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REVIEW

Genetic approach towards a vaccine against malaria

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Abstract

Malaria is a major concern for international health authorities. Millions of people contract it every year in the world due to a parasite of the *Plasmodium* genus. Due to the complexity of the parasite biology and genetics, there is currently no vaccine against the disease. However, due to the great resistance both to the medicines and to the insecticides used to combat the disease, it has become essential to obtain a vaccine as the necessary tool to prevent transmission and eliminate the disease. The bibliometric data indicate that interest in vaccines has been growing steadily since the 1980s. But nowadays, a powerful tool is used: the *Plasmodium* genome. This allows us to improve the fight against the disease. Knowing the sequences of the genes that favor the appearance of drug resistance, or those that encode for proteins with greater antigenic response, is a tool that can become fundamental. This article reviews the state of the art on vaccines and genetics, in the fight against malaria, and analyzes the fixed photo that the worldwide research on the disease poses.

Keywords Malaria · Genes · Vaccine · *Plasmodium* · Worldwide

Abbreviations

RBCs	Red blood cells
VIMT	Vaccines that interrupt malaria transmission
TBVs	Transmission-blocking vaccines
malERA	Malaria Eradication Research Agenda
CSP	Circumsporozoite protein
CelTOS	Cell-traversal protein for *Plasmodium* ookinetes and sporozoites
TRAP	Thrombospondin-related adhesive protein
AMA1	Apical membrane antigen 1
RON2L	Rhoptry neck protein 2
SEA	Schizont egress antigen

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Introduction

Malaria is a disease that has affected the human population since ancient times. Today, it remains one of the infectious diseases with the highest morbidity and mortality rates, with more than one million people dying every year from this disease worldwide [1]. It is produced by different species of a protozoan parasite of the *Plasmodium* genus distributed especially in the tropical and subtropical areas of the planet. The most widespread species are *P. falciparum* and *P. vivax*, responsible for more than 95% of cases. Four other species affect humans: *P. malariae*, *P. ovale curtisi*, *P. ovale wallikeri*, and *P. knowlesi*. Of all of them, *P. falciparum* is the species that produces the most severe form of the disease and mainly affects the African continent where the child population is the most susceptible along with pregnant women. However, cases of severe malaria caused by *P. vivax* [2] are currently being reported. This species extends to areas of the Asian continent, Oceania, and Central and South America. In some regions, there are frequent cases where both species coexist [3].

The parasite is transmitted by female mosquitoes belonging to the genus *Anopheles* that inoculate the sporozoites when they bite humans; these through the peripheral circulation reach the liver cells where they multiply and transform into merozoites that pass into the bloodstream to infect the red blood cells inside which they will develop and multiply until the breakage of the blood cell releasing new merozoites that

will invade new red blood cells (schizogonic cycle). After several erythrocyte cycles, some merozoites become the sexual stages of the parasite (male and female gametocytes) which are the forms that will pass to the mosquito when it bites a sick individual. Inside the mosquito, the zygote will form and after migration through the mosquito midgut, the sporozoites (sporogonic cycle) will develop that are transferred to the salivary glands closing the cycle. In *P. vivax* and *P. ovale*, the parasite can take a latent or dormant form; they are the hypnozoites that can become active after a period of time, transforming into merozoites that would invade the red blood cells triggering the disease.

Unlike other pathogenic organisms such as bacteria or viruses, parasites have a complex eukaryotic cell organization with complex life cycles as well as enormous antigenic variability and mechanisms capable of evading the host immune response, all of which make it difficult to obtain vaccines. It is a fact that there are currently no vaccines against parasitic protozoa such as *Leishmania*, *Trypanosoma*, or *Plasmodium* despite infecting millions of people worldwide. In the case of malaria, the emergence of resistance to the drugs used to treat it, as well as resistance to insecticides to combat *Anopheles* mosquitoes or the emergence of zoonotic species, has put research into obtaining a vaccine as the necessary tool to prevent transmission and eliminate the disease at the forefront [4].

