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Malaria vaccines and the new malaria agenda

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Abstract

The development of an effective malaria vaccine has taken many decades, but there is now a good chance that the first malaria vaccine will be licensed within the next few years. However, this vaccine (RTS,S) will not be fully effective, and more efficacious, second-generation vaccines will be needed. Good progress is being made in the development of potential vaccines directed at each of the three main stages of the parasite's life cycle, with a variety of different approaches, but many challenges remain, e.g. overcoming the problem of polymorphism in many key parasite antigens. It is likely vaccines that are effective enough to block transmission, and thus contribute to increasing drives towards malaria elimination, will need to contain antigens from different stages of the parasite's life cycle.

Keywords: Malaria, malaria eradication, malaria transmission, polymorphism, vaccines

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Introduction

There has been considerable progress in the control of malaria in recent years, as a result of increased investment, now about $2 billion a year, by organizations such as the Global Fund, the President's Malaria Initiative, the World Bank and bilateral donors. This investment has allowed scaling up of the few effective malaria control tools that are currently available: effective treatment with artemisinin combination therapy, long-lasting insecticide-treated bednets, and indoor residual spraying. When these interventions have been deployed widely, a marked reduction in the incidence of malaria has been reported from a number of countries in Asia and sub-Saharan Africa, where malaria transmission has traditionally been low or moderate [1]. These successes have led to an increased interest in the possibility of malaria elimination (Table 1) as a feasible medium-term goal in some countries. Malaria eradication, a taboo topic in the malaria community for over three decades following the failure of the first global effort to achieve this goal, is a more distant prospect.

However, despite these recent successes, malaria remains largely uncontrolled in many parts of sub-Saharan Africa and in a few countries in Asia, such as Myanmar, and still causes nearly 800 000 deaths each year, mainly in children in sub-Saharan Africa [2–4]. This large number of deaths could be reduced further by increasing coverage with existing control tools, but it is generally recognized that this would not be sufficient to achieve the high level of control that would be needed to make elimination a credible objective. Furthermore, the limited successes that have been achieved in high-transmission areas in sub-Saharan Africa are threatened by the potential spread of artemisinin-resistant strains of *Plasmodium falciparum* from Southeast Asia [5] and by the spread of strains of *Anopheles gambiae* that are highly resistant to pyrethroid insecticides [6]. Additional tools will be required to achieve effective malaria control in these high-transmission areas.

The malaria-endemic world can now be divided into two areas: those where effective malaria control has been achieved and where malaria elimination is a feasible short-term to medium-term goal; and those where malaria has yet to be brought under effective control and where elimination is a distant prospect [4,7]. Malaria vaccines have an important role to play in each of these situations, but different types of vaccine will be needed for each. In this article, we review the progress that is being made in the development of vaccines that fit these target product profiles, and discuss how they might be deployed.

TABLE 1. Definitions

Controlled low-endemicity malaria
Endemic malaria transmission has been reduced to a very low level. Transmission still occurs but does not constitute a major public health burden
Controlled non-endemic malaria
Endemic transmission has been interrupted but malaria resulting from onward transmission from imported infections continues at a level that prevents elimination
Elimination
Interventions have interrupted endemic transmission and limited onward transmission from imported infections below a threshold at which the risk of re-establishment is minimal
Eradication
Cessation of all naturally occurring transmission of one or more species of human malaria parasites

Development of Malaria Vaccines

Malaria parasites and their life cycles

Humans may be infected by five species of *Plasmodium* (Fig. 1). Both *P. falciparum* and *Plasmodium vivax* can cause severe disease, but *P. falciparum* has been the focus of most vaccine-related research. *Plasmodium ovale* and *Plasmodium malariae* are rarely considered as vaccine targets, and *Plasmodium knowlesi*, a primate malarial parasite that was used in early blood-stage vaccine studies [8,9], has only recently emerged as the cause of a naturally acquired infection in humans [10].

