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Plasmodium vivax malaria vaccines

Why are we where we are?

Arturo Reyes-Sandoval[1,*] and Martin F Bachmann[1,2]

[1]The Jenner Institute; University of Oxford; Oxford, UK; [2]Dermatology; University Hospital Zurich; Zurich, Switzerland

Malaria is one of the few diseases in which morbidity is still measured in hundreds of millions of cases every year. *Plasmodium vivax* and *Plasmodium falciparum* are responsible for nearly all the malaria cases in the world and despite difficulties in obtaining an exact number, estimates indicate an astonishing 349–552 million clinical cases of malaria due to *P. falciparum* in 2007 and between 132–391 million clinical episodes due to *P. vivax* in 2009. It is becoming evident that eradication of malaria will be an arduous task and *P. vivax* will be one of the most difficult species to eliminate and perhaps become the last standing malaria parasite. Indeed, in countries that succeed in decreasing the disease burden, nearly all the remaining malaria cases are caused by *P. vivax*. Such resilience is mainly due to the sophisticated mechanism that the parasite has evolved to remain dormant for months or years forming hypnozoites, a small structure in the liver that will be a major hurdle in the efforts toward malaria eradication. Furthermore, while clinical trials of vaccines against *P. falciparum* are making fast progress, a very different picture is seen with *P. vivax*, where only few candidates are currently active in clinical trials.

Keywords: *Plasmodium vivax*, vaccine, clinical trials, malaria, human trials

Abbreviations: PEV, pre-erythrocytic vaccines; BSV, blood-stage vaccines; TBV, transmission blocking vaccines;

*Correspondence to: Arturo Reyes-Sandoval; Email: arturo.reyes@ndm.ox.ac.uk

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Introduction

There is consensus that *Plasmodium vivax* is the most widely distributed human malaria parasite in the world and the one that presents most challenges in the route toward malaria elimination. Almost 3 billion people lived at risk of *P. vivax* malaria in 2010. This should not be surprising if we consider that the parasite is endemic in a third of the earth's land mass, spanning approximately 44 million square kilometres within 95 countries. This represents an impressive geographical range stretching over three continental zones and including both, hot and temperate climates,[1] an achievement that no other human malaria parasite has made.

Despite the tremendous disease burden caused by *P. vivax*, most efforts in terms of research and development of preventative measures have been concentrated in its sibling, *P. falciparum*. **Figure 1** shows a comparison of disease burden (**Fig. 1A**) and investment in research and global malaria vaccine pipeline (**Fig. 1B**) for each parasite. Disease burden is comparable for both parasites with a higher proportion of number of clinical cases caused by *P. falciparum* every year,[2,3] a similar proportion in number of people being at risk and a more widespread presence of *P. vivax* in the world as shown here by the number of countries where this parasite is endemic.[4,5] The picture changes and a rather uneven distribution is seen when comparing the investment on research and the number of vaccines that are currently reaching clinical trial status. Between 2007 and 2009, only 3.1% of the global funds were invested in *P. vivax* R&D, while 44.6% was destined to *P. falciparum* in a study where 52.3% of funds did not specify a particular strain.[6] The differences in the number of clinical trials seem to reflect the levels of investment for each parasite. Only two trials for a *P. vivax* vaccine are currently active according to WHO and

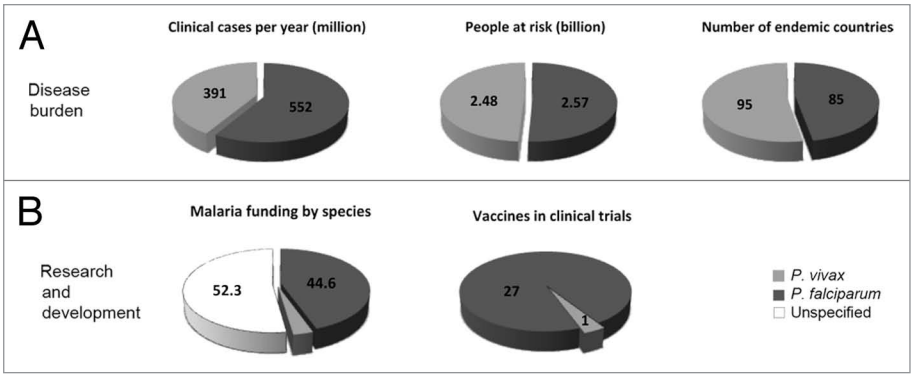

Figure 1. A comparison of disease burden (**A**) and investment in research and global malaria vaccine development (**B**) for both, *P. vivax* and *P. falciparum* between 2007 and 2009. Figures based on references 1,5, and 6.