The search for a vaccine against malaria is nothing new. In the twentieth century, it was thought that it was possible to get a vaccine when in the 1970s, volunteers inoculated with irradiated *P. falciparum* and *P. vivax* sporozoites developed full immunity (sterilizing) to the bites of infected mosquitoes in more than 90% of cases. However, the duration was moderate [5]. It has also been found that people living in malaria-endemic areas progressively acquire partial immunity that protects them from clinical disease, especially severe malaria and death [6, 7] as well as a decrease in parasitemia levels to almost undetectable levels [8]. Passive immunity was also achieved by transferring immunoglobulins obtained from the "immune" serum African individuals to Thai children experiencing recrudescence of malaria infection [9]. All this evidence suggested that a vaccine against malaria was possible and there are many different studies in this field. However, despite the many efforts made, there is still no effective vaccine to provide at least 75% protection. The fact that there are no suitable experimental models for human *Plasmodium* species makes research extremely difficult and slows down, and although large number of candidate vaccines is currently being evaluated, few are in the clinical phase.

The selection of antigens to be included in a vaccine is not easy, because, although research on the parasite proteome has provided numerous potential targets, few are selected as vaccine candidates [10, 11].

The first strategy chosen to design possible vaccines was to select antigenic targets from the different stages of the life cycle capable of inducing humoral and/or cellular response, and so pre-erythrocytic stage, blood stage, and transmission-blocking vaccines have been developed. The former tries to prevent infection by preventing the invasion to hepatocytes or by eliminating those that have already become infected, preventing any of the symptoms of the disease. Erythrocyte vaccines prevent the development of the disease by acting on circulating merozoites and preventing them from invading red blood cells. Therefore, they do not prevent the disease, although they would decrease its severity. The latter are known as altruistic vaccines because they do not prevent infection or disease in the person receiving them. These act on the sexual forms, both in the human and in the mosquito, preventing the transmission of the disease. Pre-erythrocyte vaccines and transmission-blocking vaccines have the advantage of acting on cycle bottlenecks, where the number of parasites is lower, and therefore the probability of success is higher. The primary goal of the first vaccines developed against malaria was to reduce morbidity and mortality in children living in highly endemic countries. However, in the face of the changing disease landscape, the new challenge is to eliminate and eradicate the disease, and it is essential to develop vaccines that are capable of reducing and preventing the transmission of the parasite. In this new scenario, the malERA (Malaria Eradication Research Agenda) Consultative Group on Vaccines introduces a new concept of vaccines that interrupt malaria transmission (VIMT) to replace the term transmission-blocking vaccines (TBVs) [12]. This new term includes not only vaccines directed against the vector, but also highly effective pre-erythrocytic or erythrocytic stage vaccines, and vaccines targeting parasite antigens of sexual and mosquito stages of the infection. The aim of this new strategy is to prevent infection. In 2013, malERA also recognized the need to include *P. vivax* in studies of antimalarial vaccines, as there are now regions where this species is even more untreatable than *P. falciparum*.

Advances in the knowledge of the *Plasmodium* genome and in molecular biology techniques have made it possible to better understand the vector-human and host-parasite interactions, contributing to the design of new vaccines [13]. Subunit vaccines contain part or complete antigens and recombinant DNA technology has facilitated the development of these types of vaccines in different expression systems. The first vaccines designed as recombinant protein subunit vaccines generally induce a poor cellular response. The newest generation of subunit vaccines are DNA based. DNA and recombinant viral vaccines can induce high levels of cellular response although the humoral response is low.

Subunit vaccines against pre-erythrocyte phases have demonstrated high protection against malaria infection. A truly effective vaccine should prevent the invasion to hepatocytes by inducing high antibody titers against sporozoites that would prevent their entry, and high T cell responses against