The life cycle of human malaria parasites is complex. The infection commences with the inoculation of a small number of sporozoites (usually <100) through the bite of an infected mosquito. Some sporozoites are retained in the tissues around the bite, but others pass through the circulation to the liver, where they develop into schizonts. Development in the liver involves a 10 000-fold or more increase in parasite numbers. Following an incubation period of about 10 days, schizonts rupture, leading to invasion of erythrocytes by merozoites. Progressive cycles of multiplication within erythrocytes every 48 or 72 h, according to the species, results in the clinical features of the disease. A few *P. vivax* (and *P. ovale*) sporozoites become non-dividing hypnozoites that remain dormant in liver cells for weeks, months or years before reactivating. Finally, unknown factors [11] lead to the conversion of a small number of asexual forms into the male and female gametocytes that are required to initiate development in the vector mosquito when she takes a blood meal. Attempts are being made to develop vaccines that target each stage of the life cycle [12] (Table 2).

Pre-erythrocytic vaccines

There is a clear logic to vaccination directed at the sporozoite stage of the parasite, as the number of parasites inoculated by a mosquito is relatively small [13], creating a bottleneck in the parasite's life cycle (Table 3).

The earliest successful attempts at developing a pre-erythrocytic vaccine were made in chickens injected with large numbers of sporozoites of *Plasmodium gallinaceum* inactivated by UV irradiation [14]. Subsequently, Nussenzweig *et al.* [15] showed that vaccination with X-irradiated *Plasmodium berghei* sporozoites fully protected mice against sporozoite challenge. Similar high levels of protection were obtained in humans, using multiple bites by irradiated mosquitoes infected with *P. falciparum* [16].

Immunization with the dominant sporozoite surface protein molecule, circumsporozoite protein (CSP), gave protection in rodents and, following the cloning of CSP of

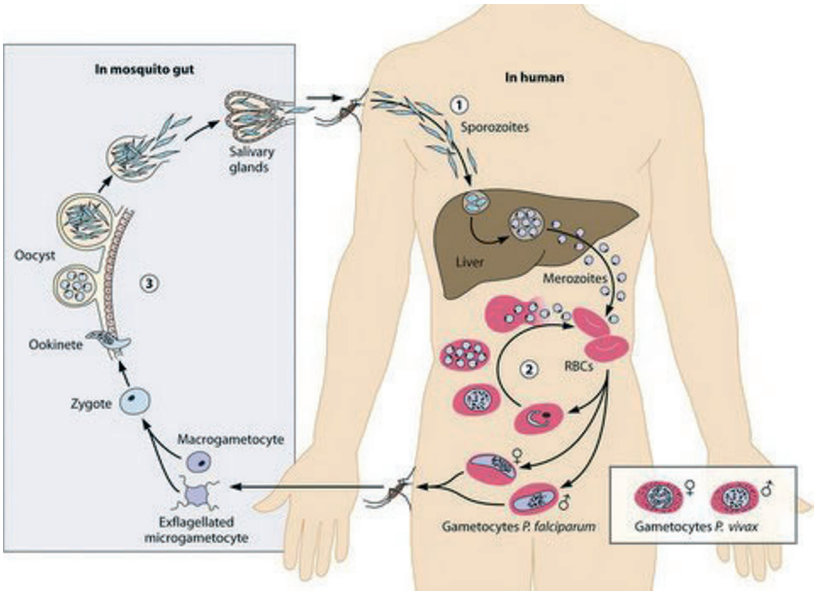

FIG. 1. Malaria life cycle showing (1) pre-erythrocytic sporozoite inoculation and invasion of the liver, (2) asexual blood stages, and (3) the sexual cycle and sporogony in the mosquito. Vaccines are being developed against each of these phases of the life cycle. Adapted from Ref. [55] and reprinted with permission.