NIH websites (http://www.who.int/vaccine_research/links/Global_malaria_vaccine_portfolio.pdf) (www.ClinicalTrials.gov).

Why Is It So Difficult to Develop a *P. vivax* Malaria Vaccine?

Progress on *P. vivax* research has been slow and has traditionally followed the footsteps of *P. falciparum*. Many reasons have been proposed to explain this and we discuss below three major factors that have slowed down progress of *P. vivax* research; lack of investment, scarcity of tools and the high cost to develop new vaccines in general.

The paucity of resources toward *P. vivax* vaccine research and development could be attributed to many causes, such as the low appreciation and knowledge of the magnitude of the disease burden, epidemiology, pathobiology, and clinical manifestations of this type of malaria,[3] which has contributed for many years to the misperception that *P. vivax* causes a "benign" malaria that does not kill per se. Nevertheless, recent reports of severe disease and mortality caused by *P. vivax* have challenged this perception and similar mortality rates for both, *P. vivax* and *P. falciparum* have been reported in hospitalized patients from Indonesian Papua or India.[7,8] These studies indicate that between 21–27% of patients with severe malaria are due to infection of *P. vivax*[8] and the risk of *P. vivax* severe disease (1/270) and death (1/3959) can be comparable to the *P. falciparum* risk of severe disease (1/185) and death (1/1742).[7] The most recent call to eradicate malaria[9] has given impetus to the research on *P. vivax*, yielding greater knowledge on the parasite and helping researchers and funding agencies to avoid "flying blind".

A second reason for the slow pace in vaccine development is the lack of tools to assess novel vaccine candidates. Access to *P. vivax* parasites is limited to endemic countries due to the lack of a reliable and continuous in vitro culture methodology, which would permit maintainance and growth of a parasite in any laboratory. Numerous attempts have been made since the first report of a methodology to culture *P. falciparum* and *P. vivax* by Charles C Bass and Foster M Johns in 1912[10] in a series of investigations at the time of the construction of the Panama Canal. More than 100 years have passed and not only a long-term culture remains elusive but research on this area is still fragmentary and disconnected.[11] To make things even more difficult for the development of a *P. vivax* vaccine, animal models to grow the parasite in vivo and to test new vaccine candidates are scarce. Nevertheless, great progress has been made in the development of a challenge model using the new world's monkey *Aotus lemurinus griseimembra* in Colombia.[12]

Another important factor to help explain the difficult process to produce a vaccine is the cost associated with such development, which is far higher than other approaches used for disease control and prevention. It is estimated that an investment of $600–800 million is required over a period of 10–15 y to create a successful vaccine.[13] In comparison, a new drug development would take 7–10 y with a cost of $150–250 million.[14] Less costly are vector control products and new diagnostic tools, which would require $60–65 million over a period of 10–12 y and $2–5 million over 3–5 y, respectively.[6] **Figure 2** shows an average of the total costs for each of the four developments mentioned above.

Plasmodium vivax Vaccines: The Leading Candidates in Clinical Trials

It is noticeable that despite the huge disease burden caused by *P. vivax*, only a few vaccine candidates have been tested in clinical trials. Several new candidates are currently being investigated pre-clinically and there are excellent reviews discussing these.[15,16] We will therefore focus on the candidates that have been tested in clinical trials up to the year 2013.

The life cycle of the *P. vivax* malaria parasite is complex and involves female anopheles mosquitoes as the definitive host, where the parasite's sexual development is completed, and humans as the intermediate host, where the parasite completes asexual development and forms infectious sexual stages (gametocytes). Furthermore, the malaria parasite's life cycle in humans alternates between intracellular and extracellular stages.

Vaccines can be designed to target the *Plasmodium* parasite at various stages of its life cycle and they can be classified as pre-erythrocytic (PEV), blood stage (BSV), and transmission-blocking (TBV) vaccines. A major factor to consider is whether the vaccine targets the parasite at an extra- or intracellular compartment since the protective immunity will rely on either, antibodies or cytotoxic lymphocytes, respectively.

So far, three PEV and one TBV have been assessed in early phase I clinical trials and from these, only one program remains with plans for further phase II trials. A timeline of vaccines tested in clinical trials is shown in **Figure 3**.