the infected hepatocytes. This would prevent the emergence of blood-borne parasites, including sexual forms, and thus prevent transmission. Two strategies have been used for the development of pre-erythrocyte vaccines: selecting an appropriate antigen and using complete sporozoites. In the first case, one of the antigens selected was the surface antigen of the circumsporozoite, CS, the largest surface protein of the sporozoite, essential for its development and expressed the intracellular hepatic stages. This protein contains three regions: an N-terminal domain, which binds to the hepatocyte; a central region; and a C-terminal domain. Although other immunogenic sporozoite proteins related to hepatocyte mobility and invasion have been identified [14], none have induced an immunogenic response comparable to CSP. Thus, the vaccine RTS,S/AS01 was designed, developed by GlaxoSmithKline as Mosquirix, which contains much of the primary sequence of *P. falciparum* CSP fused to a hepatitis B surface antigen to form a virus-like particle and these were mixed with the adjuvant AS02 [15]. This was the first first-generation vaccine to complete phase III of the clinical trial and is the first and only vaccine so far to show a protective effect against malaria in young children in clinical trials. However, the results are worse than expected, with only partial (36%) and short-term effectiveness [16–18]. It is important to note that clinical trials began in 2002 and ended in 2014 [19], so it is likely that the sequence used is based on a laboratory strain isolated decades ago that does not represent the protein of the parasites currently circulating in endemic areas [20]. It may be necessary, in the design of new vaccines, to make modifications in the design of the antigen because recent research by Tan et al. [21] and Kisalu et al. [22] have shown that most antibodies to *P. falciparum* circumsporozoite protein (PfCSP) bind to the central region of the protein, while most protective antibodies also bind to unidentified epitope, not included in the RTS,S/AS01 vaccine. These epitopes are related to hepatocyte invasion, so this region of PfCSP should be included in a future vaccine [18]. Sack et al. [20] consider that for next-generation subunit vaccine, variants representing the most prevalent genotypes should be included, as well as adding new antigens other than PfCSP, as antibodies obtained from individuals immunized with sporozoite vaccines and protected from CHMI recognize a large number of non-PfCSP antigens.

Currently, there are not many non-PfCSP recombinant PE vaccine candidates in clinical trials. A protein component of vaccines is the cell-traversal protein for ookinetes and sporozoites (CelTOS), which is localized to micronemes that are organelles for parasite invasive motility. It was the first protein identified as a potential target by Mining Genomic and Gene Expression Databases [23]. The human vaccine is in the clinical phase [17]. Another selected protein is thrombospondin-related adhesive protein (TRAP), essential for sporozoite motility and host cell invasion. Against this protein, individuals in endemic areas have high levels of antibodies, anti-PFTRAP. Different types of vaccines have been developed with this protein without good results. Bauza et al. [24] suggest that a combination of CSP and TRAP subunit vaccines could enhance protection against malaria.

The viral-vectored vaccines have now gained importance as they have shown very good results in stimulating high T cell responses. One strategy employed to improve the cellular immune response of protein-based subunit vaccines has been to use viral-vectored vaccines [24–26] using chimpanzee adenoviral vector (ChAd63) or modified vaccinia strain Ankara (MVA) in heterologous prime-boost immunization regimes [27, 28]. Some candidates are in the experimental phase with encouraging results [29–31].

Studies, both in humans and animals, show the possibility of achieving sterile immunity by vaccination [5, 32]. McCarthy and Clyde [32] have led to the development of vaccines with complete sporozoites (PfSPZ) attenuated by different mechanisms. These types of vaccines induce a broad cellular and humoral response. Several candidate vaccines are currently being investigated, including PfSPZ-attenuated sporozoites by irradiation [33], vaccines in which the sporozoites are fully infectious and are attenuated by the joint administration of the antimalarial drug PfSPZ-CVac [34], and vaccines with attenuated sporozoite by the knockout gene PfSP-GA1 [35]. There are 43 research groups in 15 countries, collaborating in the advancement of this type of vaccine manufactured by Sanaria and that have been shown to induce a broad cellular and humoral response [31, 36].

The erythrocyte or blood-stage highly effective vaccines are also included in the new concept of ITVL because by lowering the density of asexual forms below the threshold required for infectious gametocyte production, they block the transmission from the parasite to the vector. Pre-erythrocyte vaccines rely on the reduction of parasitemia and clinical symptoms in children with severe malaria who were given serum from hyperimmune adults [9, 37]. The proteins expressed on the surface of the merozoite, the blood form of the parasite, represent the first targets due to repeated exposure in the host immune system [11]. Some of these were correlated with immune protection in endemic regions or identified by screening expression libraries with hyperimmune sera. The proteins present on the surface of parasitized red blood cells also represent potential targets, and they are associated with natural immunity to malaria, but their high diversity is a major drawback to vaccine design. The blood-state vaccines have been deprioritized in favor of targeting pre-erythrocytic and mosquito stage parasites. The main obstacles to its development are the antigenic polymorphism of both the merozoites and the surface proteins of infected red blood cells, the redundancy in the merozoite invasion pathways [38], and the difficulties in producing natively folded proteins. The main blood-stage vaccine candidate currently is PfRh5, a merozoite protein, essential for red blood cell invasion [39–41]. A first