TABLE 2. Malaria vaccine targets

Pre-erythrocytic stages
These vaccines are directed against either the invasive sporozoite or the early stage of development in the liver
Asexual blood cycle
These vaccines are designed to stop invasion of erythrocytes or to kill intra-erythrocytic parasites
Sexual cycle
These vaccines aim to stop mosquitoes becoming infected by preventing parasite fertilization and/or by inhibiting early development in the insect vector

TABLE 3. Pre-erythrocytic-stage vaccine strategies

Subunit vaccines based on the sporozoite surface proteins circumsporozoite surface protein (CSP) and thrombospondin-related adhesion protein (TRAP)
Viral particle coexpression of CSP, e.g. RTS,S
Viral vector, e.g. adenovirus, expression forms of CSP and TRAP
Recombinant liver-stage antigens LSAI and LSA3
Sporozoites attenuated by irradiation or genetically
Sporozoite infections controlled by chemotherapy

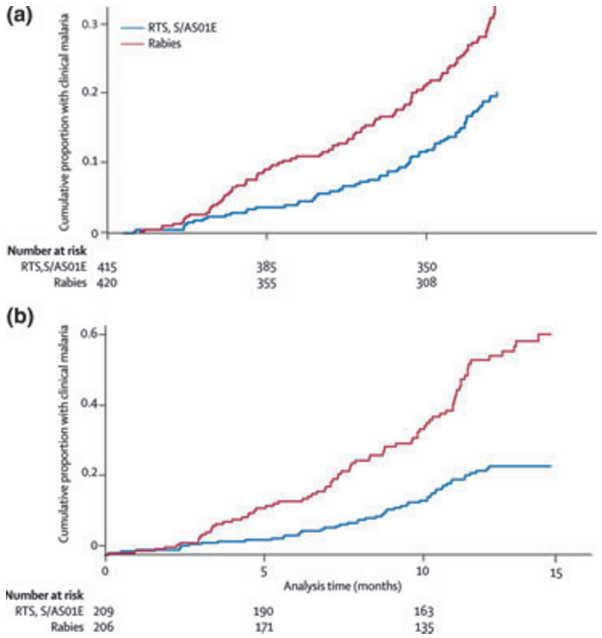

FIG. 2. Kaplan–Meier curves showing the cumulative proportion of clinical *Plasmodium falciparum* cases after vaccination of children with RTS,S/ASOIE as compared with controls given rabies vaccine in (a) Korogwe, Tanzania and (b) Kilifi, Kenya [18].

P. falciparum, a series of recombinant and synthetic candidate vaccines based on parts of CSP were tested in small-scale human trials. Most gave little or no protection, with the notable exception of one called RTS,S, a construct of CSP with the hepatitis B virus surface antigen [17]. A series of trials with this construct, given with a powerful adjuvant over a period of 15 years, showed 30–50% protection against clinical episodes of malaria (Fig. 2) [18] and, in infants, there was a strong correlation between anti-CSP antibodies and the risk of clinical malaria. This vaccine is now undergoing a phase 3 trial at 11 sites in seven countries in Africa, involving over 15 000 children, and is likely to be the first malaria vaccine to be licensed. A key to the success of RTS,S has been its use with the powerful adjuvant AS02, an oil in water emulsion containing monophosphoryl lipid A and Quil A saponin, or with AS01, which consists of monophosphoryl lipid A, Quil A saponin and liposomes.

As illustrated by RTS,S, it has proved difficult to induce high enough immune responses to several malaria antigens to provide protection, and the lack of availability of powerful adjuvants that can be used safely in humans and that are not protected by company patents has hindered malaria vaccine development by academic groups. However, steps are being taken to overcome this by making new adjuvants more widely available through non-profit organizations, such as the Infectious Disease Research Institute in the USA and TRANSVAC in Europe.

Despite its early success, utilization of whole sporozoites as a vaccine was initially considered to be an impracticable approach, because of the problems of obtaining a sufficient number of sporozoites free enough of contaminants to be used as a vaccine. However, outstanding progress has been made recently in producing an irradiation-attenuated sporozoite vaccine that is compliant with regulatory requirements for clinical use [19]. The initial clinical trial using sporozoites given intradermally or subcutaneously showed limited protection, but intravenous inculation may be more successful. An alternative approach is to produce genetically attenuated parasites. One or two genes are deleted, making the parasite capable of only limited development in the liver. Experimentally, double knockouts have proved to be highly effective vaccines, and *P. falciparum* genetically attenuated parasites gave good protection in a humanized mouse model [20,21].