Pre-erythrocytic vaccines

The pre-erythrocytic (PE) stage has traditionally been the most attractive phase to develop a vaccine and is currently

the leading vaccination strategy for *P. falciparum*.[17] Targeting the sporozoite is attractive for a number of reasons and perhaps the most important comes from the fact that this is the only stage where sterile protection has been achieved, as first shown 46 y ago in the seminal work by Ruth S Nussenzweig, in which sterile protection in mice was induced by using radiation-attenuated sporozoites (RAS) as vaccines.[18] Moreover, the PE stage is a bottleneck where the number of sporozoites (10^2) and/or infected hepatocytes (10^1–10^2) to attack is low, compared with the number of parasites during asexual (10^{11}) or sexual (10^9) blood stages.[19] The PE stage is also clinically silent and stopping the parasite at this stage can prevent severe symptoms and death.

Despite being considered as a single stage, PE vaccines attack the parasite in two very different ways. Immune responses can be elicited (1) against the extracellular sporozoites for which antibodies can play a major role in stopping the parasite before reaching the liver (anti-sporozoite vaccine) or (2) against the infected hepatocytes whereby cytotoxic lymphocytes play a major role by destroying cells that express parasite antigens within the context of MHC class I molecules (bona fide liver-stage vaccine). An optimal vaccine should induce both, antibodies and $CD8^+$ T cells against an antigen that can be a target at both, the sporozoite and the infected hepatocyte stages. Recombinant viral vectors are particularly efficient at eliciting such dual responses.[20–22]

Plasmodium parasites are notorious for constantly escaping the immune responses, in particular at the blood stage. In contrast, after a mosquito bite, the free sporozoites are present in the blood for only a few seconds to minutes before infection of liver cells. The immune system is therefore usually inefficient to respond to pre-erythrocytic antigens and the parasite is least expected to evolve strategies to avoid immune responses against such antigens, which makes them highly attractive candidates for vaccine development. Thus, it is not surprising that the sporozoite-specific circumsporozoite protein (CSP) is the leading target for vaccine development (see next section).

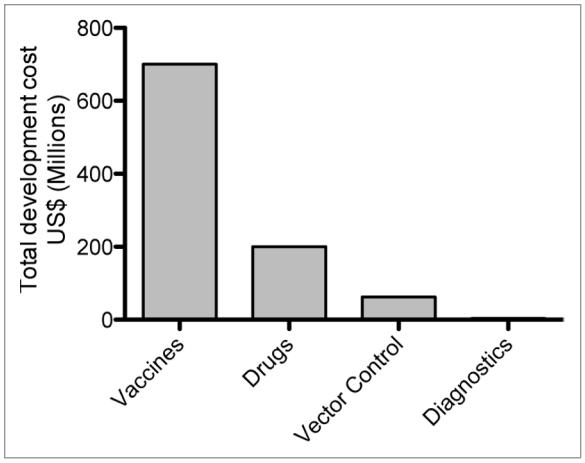

Figure 2. Costs associated with research and development of various novel control measures for infectious diseases and malaria.[6,13,59]

Plasmodium vivax Pre-erythrocytic Vaccines in Clinical Trials

The leading *P. vivax* vaccine candidate is a pre-erythrocytic vaccine that targets the circumsporozoite protein (CSP), which is considered to be the most abundant protein on the surface of mature sporozoites[23] and has a vital role for the parasite by mediating gliding motility and invasion of both, hepatocytes and mosquito salivary glands.[24–26] In contrast to *P. falciparum*, a higher diversity is seen within the CSP molecule of *P. vivax* and two types are present around the world, VK210 and VK247, which differ in the central repetitive region of the molecule.[27]

Vivax-1 vaccine

This vaccine has been developed by joint efforts between the University of Maryland, New York University and Chiron. Vivax-1 consists of a recombinant *P. vivax* CSP antigen (rPvCS) containing 234 amino acids (aa) out of 373 present in the Belem strain of *P. vivax*. The protein was expressed in *Saccharomyces cerevisieae* and contains 15 aa of the N-terminal region, all the aa of the central repeat region (VK210) and 48 aa of the C-terminal end. The antigen was purified from yeast cell lysate and adsorbed onto aluminum hydroxide (Alhydrogel) to formulate the vaccine. Initial evaluation of Vivax-1 in Swiss-Webster mice resulted in high antibody titers that were able to recognize the native CS protein and inhibit in vitro invasion of hepatocytes by sporozoites.[28]