generation of this vaccine is being tested in clinical trials in Oxford and Tanzania [42]. Another vaccine being developed is the AMA1 (apical membrane antigen 1)-RON2L (rhoptry neck protein 2) complex, which is targeted against the invasion of merozoites. Both form a complex essential for merozoite invasion during the blood stage of infection [43]. Attempts at vaccines with AMA1 alone have failed [44], while forming a complex with RON2L improves the immunogenicity of some preserved AMA1 epitopes. Another antigen showing preclinical promise is schizont egress antigen-1 (SEA-1) [45]. SEA-1 is a parasite antigen expressed in schizont-infected red blood cells (RBCs) that is essential for schizont rupture in the blood state. Antibodies to PfSEA-1 are associated with a reduction in the incidence of severe malaria. It is not known whether these antibodies would also block the exit of the liver schizont, but it should be investigated as this would mean that a single vaccine would block an essential process in multiple states [46].

Finally, malaria transmission can also be interrupted with vaccine to disrupt parasitic infection of the host mosquito, also identified as a transmission-blocking vaccine. There are currently two vaccine candidates. The first of these is directed against the post-fertilization antigen, Pfs25, expressed in zygotes and oocytes. The second is directed against the preferred antigen, Pfs230, a protein expressed in gametocytes. In ongoing trials, these have been combined with an exoprotein (EPA) to increase their immunogenicity. Currently, the Pfs25-EPA/alhydrogel conjugate vaccine is in phase 1 clinical trials in the USA and Africa [47]. The recombinant Pfs230D1M protein is also being investigated and is considered a promising option for a blockade of transmission vaccine [48].

Another potential target is Pfs47, a protein that makes parasites "invisible" to mosquitoes' immune systems [49]. Recent research suggests that Pfs47 acts as a "lock and key" because if *P. falciparum* has Pfs47 haplotype compatible for a given anopheline mosquito, this is sufficient for the parasite to evade mosquito immunity [50]. Therefore, Pfs47 can be considered as a vaccine candidate.

The next generations of vaccines must combine several potential targets from different stages in the *Plasmodium* life cycle to achieve greater effectiveness, multi-state, and multicomponent vaccines. In this regard, knowledge and use of genomic resources is essential, as has been demonstrated by various trials, such as those that accelerate genome-wide functional approaches in mice with the aim of accurately reproducing certain aspects of the pathogenesis of human infectious diseases [51].

Materials and methods

In this analysis, the search query [TITLE-ABS-KEY (malaria AND vaccine AND gene)] in the Elsevier Scopus database was performed. As a result, 2254 documents met the selection parameters. It must be considered that using alternative search parameters, the results obtained can be different. It should also be borne in mind that Elsevier Scopus is a dynamic database that can include, delete, or move a document from 1 year to another, depending on its own parameters. This dynamic nature of the database may result in minimal changes in the result of a search carried out at two different times but does not alter the meaning of the analysis and the conclusions that can be drawn from it.

In the analysis of the keywords, these were processed by grouping those that had the same meaning, but a different spelling, as is the case, for example, of *Plasmodium falciparum* and *P. falciparum*. In addition, those whose consideration did not contribute anything to the analysis, such as, *Article*, were discarded.

The aspects that have been studied are number of publications per year, distribution of publications by country and by institutions, main authors, and keywords. The set of articles obtained in the main search is represented by a network with nodes and links between them. Nodes are their keywords and their importance is represented by the size of the node and its centrality in the network. The size of the connection between two nodes represents the number of relationships between the two keywords, so the larger the relationship between those two keywords. If it is desired to know around which topics the articles are grouped, those parts of the network that are more interconnected with each other, it is necessary to use a community detection algorithm. Finally, community detection algorithm is applied to the network of the main query to group the global research trends to the search criteria. As less strongly related elements are associated with this nucleus, the peripheral elements are structured. In addition, community detection was carried out using a VOSviewer software tool, which allows the generation of graphs. In these graphs, the collaborations between countries and keywords, which are represented by nodes, are displayed.