Recently, another way of inducing protective immunity in humans with sporozoites has been reported. A high level of protection was achieved when volunteers were exposed to bites of *P. falciparum*-infected mosquitoes and given chloroquine at the same time; immunity persisted for more than 2 years in four of six volunteers [22]. Further investigations need to address issues of strain specificity, efficacy in endemic populations, and whether the immunosuppressive effects of blood-stage infections underlie the reason why this level of protection is not achieved during natural infection [23]. Elucidating the mechanisms of immunity induced in this experiment may provide important information to guide future vaccine development.

Blood-stage vaccines

The fact that it is possible to develop some degree of protective immunity to the blood stages of *P. falciparum* was established many years ago, when it was shown that immuno-

TABLE 4. Asexual blood-stage vaccine strategies

Recombinant merozoite proteins involved in invasion of erythrocytes. Most are derived from
Merozoite surface protein (MSP)-1
Apical merozoite antigen 1 (AMA1)
also
Erythrocyte-binding antigen 175
MSP3
Blood-stage combinations, e.g. MSP1 + MSP2 + ring-infected erythrocyte surface antigen
Long synthetic peptides
MSP3 + glutamate rich protein
Viral vector prime-boost strategies, e.g.
Chimpanzee adenovirus AdCh63/AMA1^{+}
Modified vaccinia antigen/AMA1
Combinations from different life-cycle stages, e.g.
Viral vector expression of thrombospondin-related adhesion protein + AMA1 + MSP-1
CSP + AMA1 mimetopes

globulin obtained from adults who were semi-immune to *P. falciparum* cleared parasites when given to infected children [24]. Building on this finding, attempts have been made to develop vaccines against the asexual blood-stage parasites by inducing an immune response that either blocks invasion of erythrocytes or inhibits their subsequent multiplication. Although a large number of blood-stage vaccines have been developed and tested in preclinical and clinical trials [25,26] (Table 4), almost all have been based on a small number of antigens expressed on merozoites [3,27]. Although protection has been achieved in experimental animals, little clinical success has so far been achieved in humans with blood-stage vaccines.

The challenges in the development of an effective asexual blood-stage vaccine are considerable. First, there is the difficulty in deciding what type of immune response needs to be induced. Should this be directed at just inducing antibodies that inhibit invasion or parasite growth with or without the cooperation of phagocytic cells, or is it also important to induce an antibody-independent proliferative T-cell response? Second, nearly all of the blood-stage molecules investigated so far show genetic diversity (polymorphism), so that the immune response induced by a vaccine may be only strain-specific [28]. Finally, there is considerable redundancy, giving the parasite alternatives when, for example, one route of invasion is blocked by vaccination.

There is now strong evidence that *P. falciparum* erythrocyte membrane protein I, expressed on the surface of *P. falciparum*-infected erythrocytes, plays major roles in both the natural acquisition of immunity and the sequestration of this parasite in small blood vessels, causing tissue damage. This molecule is therefore an attractive vaccine candidate. However, the ability of this antigen to undergo sequential antigenic variation complicates its use as a vaccine [29]. There are, however, two situations in which this is worth further investigation. There is evidence that selected *P. falciparum* erythrocyte membrane protein I variants are associated with severe malaria, and vaccination against this subgroup could conceivably be beneficial. In addition, there is evidence that a very select group of variants, notably one termed VAR2CSA, is responsible for sequestration of *P. falciparum* in the placenta, raising the intriguing option of developing a vaccine specifically designed to protect pregnant women [29,30].

As in the case of pre-erythrocytic vaccines, a whole parasite approach has also been used to induce immunity against blood-stage parasites. Naive volunteers who were infected three times with about 30 *P. falciparum*-infected erythrocytes and treated before the infection was patent were protected against subsequent challenge. Protection was not associated with antibody production, but volunteers mounted a strong proliferative T-cell response [31].

Vaccines that impact on transmission

With the prospect of malaria elimination in an increasing number of countries, vaccines that interrupt transmission, recently termed vaccines that inhibit malaria transmission [32], are attracting increasing attention.

Two approaches can be taken to the development of vaccines that block transmission. Any highly effective pre-erythrocyte-stage vaccine will reduce transmission, as it will greatly reduce the chance that the parasite will invade erythrocytes and hence be able to produce gametocytes and transmit the infection. Blood-stage vaccines are less likely to be able to achieve this unless they are almost totally effective, as even a low level of blood-stage infection may allow production of a sufficient number of gametocytes to sustain transmission. The second approach is to target the sexual stages of the parasite in the human host and early developmental stages found in the mosquito. Vaccines based on the gamete surface antigens, P230, P48/45 and HAP2, prevent fertilization in the mosquito midgut, whereas those based on the ookinete antigens, P25 and P28, induce antibodies that prevent ookinetes from migrating across the midgut wall (Table 5).

A positive aspect of this type of transmission-blocking vaccine is that it attacks the parasite at a vulnerable stage of its

TABLE 5. Sexual-stage vaccine strategies

Antigens expressed in gametocytes and gametes, and involved in fertilization, notably P48/45, P230 and HAP2, are used to induce immune responses that prevent fertilization in the mosquito midgut. Antibodies against these proteins are induced during infections, and this might serve to boost a response to vaccination
Antibodies against P25 and P28 expressed only on zygote/ookinete stages in the mosquito prevent invasion and penetration of the midgut wall and any further development of the parasite
A similar effect can be produced experimentally by immune responses induced against components of the mosquito gut wall

life cycle, when it has to transfer from one host to another and when its numbers are very small [13]. Early clinical trials have been conducted with vaccines based on the ookinete antigens of *P. falciparum* and *P. vivax* [33,34] with induction of transmission-blocking antibodies, but no large-scale clinical trial of a transmission-blocking vaccine has yet been undertaken. Another interesting experimental approach to transmission blocking is to focus on the vector rather than the parasite, by vaccinating with components of mosquito gut or salivary glands that kill the feeding mosquito or reduce its fecundity or lifespan, and hence its ability to transmit [13,35].

Alternative vaccine strategies

Most of the studies of vaccines described above have relied on conventional vaccine technologies—the use of whole organisms or proteins expressed in bacteria or yeasts. However, several other strategies have been explored. Studies with DNA vaccines gave promising results in rodent malarias but, in humans, DNA vaccines have proved to be less immunogenic [36], and this approach is now receiving less attention. A more promising approach has been expression of parasite antigens in viral vectors given in heterologous prime-boost regimens, with a viral-vectored vaccine followed either by a protein vaccine or by a different viral vector. High-level T-cell responses and protection in some volunteers have been achieved with vaccines based on the sporozoite antigen thrombospondin-related adhesion protein given with two different viral vectors—fowlpox and modified vaccinia virus [37]. More recently, even higher T-cell responses have been produced with thrombospondin-related adhesion protein expressed in a chimpanzee adenovirus vector [38].

Adenovirus-based vaccines have also been shown to induce strong antibody responses to the blood-stage and sexual-stage parasites [39–41]. Microarray technologies [42] provide opportunities to investigate many new antigens as potential vaccine candidates [43,44].

Evaluation of Malaria Vaccines

The use of new techniques has accelerated the rate at which new vaccine candidates can be developed in the laboratory. A major challenge now is selecting those that warrant further clinical evaluation.

Animal studies

The initial stage in the evaluation of a new vaccine candidate is usually the demonstration of immunogenicity, either humoral or cellular, in a small-animal model. The value of these initial studies is enhanced if the immune response is associated with protection. In the case of malaria, the best developed functional assay is one that measures the ability of antibodies to block infection of mosquitoes fed on blood containing gametocytes [45]. Measurement of antibodies that inhibit the growth of cultured blood-stage parasites *in vitro*, either alone or in combination with white blood cells, has also proved to be a useful screening assay for blood-stage candidate vaccines [46]. It has proved more difficult to develop reproducible bioassays for pre-erythrocytic vaccines. *P. vivax* presents a particular problem, as it is difficult to culture this parasite *in vitro*, and no functional assay has yet been developed for *P. vivax* hypnozoites. Humanized mice that can be infected with parasitized human erythrocytes [47] or that can support human liver stages [21] provide a model that falls between *in vitro* assays and animal challenge studies.

Whether non-human primate challenge studies constitute an essential step in malaria vaccine development is debated [48], as all non-human primate models have weaknesses, and there is now a trend towards moving more rapidly into human studies than has been the case in the past.

Evaluation in humans

Evaluation of malaria vaccines in humans follows the traditional pathway from phase 1 safety and immunogenicity studies in a small number of subjects through medium-sized phase 2 efficacy studies to large-scale phase 3 registration trials.

An unusual feature of malaria vaccine development has been the increasing use of phase 2a challenge studies in non-immune volunteers. Experimental infections can be induced with either bites by experimentally infected mosquitoes [49] or inoculation of a small volume of infected blood obtained from a very carefully screened donor [50], the latter giving more consistent infections. The challenge model, now used in over 1000 volunteers, has proved to be remarkably safe, and when PCR is used to detect early infections [51], many volunteers receive treatment before developing significant symptoms. Challenge studies with the RTS,S vaccine have been good predictors of the outcome in field trials [17], but with some other vaccines this has not been the case [52].

Because of safety concerns, early malaria vaccine candidates were taken through a series of age de-escalation trials, but there is now an increasing willingness to move more rapidly into trials in young children, the primary target in endemic areas, once immunogenicity and safety have been demonstrated in adults from an endemic area. Evaluation of the efficacy of malaria vaccines in reducing transmission will be challenging. Such studies will need to be community rather than individually randomized trials, and

such trials will be large and expensive. If the membrane feeding assay can be standardized [53] and shown to be a reliable predictor of success in the field, this will greatly facilitate the development of vaccines directed at sexual stages of the parasite.

How will Malaria Vaccines be Used?

It is likely that the first malaria vaccine to be licensed will be RTS,S/ASO1. Initial trials suggest that RTS,S will give about 50% protection against both uncomplicated and severe episodes of malaria that persists for at least 2 years, and the vaccine therefore has the potential to prevent many deaths [17]. It is not likely that RTS,S, deployed in a limited segment of the population of high-transmission areas, will have any significant impact on malaria transmission. How, therefore, might this vaccine best be deployed? A sensible strategy for the introduction of RTS,S will be to give priority to the areas where malaria is not yet controlled because existing control measures cannot be deployed effectively or are ineffective. In these areas, a vaccine that provided 50% protection for a period of 2 years would be a sound investment. However, in areas where malaria transmission persists but is well controlled with existing measures and causes little morbidity or mortality, introduction of a vaccine such as RTS,S, directed primarily at the prevention of clinical disease, would be less cost-effective. Where the boundary between these two epidemiological situations lies is uncertain, and may be influenced by the costs of the vaccine.

It is currently proposed to give RTS,S at the ages of 2, 3 and 4 months, possibly with a booster dose during the second year of life. This schedule was chosen partly because it fits in well with the current schedule of administration of routine paediatric vaccines, and partly because the main burden of severe malaria in highly endemic areas is in children during their first 2 years of life. However, as the level of malaria transmission declines, the age of cases increases, so that an increasing proportion of cases of malaria are seen in older children. In such circumstances, focusing malaria vaccination on just the first year of life may no longer be the most appropriate strategy, unless the vaccine gives prolonged protection, and infant immunization may need to be supplemented by additional catch-up strategies, including mass vaccination campaigns for older children. Selected groups of high-risk subjects, such as pregnant women, patients with human immunodeficiency virus infection and those with haemoglobinopathies, may also warrant priority.

In areas where malaria is reasonably well controlled, the primary objective of malaria vaccination will be to reduce and, eventually, interrupt transmission. This will require vaccination of the whole population. The level of coverage required to interrupt transmission will be determined by local epidemiological circumstances, but modelling suggests that, in many circumstances, 90% coverage of the whole population with a vaccine with an efficacy of over 90% will be required to completely interrupt transmission, a daunting challenge.

Challenges for the Future

Future malaria vaccine development faces a number of major challenges, the biggest one of which is probably financial (Table 6).

Technical challenges

The increasing ability to make large numbers of vaccine constructs quickly with viral vector or other technologies is removing one of the constraints faced by early vaccine developers. This ability is especially important in the development of vaccines based on parasite antigens that shows substantial antigen polymorphism, as is the case for many erythrocyte antigens being considered as potential vaccine antigens. Another important technical challenge for the coming years will be finding the optimum ways of identifying the most promising antigens to take forward into clinical development. This down-selection process will probably have to depend primarily on *in vitro* biological assays, and it is important that research continues on improving the biological relevance, reproducibility and high-throughput features of such assays for each of the stages of the parasite's life cycle.

Once a decision has been made to take forward a promising candidate to clinical development, a major challenge for academic investigators, the group most likely to have undertaken this work, is production of the good manufacturing practice material that allows first-into-human studies to be undertaken. A number of ideas have been suggested with regard to how this process could be facilitated, such as the creation of publicly supported good manufacturing practice facilities that could be used by academic groups to facilitate

TABLE 6. Approaches to the acceleration of malaria vaccine development

Focusing on new antigens characterized by genomic analysis as being likely to have vaccine potential, e.g. surface expression. Most efforts so far have focused on antigens characterized by protein chemistry decades ago
Provision of multiple-use good manufacturing practice facilities that will allow the production of test batches of vaccine for academic investigators quickly and at affordable cost
Optimization of *in vitro* assays that will allow rapid, biologically meaningful screening of vaccine candidates, e.g. transmission-blocking growth inhibition assays
More flexible regulatory processes

the development of vaccines against several neglected diseases [54].

Regulatory issues

It is uncertain how the second generation of disease-control malaria vaccines will achieve licensure. Assuming that RTS,S becomes licensed, will the regulators require manufacturers to show that the new vaccine is superior to or at least non-inferior to RTS,S in an efficacy trial? The sample size required for such a trial would be very large. In the case of other infections, such as those caused by pneumococci or meningococci, second-generation vaccines have been licensed on the basis of an immunological assay. Whether this will ever be possible for malaria vaccines is uncertain.

It is unlikely that any of the early generation of malaria vaccines based on a single antigen will have the level of efficacy needed to interrupt transmission in highly endemic areas, and this will probably require combination vaccines. These might be vaccines containing multiple antigens of a similar type, a combination of two different formulations of the same antigen inducing different kinds of immune response, or a combination of antigens from different stages of the parasite's life cycle. Finding the best way of combining individual vaccines that have each shown partial protection is going to be extremely challenging.

Financial issues

The development of RTS,S has been a long and expensive process, and has only been possible because of the generosity of the Bill and Melinda Gates Foundation, GSK and other donors. Whether other promising vaccine candidates will be able to attract financial support on this scale is uncertain. Malaria vaccines are likely to be relatively expensive in relation to other vaccines, especially if multiple components produced with different technologies are required. Thus, even if these vaccines are sold at or near to the price of manufacture, there are likely to be major financial constraints on the rapid deployment of malaria vaccines in the areas where they could contribute most to malaria control or elimination. The international community is already struggling to find the financial resources to support the introduction of highly effective meningococcal, pneumococcal and rotavirus vaccines in poor countries, and addition of malaria vaccines to this list will provide an additional major challenge, but ways must be found of doing this. The recent, renewed commitment of the international donor community to the Global Alliance for vaccines and Immunization is encouraging it would be a major tragedy if, after 50 years of scientific endeavour, the first generation of successful malaria vaccines failed to reach those in the poorest countries of the world, where effective protection against malaria is needed most.

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