Vivax-1 was further assessed pre-clinically in a study where immunized squirrel monkeys *Saimiri sciureus boliviensis* were challenged with *P. vivax* Salvador I sporozoites. Results indicated that the vaccine was safe and induced high levels of anti-sporozoite antibodies and complete protection against the challenge was elicited in 67% of the vaccinated monkeys. Even though there were not statistically significant differences between groups, this study supported further tests of the vaccine in clinical trials.[29] Vivax-1 was tested in a phase I trial to assess safety and immunogenicity in humans using escalating doses of 50, 100, 200, and 400 ug of vaccine formulated in alhydrogel. Despite three consecutive immunizations, antibody responses were low or absent even in the group receiving the highest dose, which indicated that other formulations or adjuvants would be required to warrant further testing of a pre-erythrocytic CSP vaccine.[30] This marked the end of the development of the Vivax-1 vaccine by joint efforts of the University of Maryland, New York University, and Chiron.

Long synthetic peptides (LSP) vaccine

Important efforts have also been made in the development of a pre-erythrocytic vaccine using solid phase peptide synthesis technologies to produce long peptides using sequences of the CSP molecule.[31] The LSP vaccine consists of three peptides

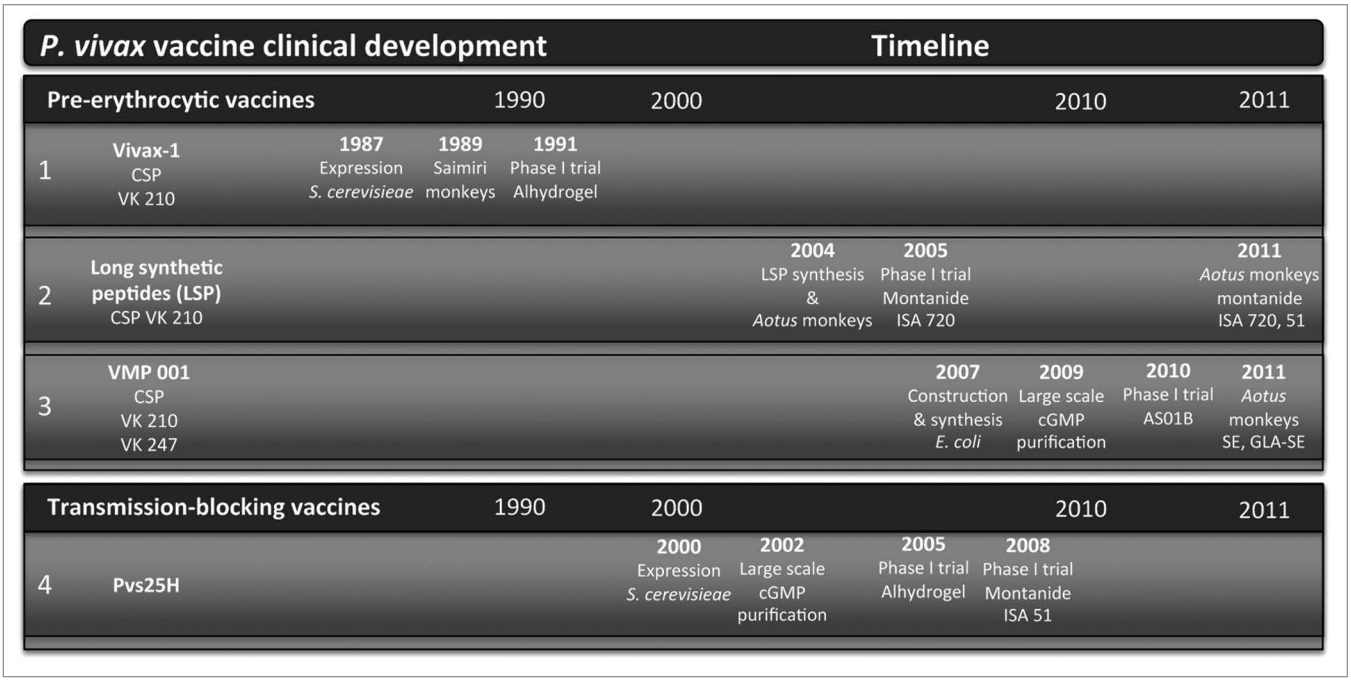

Figure 3. Timeline in the progress of vaccines tested to completion in clinical trials. (1) Vivax-1 vaccine.[28–30] (2) LSP vaccine.[31–33] (3) VMP001.[34–38] (4) PVS25H.[46–49]