The results obtained were processed using OpenRefine (http://openrefine.org/), an open source tool. OpenRefine "is a standalone desktop application initially developed by Google for data cleansing and transformation to other formats." This methodology facilitates the massive analysis of disorganized data that would otherwise be very difficult to understand, given the large size of the database used. This methodology has been used successfully in other bibliometric studies [52]. The results obtained by OpenRefine were tabulated in spreadsheets.

Results

Evolution of scientific output and subject area

Figure 1 shows the evolution of the number of publications on vaccines and genes in malaria worldwide around 1979, the

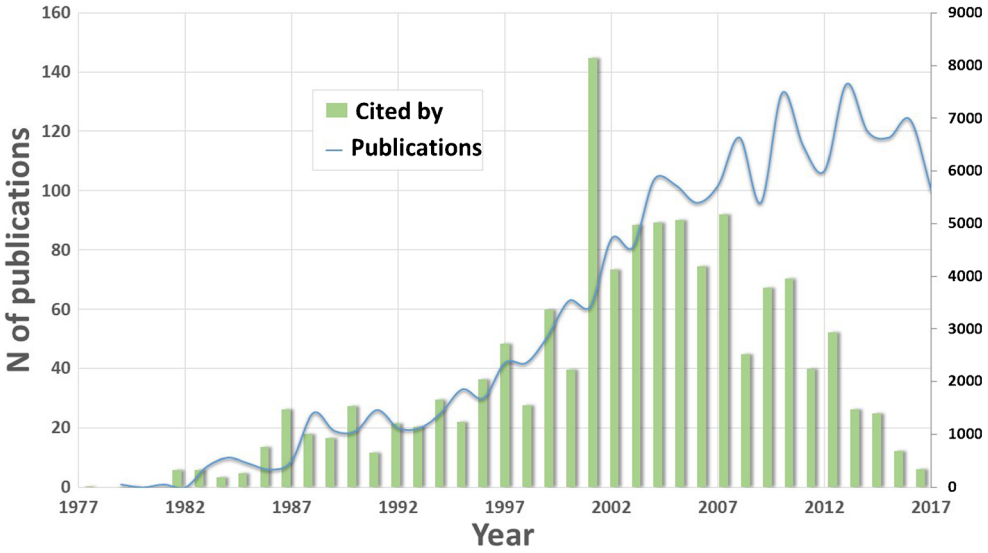

Fig. 1 Trend of the number of publications from the years 1977 to 2017 and the cites received for articles published each year

year in which the first article on this subject was published, and 2017, the last full year for which data are available. The results show a remarkable growth in the number of publications, from an average of 1.8 publications per year in the first 5 years to an average of 120 publications in the last 5 years. But there is no linear growth with a constant increase in the number of articles published each year, but a typical pattern of sawtooth growth can be observed in the graph, with relative minimums and maximums appearing in a high number. In any case, there is no doubt about the significant increase in the number of publications over the period, with an average growth of 3.5 documents per year. Throughout these almost 40 years, 2254 documents have been published with this theme. Figure 1 also shows the number of cites received for articles published each year—in green bars. In this case, it can be observed that the evolution of the growth in the number of citations is proportional to the growth in the number of articles, until 2008. After this one, there is no time perspective big enough to keep it that way. There is a year that breaks the rule. It is the year 2002. This year's articles have received, in total, more than 8000 cites. This is due to the fact that in that year, two of the five articles in this series that have received the most citations were published. Of these, the article in which the sequencing of the *P. falciparum* genome was published stands out [53], with more than 2700 cites.

Subject area analysis establishes that the three areas in which most articles are classified are immunology and microbiology, medicine, and biochemistry, genetics, and molecular biology, with 55, 51, and 40%, respectively, of the publications classified in these. But if the data are analyzed according to the evolution in the classification by subject area in each of the four decades into which this study can be divided, it can be seen that these values have not been constant over time (Fig. 2). This fact is especially notable in the area of medicine. While in the 1980s, only 36% of articles were classified in this area and in the last decade, the value has increased to more than 60%, demonstrating the great interest that the development of a vaccine against malaria generates in the field of human health.

Publication distribution by countries and institutions

Eleven institutions have been found to have published at least 50 publications on vaccines and genes in malaria. The National Institute of Allergy and Infectious Diseases and the National Institutes of Health with 163 and 158 publications, respectively, stand out from the rest. Among these are five North American institutions; four European—three British and one French; one Australian; and one Latin American, from Brazil. These data are consistent with the results of the analysis by country, not by institution. In this instance, the country with the most articles published on this subject is the USA, with 933, followed by the UK, with 363. At a considerable distance are the rest of countries such as Australia, with 196 publications or France, with 162. The remaining countries with at least 100 publications on vaccines and genes in malaria are India, Japan, Germany, Brazil, Holland, Switzerland, and the Netherlands.

Figure 3a shows a world map. In this, each country's scientific production of vaccines and genes in malaria is highlighted in color. It is observed that the countries with the most publications in this field are the USA and the UK. At a second level are countries with incidence in this disease, such as Brazil or India, but also France, Germany, Japan, or Australia. And on a third level of importance, there are countries like Colombia, China, and Kenya. If this analysis is carried out with the publications on vaccines and genes with respect to the total number of malaria publications and these are represented in %, Fig. 3b is obtained. It is now clear that

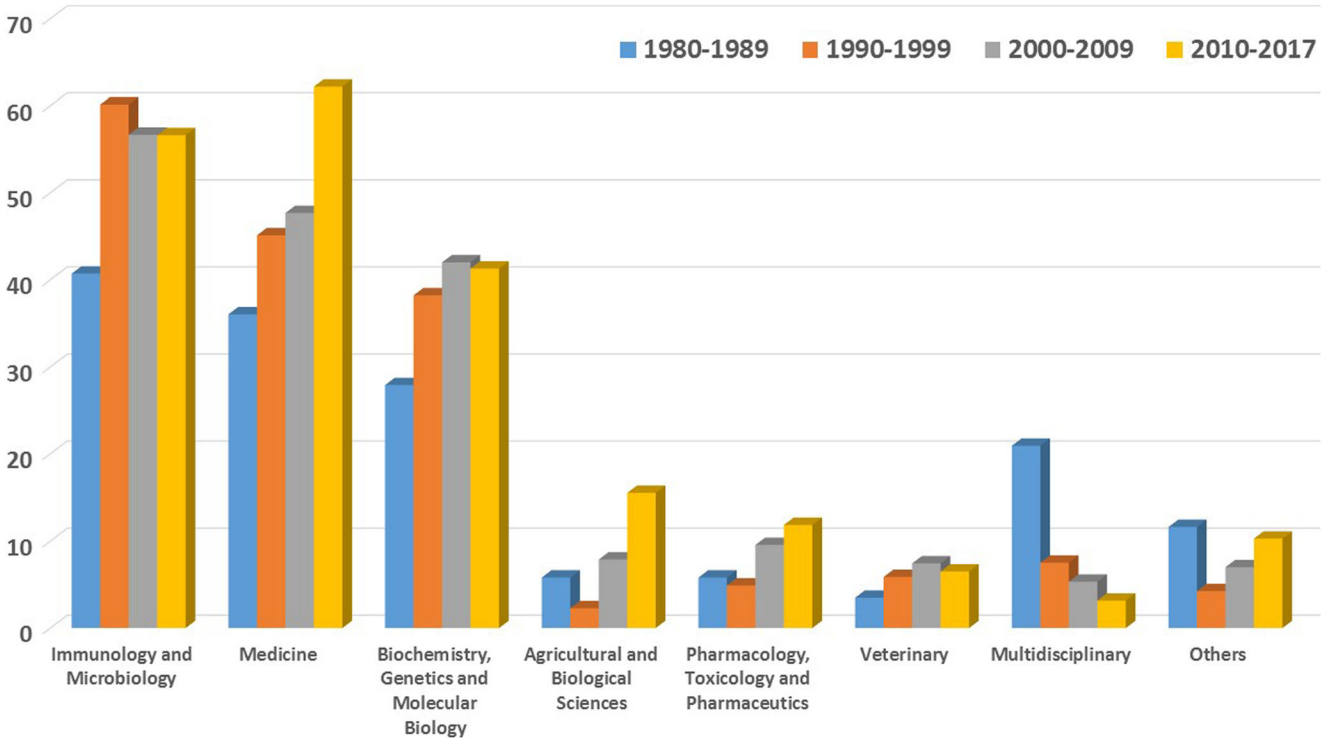

Fig. 2 Evolution of the main subject areas in which publications about vaccines and genes in malaria are classified

some countries have focused their scientific efforts in this field, like the Dominican Republic, where 40% of its malaria publications are on this subject. In this new ranking, there appear countries that in absolute value did not have great scientific production; however, in these relative terms, they appear at the same level as Colombia. These are Honduras, Nicaragua, or Papua New Guinea. On the other hand, it is striking that Australia is still in this ranking and is one of the countries with a large scientific production in this domain, which shows that they also invest a great effort in this field within the malaria studies.

Figure 4a shows a distribution in communities of the countries that have published at least 25 articles. The 24 countries that appear in the distribution are grouped into five clusters. The first of the clusters, highlighted in red in figure, is made up of Italy, Colombia, Papua New Guinea, Spain, Switzerland, and Australia, as the central country. The second of the clusters, highlighted in green, is made up of Brazil, China, Japan, South Korea, Thailand and the USA, as the central country. Denmark, Kenya, the Netherlands, Sweden, Tanzania, and the UK, as the central country, can be found in the third cluster, highlighted in blue in figure. The remaining are two smaller clusters, with three countries each; Belgium, Canada, and India on the one hand, highlighted in yellow, and France, Iran, and Germany on the other, highlighted in violet. In addition, the relationship between two countries is represented by the thickness of the joint line of two nodes.

Main authors

Twenty-one authors have been found to have published at least 25 publications on vaccines and genes in malaria. Most of these (8 out of 21) are from the USA. In addition, there are three authors from the UK, three from Australia, and three from Japan. There are also four other countries with one author among the main authors: Colombia, Brazil, Thailand, and the Netherlands. Of all these authors, SL Hoffman and AVS Hill stand out from the rest, with 57 and 53 articles, respectively. The first of them participated in the sequencing of the *P. falciparum* genome, and also stood out for its genomic studies of the malaria parasite and DNA vaccines, while the second one focuses its research, above all, on the study of the immunogenicity of some vaccines.

Figure 4b shows a graph of the relationships established by the main authors with at least 25 published articles on vaccines and genes in malaria. From this type of representation, it is possible to deduce the links that each author establishes with other researchers. This analysis facilitates the establishment of relationships such as those of Americans SL Hoffman with DL Doolan or M Sedegah, who have co-published 54 articles related to the immunology of malaria, or those of the British AVS Hill and SC Gilbert, who have co-published 131 papers, also related to the immune response to malaria. But it is even more remarkable that the only author who does not appear in the

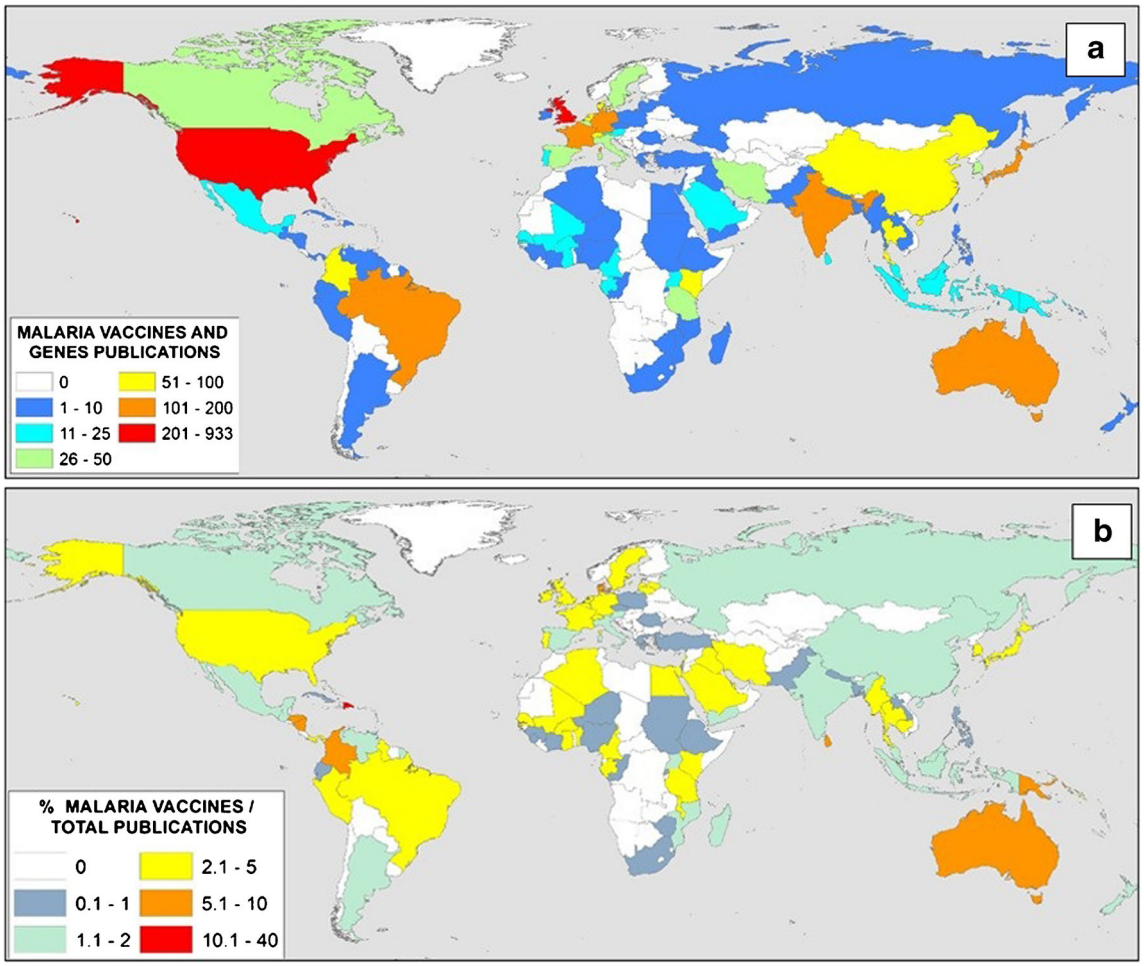

Fig. 3 World map of publications of vaccines and genes related to malaria

graph is the Latin American, MA Patarroyo. This is because it lacks links with the other main authors. This should not be interpreted as a meaning that he does not investigate. In fact, according to the Scopus database, this author has published almost 200 articles to date, which have received more than 1800 citations. The only thing that can be deduced is that their collaborations are developed in a different line to the object of this study.

Table 1 shows the 11 institutions with, at least, 50 publications on vaccines and genes in malaria. Of these, two institutions stand out: The National Institute of Allergy and Infectious Diseases, and the National Institutes of Health, with 163 and 158 publications, respectively. These institutions are also the ones with the highest H-index and their articles have the highest number of cites. If the attention is paid to the average number of cites received per article, it can be observed that the Naval Medical Research Center has a very high number, 92.2 compared to the average value of these 11 institutions, which is 54.4 cites per article. This is because the researchers at this center were responsible for some of the most cited articles [53, 54].

Keywords analysis

To carry out the analysis of the keywords, the terms that do not contribute anything to the analysis have been discarded. For example, "article," "journal," or "review." In addition, different keywords with the same meaning, such as "*Plasmodium falciparum*" and "*P. falciparum*," were considered as a single keyword. The results shown that the 20 keywords that appear most frequently in articles on vaccines and genes in malaria ranged from 1625 for "Nonhuman" to 423 for "Immune Response." The other terms that appear in this list are Human, Animals, Malaria, *Plasmodium Falciparum*, Malaria Vaccine, Parasite Antigen, Controlled Study, Protozoan Proteins, DNA Sequence, Unclassified Drug, Antigens Protozoan, Female, Genetics, