of a careful and transparent process of project selection and down-selection.

A partially effective vaccine will add to the currently available interventions. More effective vaccines and vaccines targeting malaria transmission are the tools needed to effectively address the challenge of elimination and eradication.

Strategic considerations

Plasmodium lifecycle bottlenecks

When considering the *Plasmodium* lifecycle, three main bottlenecks can be identified [1,2]. A bottleneck is defined as a moment in the cycle when the parasite could be considered vulnerable. The number of sporozoites inoculated during a blood meal by the *Anopheles* female is considered to be low, and does not compare in any way to the large number inoculated during a clinical challenge trial. One of the principal challenges met by a vaccine targeting the pre-erythrocytic stage is the very short period of time spent by the sporozoites in the blood stream prior to hepatocyte invasion. Circulating antibodies are the main actors at this stage (Figure 1).

Strategic questions to be answered

When approaching the development of a malaria vaccine, one could divide the areas of investment into four categories (Figure 2).

Antigens

Most of the antigens that are used today in vaccine development were discovered 10–30 years ago [3]. Using more recent developments in the genomics and proteomics of *P. falciparum* and *P. vivax,* several research groups have identified additional pre-erythrocytic and liver-stage antigens, the down-selection of which could make possible the incorporation of new antigens to complement CSP in a multiantigenic approach. However, the benefits of additional antigens have yet to be confirmed.

Platforms

Further increasing the efficacy of RTS,S and inducing a stronger cellular immune response will require the use of vectors, such as replication incompetent adenoviruses. Other interesting vector options are also being explored, from an attenuated Yellow fever virus to biodegradable nanoparticles.

Adjuvants & formulations

RTS,S has demonstrated that a potent adjuvant system such as AS01 or AS02 is pivotal to the generation of increased, albeit still partial, efficacy. Such adjuvant systems could be used for many other proteins, but their use is limited by intellectual property considerations. The malaria vaccine community has long relied on what was freely available – that is, aluminium-based adjuvants or montanide. The Infectious Diseases Research Institute [4], with funding from the Bill & Melinda Gates Foundation, is working on several options, the most advanced of which, the glycopyranosyl lipid adjuvant (GLA), should fill this gap. In parallel to access to potent adjuvants, great emphasis is placed on the quality of the formulation and the quality of the protein for those approaches using recombinant proteins. MVI has selected CSP as the antigen of choice for the evaluation of vectors and carrier platforms.

Evaluation technologies

The malaria vaccine field suffers from a lack of surrogates of protection. The RTS,S Phase III trial offers the unique opportunity of a placebo-controlled trial to better understand correlates of protection.

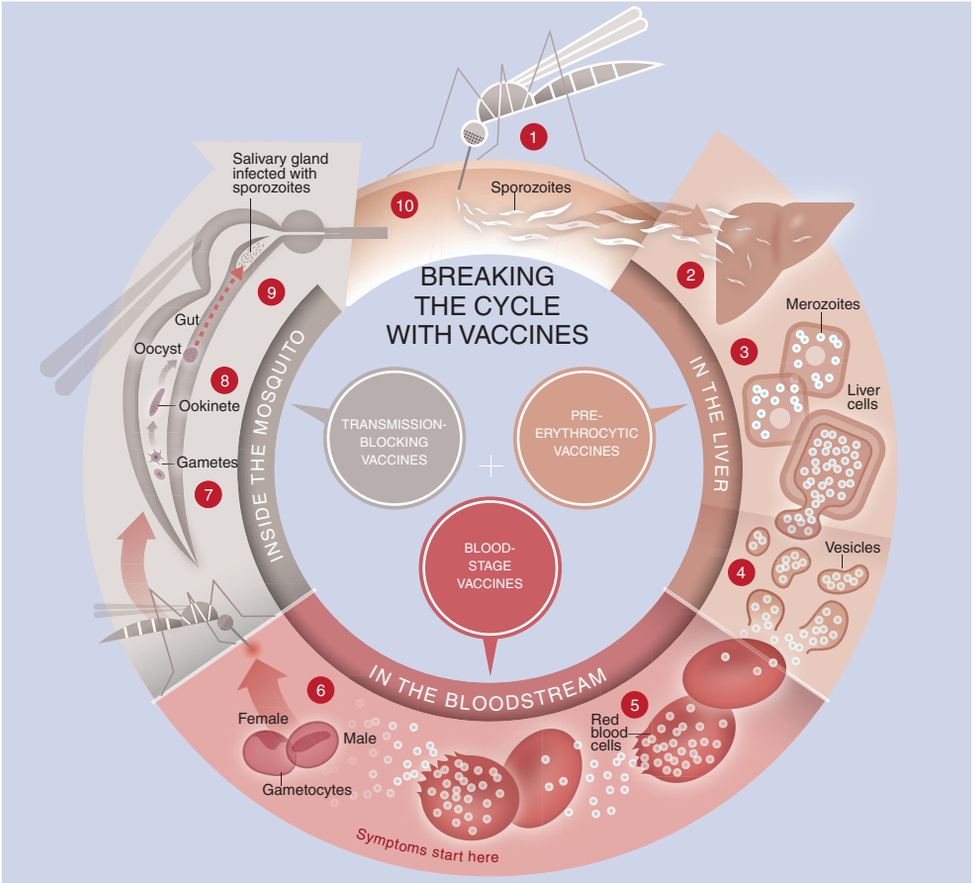
Fortunately, however, a clinical challenge model in immunologically naive adults is available to assess the efficacy of pre-erythrocytic vaccine candidates, and we consider the challenge trial a critical milestone prior to moving into endemic countries [5–7]. A new center is under development at the Seattle Biomedical Research Institute (WA, USA) to expand the recruitment capacity for such trials.

The development and the standardization of assays is another area of significant investment; these include the growth inhibition assay, T-cell assays, ELISA and immunofluorescence assays. The creation of reference centers has allowed the development of standard operating procedures and their transfer to other centers.

Currently available assays do not predict vaccine efficacy, but may result in projects being advanced to the field. Thus, the most reliable model in malaria remains the field trial in endemic conditions. The history of vaccine development has been a long succession of empirical developments often confirmed by immunological studies.

Two important paradigm shifts

For the past three and a half decades – with the exception of work carried out on transmission-blocking vaccines – the aim of malaria vaccine



1. Malaria infection begins when an infected female *Anopheles* mosquito bites a person, injecting *Plasmodium* parasites, in the form of sporozites, into the bloodstream.

2. The sporozoites pass quickly into the human liver.

3. The sporozoites multiply asexually in the liver cells over the next 7–10 days, causing no symptoms.

4. The parasites, in the form of merozoites, burst from the liver cells in vesicles, journey through the heart and arrive in the lungs, where they settle within lung capillaries. The vesicles eventually disintegrate, freeing the merozoites to enter the blood phase of their development.*

5. In the bloodstream, the merozoites invade red blood cells (erythrocytes) and multiply again until the cells burst. Then they invade more erythrocytes. This cycle is repeated, causing fever each time parasites break free and invade blood cells.

6. Some of the infected blood cells leave the cycle of asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called gametocytes, that circulate in the bloodstream.

7. When a mosquito bites an infected human, it ingests the gametocytes, which develop further into mature sex cells called gametes.

8. The gametes develop into actively moving ookinetes that burrow into the mosquito's midgut wall and form oocysts.

9. Inside the oocyst, thousands of active sporozoites develop. The oocyst eventually bursts, releasing sporozoites that travel to the mosquito's salivary glands.

10. The cycle of human infection begins again when the mosquito bites another person.

Figure 1. The lifecycle of *Plasmodium falciparum*.
*As discussed in [28].

development has been a vaccine with efficacy equivalent to some of the existing viral and bacterial vaccines. More recently, the Malaria Vaccine Technology Roadmap defined two milestones: a vaccine with 50% efficacy against severe malaria by 2015, and a vaccine with 80% efficacy against clinical malaria by 2025 [101]. The increase in coverage of insecticide-treated

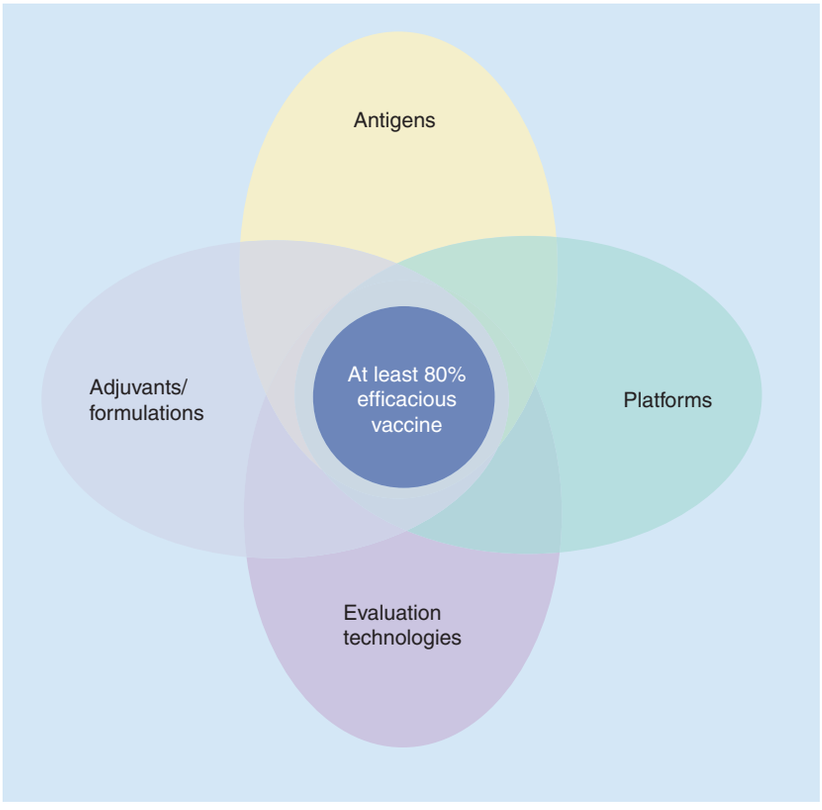


Figure 2. The four axes of malaria vaccine research & development.

bed nets (ITN), in-house spraying of insecticides, artemisinin-based combination drugs, and of intermittent preventive therapy in pregnant women and infants (ITPi) are expected to have a major impact on the epidemiology of malaria [8]. Considering this new environment, should these long-term goals not be revisited? With robust modeling, an extrapolation of the epidemiological situation of malaria in 2025 should help define the profile of the vaccine required at that time and guide investments made today.

The public health community has potent tools to combat malaria, a key difference between malaria and those diseases that have been eradicated or controlled through vaccination and have few other interventions available. A malaria vaccine should not only be benchmarked against other vaccines, but also against other malaria interventions, in addition to considering its cost, given that its use will be mostly in endemic countries where cost is an important factor in prioritization of health interventions.

Current situation

A vaccine candidate in Phase III: RTS,S

A pivotal Phase III trial of GlaxoSmithKline (GSK) Biologicals' (Rixensart, Belgium) RTS,S is now underway at 11 sites across sub-Saharan Africa. This trial is designed to confirm the efficacy and safety observed in earlier Phase II studies. A follow-up of 30 months should allow for a reliable estimate of the candidate's public health impact on malaria and other co-morbidities. The trial will measure efficacy under various epidemiological conditions.

RTS,S is a hybrid protein comprising the C-terminal portion of the CSP of *P. falciparum* linked to the surface antigen of hepatitis B virus and co-expressed with the S antigen from hepatitis B virus in *Saccharomyces cerevisiae*. It is combined with a GSK proprietary adjuvant known as AS01, made up of monophosphoryl lipid A (MPL) and QS21 (purified saponin from *Quillaja saponaria*) in a liposome formulation (AS01). AS02 (MPL plus QS21 in an oil-in-water emulsion) was used in most of the Phase II trials. The vaccine candidate is presented in two clipped vials, one containing RTS,S in a lyophilized form and the second one containing the adjuvant that is used as a diluent at the time of inoculation. Each vial contains two doses and can be stored under current vaccine cold chain conditions.

The Phase III trial is driven by a partnership among GSK Biologicals, MVI, 11 research centers in Africa, and several organizations in Africa, Europe and the USA. The vaccine will be studied in two populations: 5–17 months of age, and infants of the Expanded Program on Immunization (EPI) age group. Each population will be divided in three groups, two groups receiving RTS,S (one with a booster at month 20, one without booster) and a control group. In the 5–17-month age group the placebo will be a rabies vaccine. In the EPI age group, the placebo will be a meningitis C conjugated vaccine. Efficacy against clinical malaria is the primary end point, and nine secondary end points will be considered: severe malaria, malaria hospitalization, anemia, clinical malaria in different epidemiological settings, duration of efficacy, booster requirement, fatalities and other co-morbidities. Each child will be followed for a total period of 30 months. A primary analysis will be conducted 12 months after the third dose in each group and at the end of the study.

The 11 African sites are located in Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique and Tanzania. The 11 sites were selected through an open application process, and have seen their facilities and equipment upgraded; staff members have participated in the necessary trainings.

Information on other malaria interventions used by the households in which the study participants reside will be collected, including use of ITN, in-house spaying, and use of preventive therapies.

In parallel to the main study, ancillary studies will be conducted to measure immune responses, to try to define correlates of protection, to assess transmission intensity, and to assess through genotyping studies the possible impact of vaccination on the parasite strain.

The first breakthrough study of RTS,S was conducted in Manhiça (Mozambique) and published in 2004/2005 [9,10]. In a population of children aged 1–4 years, the vaccine was demonstrated to have 45% efficacy against infection over a 6-month period, 35% efficacy against clinical malaria expressed as time to first infection and 49% efficacy against severe malaria over a period of 18 months. The results of long-term follow-up (45 months) were published recently and demonstrated 30.5% efficacy against clinical malaria (time to first or only episode) and 38.3% against severe malaria [11]. The prevalence of *P. falciparum* was found to be 37% lower in the RTS,S group when compared with the control group. The vaccine candidate safety profile was found to be acceptable.

Following the initial findings, several Phase II trials were conducted to identify the optimum schedule, confirm the selection of AS01 versus AS02 as adjuvant, and confirm that the vaccine could be given concomitantly with EPI vaccines [12]. The change of adjuvant led to a new proof-of-concept study conducted in children 5–17 months of age in Kenya and Tanzania. The efficacy against clinical malaria increased to 53%, confirming the decision to use AS01 [13].

A partially effective vaccine has little interest for travelers and the military, but would complement currently used malaria interventions in endemic countries. Phase I and II trials of RTS,S were conducted under an Investigational New Drug Application (IND) of the US FDA. As the product will be manufactured at the GSK Biologicals facility in Belgium, the licensure process will be completed through the European Medicines Agency (EMEA) under Article 58. According to European regulations, vaccines made exclusively for use outside Europe are no longer licensed *per se*. Under Article 58, which was established in consultation with WHO, EMEA gives a Scientific Opinion, WHO experts provide input on eligibility for consideration under Article 58 and also during the review process. RTS,S will be the first vaccine to follow this path.

Challenges facing RTS,S

The regulatory pathway for vaccines is complex, and vaccine programs are highly sensitive to public perceptions of safety. Thus, regulators are placing great emphasis on the number of study participants so as to support an appropriate assessment of any major potential safety issues that could affect vaccine programs already in place.

Regulatory approval of a malaria vaccine involves four critical steps: a positive scientific opinion under Article 58, prequalification by WHO, licensure by country regulatory authorities, and a recommendation for use by WHO (WHO Strategic Advisory Group of Experts [SAGE] on Immunization). A recommendation for use in national programs may require additional data that could result in changes to the clinical development plan currently in place.

The introduction of RTS,S is based on an expectation of using existing national immunization programs. However, history has taught us that introduction of new vaccines takes years, and as long as a decade or more. To address this potential bottleneck, a process of consultation at national, regional and global levels has led to the development of a framework for decision-making (labeled the decision-making framework [DMF]) that is now being implemented in Africa. Among the steps outlined in the DMF process is the setting up of national-level technical advisory groups to identify, help generate and analyze the data required to make a decision to use or not use RTS,S, and to ensure that preparations are completed early enough to facilitate rapid vaccine implementation. This effort is crucial to ensuring maximum public health impact of this intervention.

Finally, several African countries appear to have controlled or are close to controlling malaria, and may question the additive impact of a vaccine. In other places, clinical malaria is observed in older age groups for which a pediatric vaccine is not indicated. Results of Phase II studies seem to indicate that RTS,S does well in a range of malaria incidence settings. Questions have also been raised on the cost-effectiveness of a partially effective vaccine. These are challenges that require thoughtful consideration and highlight the importance of robust modeling and the final price of the vaccine.

Building on RTS,S results

Prime-boost approach

A study in rhesus macaques demonstrated that the association of a replication-defective

human adenovirus serotype 35 vector encoding CSP followed by two injections of RTS,S/AS01B induced comparable anti-CSP antibody levels to three doses of RTS,S/AS01B, but dramatically higher numbers of CSP-specific CD4 T cells producing interferon-γ [14]. The prime-boost approach is considered as a priority in other areas such as TB and HIV vaccine development.

Attenuated sporozoite approach

Three and a half decades ago, radiation attenuated sporozoites inoculated through mosquito bites to malaria naive volunteers induced protection against infectious bites, giving hope that a vaccine could be developed [15–17]. It was felt at that time that technical issues would not allow any product development, and priority was given to other protein-based approaches. In 2003, a biotech company, Sanaria Inc. (MD, USA), was created to establish the manufacturing process of a live attenuated vaccine approach. Less than 6 years later, and to the credit of the Sanaria team, a product coming out of the pilot plant inaugurated in October 2006, entered into Phase I/IIa of its development [18,19]. Irradiated attenuated sporozoites have been inoculated using syringes and needles in three different dosages and two routes of inoculation. The results of this first challenge trial will guide further product development.

From a technical perspective, the developments achieved at Sanaria are impressive. However, while irradiated sporozoites inoculated by mosquitoes have been demonstrated to be protective, will irradiated sporozoites inoculated using syringes and needles generate the same level of protection? What will be the total number of sporozoites required for inoculation to generate protection, if it proves possible? The answers to these questions will guide future product development.

The development of a unique manufacturing tool that is able to generate attenuated sporozoites, or not 'in the bottle', and the development of assays related to attenuated sporozoites approaches will be a very valuable tool for research beyond vaccines.

While the radiation-attenuated approach is further along in development, a second approach using genetically attenuated sporozoites by double gene knockout is close to a first clinical challenge trial in humans [20].

These two live attenuated approaches, if proven effective and sterilizing, could have an impact on transmission.

Heterologous prime boost & access to other adenovirus serotypes

Recombinant adenoviruses are potent inducers of cell-mediated immunity. Unfortunately they are facing two major issues – a possible pre-existing immunity to the adenovirus, or the generation of immunity after the first inoculation that will negatively affect the efficacy of subsequent injections. One way to circumvent this problem would be to use two different serotypes of adenovirus. Crucell (Leiden, The Netherlands), a Dutch biotech company, is developing several replication-incompetent recombinant human adenoviruses (35 and 26) expressing the *P. falciparum* gene encoding the CSP surface antigen that will be used in a heterologous prime-boost clinical challenge in human volunteers to assess the interest of this approach.

Access to better serotypes of human adenoviruses, in terms of both immunogenicity and manufacturability, is of interest, as are technologies allowing the adenovirus vector to encode for more than one antigen.

Blood-stage antigens

Significant investments have been made in trying to develop a vaccine based on blood-stage antigens. Results obtained so far have been relatively disappointing. Contrary to the pre-erythrocytic approach, this approach does not have a defined clinical challenge model. Attempts have been made to develop a model that would inform antigen selection and development. MVI's *P. falciparum* blood-stage vaccine effort is now limited. One promising approach from the Walter and Eliza Hall Institute of Medical Research utilizes a combination of erythrocyte-binding protein (EBA) and reticulocytes-binding protein (Rh) that could be very effective at blocking merozoite re-entry in erythrocytes [21].

Additional investments are also made in the apical membrane antigen 1 (AMA1) to evaluate the possibility of generating constructs that would elicit antibodies effective against a broad range of parasite genotypes and with the minimum number of alleles required to provide broad immunity. Although several approaches have been considered, no clinical challenge model is availalable, and endemic-country trials are often the only evaluation option [22].

Targeting *Plasmodium vivax*

The development of a vaccine that will support elimination and eradication justifies investments in the development of a vaccine targeting *P. vivax*. Two leading target antigens are

CSP, a chimeric circumsporozoite construct expressed in *E. coli* developed by the Walter Reed Army Institute of Research, and *Pv*RII, a receptor-binding domain of the duffy binding protein critical to merozoite invasion of erythrocytes developed by the International Centre for Genetic Engineering and Biotechnology in New Delhi, India [23–25]. A multistage approach in which these two antigens are combined and formulated with a common adjuvant may offer the best opportunity for success. The absence of a culture system for *P. vivax* continues to impose challenges on *P. vivax* vaccine development, but successful development of a clinical challenge model in Colombia would be a valuable resource for evaluating the initial clinical efficacy of these promising new approaches [22].

Transmission-blocking vaccines

Transmission-blocking vaccines (TBVs) do not aim to protect the vaccine recipient from infection and clinical disease, but rather act at a community level by interrupting the cycle of transmission from humans back to mosquitoes. This may be achieved via antibodies directed at the sexual stage by targeting gametocytes, or at the mosquito level by targeting receptors on the mosquito mid-gut. TBVs would complement other malaria interventions. The requirement here is to induce a long-lasting serological immune response. Two approaches have been identified as promising, and have the potential to target *P. falciparum* and *P. vivax* together, at the same time. The first approach (Pfs 48/45) targets Pf gametocytes; it is a prefertilization antigen, unlike another leading target, Pfs 25, that is a post-fertilization antigen [26]. Issues in expressing a properly refolded Pfs48/45 protein have been resolved at the laboratory level, and studies in mice and nonhuman primates induced a high level of antibodies that were found effective in the membrane feeding assay, with antibodies induced after a single immunization exhibiting high reductions in mosquito parasite density, which increased to nearly complete blocking after two doses [26]. Another leading approach targets an *Anopheles* mosquito mid-gut aminopeptidase (AnAPN1), which is used to invade the mosquito mid-gut. Antibodies directed at this target have likewise demonstrated excellent preliminary results in the membrane feeding assays [27].

The development and introduction of TBVs pose unique challenges. Vaccines considered primarily as altruistic rather than protective have never been introduced before. If they are not combined with antigens with clinical disease as their target, they will not bring any immediate benefits to the recipients. Safety is therefore a major consideration in the profile of such vaccines. A public health intervention like a TBVA transmission-blocking vaccine also requires creative thinking as far as policy and regulatory pathways are concerned. The definition of surrogates of protection in agreement with regulatory authorities could lead to a fast conditional introduction, keeping in mind that safety is paramount. This could be feasible if large-scale Phase IV studies were part of the development plan. As such, a vaccine might have to be administered to large segments of populations living in endemic countries; its price would have to be kept low, which could have implications for adjuvant selection.

Project classification in the development process

To appropriately reflect the different types of malaria vaccines under development (*P. falciparum*, *P. vivax*, *P. falciparum* and *P. vivax* combinations, transmission-blocking and so on), MVI has generated a series of target product profiles that will serve as a roadmap to guide our project selection and subsequent development efforts. Furthermore, in the world of the vaccine developer, clarity of classification with respect to projects helps ensure that the criteria for their evaluation are appropriate. The development of a vaccine product is a complex process that is reflected in our portfolio by classifying projects into three major stages. This classification supports the development and application of a clear list of deliverables and go/no-go criteria for each stage. Based on models commonly accepted in industry, we have established three stages of development – namely, preclinical feasibility study, translational project and vaccine candidate.

When a research project has been identified as being of strategic interest to MVI, a certain number of questions often remain to be answered; these may include application of a novel vector or adjuvant to malaria vaccine development or manufacturability of a candidate that has yielded promising research results. Such feasibility projects typically have a duration of 6 to 18 months, a limited scope and deliverables, and a cost of less than US$1.5 million.

Once a feasibility study has successfully delivered on its milestones, a translational project may be initiated. A translational project brings a project from the preclinical phase to proof-of-concept via

successful execution of a set of precise go/no-go criteria. Translational projects are of greater scope and duration and call for larger financial investments compared with preclinical feasibility studies. The scope of translational projects typically includes good laboratory practice toxicology, current good manufacturing practices, Phase I safety studies and Phase II proof-of-concept efficacy trials. The key criteria for further advancement to vaccine candidate status include successful demonstration of a minimum efficacy requirement in Phase IIb trials in endemic countries and proven manufacturability; in addition, its presentation and composition are close to being final. A certain number of additional Phase II trials may be required prior to moving to Phase III to address questions such as optimum dosing schedule, co-administration with EPI and efficacy in other age groups. At this stage the candidate is fully characterized and will not change substantially. Costs related to completion of this phase are substantial and require a careful strategic selection of criteria that will justify its initiation.

The above classification has important strategic consequences in terms of prioritization, investment and expectations. We believe that this type of project classification is necessary to ensure that external expectations are appropriately aligned with product development status.

Conclusion

MVI has completed a re-prioritization of its portfolio based on the recent successes observed with RTS,S. The coming years will confirm or dispute the usefulness of RTS,S, and the possibility of increasing its efficacy with a prime-boost approach. While working on TBVs and a limited number of blood-stage approaches, MVI will have to collaborate with the malaria community as a whole to define and refine the vaccine profiles that will be required to support other interventions over time.

Financial & competing interests disclosure

Dr Loucq holds a small number of shares in GlaxoSmithKline. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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