of more than 70 aa each, spanning the N-terminal (N), C-terminal (C) and the central repeat region (R), the latter as a hybrid molecule containing a B-cell epitope and a universal T helper epitope from the tetanus toxin.[32] Development of an LSP has been possible due to the steady improvement in chemical techniques that allow production of longer peptide sequences with disulfide bonds that permit correct folding similar to the native structures. LSP vaccines have advantages for their clinical application, such as the peptide stability in the absence of proteases, lack of contamination with biological agents and fast production with good inter-batch reproducibility and the facility to be produced under good laboratory practice (GLP).[32]

The LSP vaccine has been shown to immunogenic in new world monkeys of the genus *Aotus lemurinus griseimembra* when administered three times at a dose of 100 ug formulated in montanide ISA720 or Complete Freunds Adjuvant (CFA).[32] Results warranted a further phase I clinical trial to assess safety, tolerability, and immunogenicity of the three synthetic peptides in malaria-naive volunteers in Cali, Colombia.[33] Volunteers were immunized three times with escalating doses of the LSPs (10, 30, or 100 ug/dose) formulated in montanide ISA720. Optimal antibody and cellular responses were obtained at the highest dose of 100 ug of LSP and future phase II clinical trials are planned.

VMP001 vaccine

The vivax malaria protein 1 (VMP001) is a recombinant vaccine that encodes a chimeric form of the *P. vivax* CSP. The chimeric protein has been expressed and purified from *E. coli* for early pre-clinical test[34] and for large-scale production under current good manufacturing practices (cGMP).[35] The novelty of this vaccine is that it contains the sequences of the two *P. vivax* CSP variants that circulate around the world, raising the possibility of providing protection against a wider range of *P. vivax* strains and hence becoming a vaccine for global use.[35] The CSP molecule of *P. vivax* isolates can be of two types, VK210 and VK247, which differ in the central repetitive region of the molecule and are present in isolates around the world.[27]

The VMP001 vaccine has been extensively evaluated in pre-clinical models in combination with various novel adjuvants with the objective to enhance immune responses. Rhesus macaques have been immunized with the VMP001 adjuvanted with a stable emulstion (SE) or the TLR 4 agonist glucopyranosyl lipid A (GLA) in SE (GLA-SE)[36] and the TLR 7/8 agonist R848.[37] Moreover, VMP001 has also been conjugated to lipid-enveloped poly(lactic-co-glycolic acid) (PLGA) nanoparticles (VMP001-NPs), which enhanced germinal center formation in mice and generated high and durable antibody titers with increased affinity to the CS repeat domains.[38]

Importantly, in July 2012, a phase I/IIa clinical trial started to determine the immunogenicity and protective efficacy of the VMP001 formulated with the GSK adjuvant system AS01B (monophosphoryl lipid A "MPL" plus the *Quillaja saponaria* plant extract QS21 in Alum). The study incorporated a dose-escalation phase with doses of 15, 30, and 60 ug of VMP001[39] (http://clinicaltrials.gov/ct2/show/NCT01157897). Results are yet to be published.

Transmission blocking vaccines (TBV)

The anti-malarial transmission-blocking vaccine (TBV) strategy aims to induce

an immune response in humans that will then disrupt the parasite's life cycle within the mosquito, thus preventing fertilization and development of ookinetes and oocysts. In other words, TBVs stop the mosquitoes from being infected by preventing the completion of sexual development of the parasite.

Similar to the PE vaccines, TBV are attractive because this also constitutes a bottleneck in the life cycle where a small number of parasites can be targeted and limited mechanisms have been developed to escape the immune response (target antigens are expressed in the mosquito and not humans), although some degree of polymorphism in Pvs25 has been found in isolates from Iran.[40] It is considered that infected mosquitoes in the field usually have 2–5 oocyst in the midgut, thus being the smallest parasite population in the entire life cycle.[19]

TBVs are considered altruistic or delayed impact vaccines because they would not prevent vaccine recipients from contracting malaria and developing symptoms but they would stop passing the parasite to someone else through a mosquito bite. The term 'community vaccine' is sometimes preferred because deployment of TBVs would benefit a whole community.[41] More recently, the term "vaccines that interrupt malaria transmission" (VIMTs) has been introduced to include any pre-erythrocytic or blood-stage vaccine having an effect on parasite transmission. The inclusion of multiple parasite life stages within the VIMTs is of particular importance for *P. vivax* control due to the extreme difficulty to reduce transmission of this parasite because of the hypnozoite development and the early formation of gametocytes that allow transmission before the clinical symptoms appear.[42]

Geographically, TBVs have a special niche for deployment. There are two major areas within the malaria-endemic world: (1) Countries where malaria has not been brought under control and where elimination is still a distant target and (2) countries where malaria has been brought under control and elimination is a short-term goal.[43] The main objective in areas where malaria is well controlled will be to reduce and interrupt transmission using TBVs. In contrast, pre-erythrocytic vaccines will be a better investment in areas where malaria has yet to be controlled in order to contribute to the decrease in cases but where attempting transmission interruption would not be cost effective.[44]

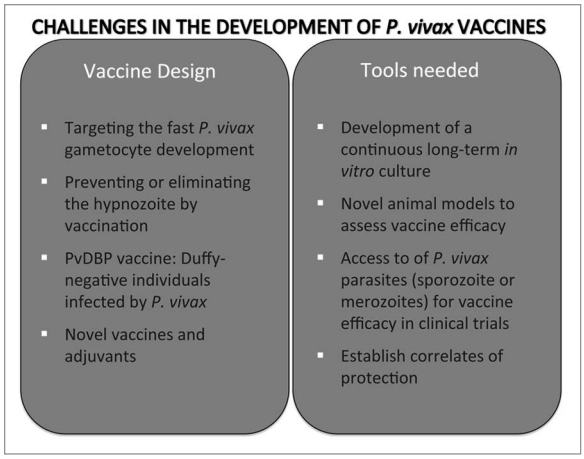

Figure 4. Challenges in the development of *P. vivax* vaccines.

Pvs25H

Pvs25 is an ookinete surface protein that is required for midgut invasion and mediates further oocytst development.[45] P25 proteins from both, *P. falciparum* (Pfs25) and *P. vivax* (Pvs25) are the leading TBV candidates.[46]

The candidate vaccine Pvs25H is a recombinant protein of 20.5 kDa encoding the aa 23–195 of the Pvs25 from the strain Salvador I of *P. vivax*.[47] The protein was expressed in *Saccharomyces cerevisiae* and has been manufactured under GMP at the Walter Reed Army Institute of Research (WRAIR).

Pre-clinical studies in mice, rabbits, and rhesus monkeys have indicated that Pvs25H formulated in Alhydrogel induces antibodies that block development of *P. vivax* in mosquitoes in ex vivo membrane feeding assays.[48,49]

There have been two phase I clinical trials using this vaccine. In the first one, the Pvs25H was adsorbed onto Alhydrogel and administered three times in a dose-escalation study by injecting either, 5, 20, or 80 ug to 10 volunteers per group. The study was designed to assess safety, tolerability, immunogenicity, and transmission blocking activity.[47] The vaccine was safe, well tolerated, and induced antibody responses and transmission blocking activity. Nevertheless, results indicated that higher levels of transmission activity would be required for a deployable vaccine and the following attempts focused on optimizing the formulation and vaccination regimen to induce higher antibody levels.

In the second phase I vaccine trial, Pvs25 was formulated as water-in-oil emulsions using Montanide ISA 51 in an attempt to enhance immunogenicity.[46] The study was designed to evaluate safety and immunogenicity of two malaria vaccine candidates, Pfs25/ISA 51 and Pvs25/ISA 51. The study intended to administer the vaccines at escalating doses of 5, 20, and 80 ug of protein in adjuvant. However, two volunteers who received 20 ug of Pvs25/ISA 51 developed erythema nodosum, due to this and other adverse events, vaccinations stopped and the study was closed to enrolment.

Future Challenges and Opportunities for the Development of a *P. vivax* Vaccine

For an infectious disease causing such a substantial burden, it is surprising that only four *P. vivax* vaccine candidates have been tested in early phase I clinical studies and from these only one might continue to Phase II trials. This reveals both the limited investments that are made in the area, and the complexity of the development of a vaccine for this parasite. *P. vivax* has evolved extraordinary mechanisms that have permitted its expansion over the largest territory on earth, compared with other human malaria parasites. The

hypnozoite, an apparently insignificant structure formed during the life cycle of the parasite, complicates dramatically the epidemiology as it permits adaptation of the parasite to temperate climates where no other human *Plasmodium* can survive. Hypnozoites make this disease extremely difficult to eliminate from endemic places and when both, *P. vivax* and *P. falciparum* co-exist, attempts to eliminate the parasite have succeeded only for the latter. The ability of *P. vivax* to remain dormant for up to two years places this pathogen at a similar level of difficulty to eliminate as the latent infections produced by *Mycobacterium tuberculosis* or HIV. Therefore, it would be ideal to develop vaccine approaches to prevent the establishment of the hypnozoite, or with the ability to attack and destroy hypnozoite-bearing hepatocytes, provided that the hypnozoite is "visible" to the immune system. In this context, for the prevention of hypnozoites, pre-erythrocytic vaccines have the best chance to achieve this by neutralizing the sporozoites before reaching the liver or by destroying infected hepatocytes through the deployment of cytotoxic lymphocytes to the liver, as has been described with the use of persistent recombinant adenoviral vectored vaccines.[22]

Despite being the most intriguing structure of *P. vivax*, the hypnozoite is not the only notable attribute of the parasite that makes diagnosis, treatment and elimination very difficult. *P. vivax* has a preference for reticulocytes[50] and this has impeded the development of continuous in vitro culture techniques. A third important feature of the *P. vivax* cycle is the ability to quickly develop gametocytes,[51] which has prompted the use of the expression of a fast and furious sexual life strategy.[52] Blood gametocytes can be present within 8 d after mosquito inoculation, which is followed by a continuous gametocyte production and circulation—as opposed to *P. falciparum* which has a tendency to adhere to endothelial walls of blood vessels—making *P. vivax* transmission a very effective and dynamic process.[52]

Another challenge can potentially present during the development of blood-stage vaccines. The Duffy-binding protein (DBP), PvRII, has been considered to be an attractive vaccine candidate against the asexual blood stages of the parasite because of the "absolute requirement by the *P. vivax* DBP for the DARC receptor" (Fy glycoprotein or CD234) on the surface of the red blood cells.[53] Recent evidence, however, indicates that *P. vivax* can infect Duffy blood-group negative people and clinical malaria is commonly observed in Duffy-negative people,[54] thus raising the possibility that additional vaccine strategies might be required to halt the parasite at the blood stage. A list of these and other current challenges is shown in **Figure 4**.

In summary, *Plasmodium vivax* will be the biggest hurdle toward malaria eradication. For an infection with such a substantial disease burden there has been remarkably little vaccine development and very few laboratories have achieved to assess new vaccine candidates in clinical trials. Partially responsible are the lack of tools to assess new vaccine candidates and the high cost associated with vaccine development and clinical trials. Nevertheless, inspired by the Gates malaria forum in 2007, the goal to eradicate malaria has come back to the global health agenda 40 y after the initial commitment by the WHO to eradicate this disease and thus, efforts to prevent and cure the most prevalent and neglected form of malaria caused by *P. vivax* has increased as a result of this renewed interest and new vaccine candidates are making good progress toward clinical assessment.

Finally, it is highly likely that we will see new clinical developments very soon and ideally these should maximize immunogenicity without compromising safety. This could be achieved by designing vaccines to mimic pathogens without causing disease. Recombinant viruses and particle vaccines are good examples of platforms that can achieve this by simulating key features of pathogens, such as size, repetitiveness, and toll-like receptor ligand stimulation resulting in an enhancement of the vaccine efficacy.[55,56] Therefore, it will be very interesting to see recombinant adenoviruses and MVA entering the arena as a pre-erythrocytic, blood-stage, or transmission blocking vaccines. Such platforms have demonstrated an unparalleled ability to stimulate both, antibody and T-cell responses in pre-clinical and clinical studies for *P. falciparum*, while keeping a good safety profile.[20,21,57,58] Other platforms of great interest that would certainly make a substantial addition to the vaccine pipeline are the antigens delivered as particle vaccines, such as virus-like particles (VLPs) and RTS, S-like vaccines for *P. vivax*, which could enhance efficacy in comparison to a protein-based vaccine. Such platforms could be used in combination as a multi-antigen or multi-stage vaccine, mixing pre-erythrocytic candidates or even combining these with blood-stage or transmission-blocking vaccines.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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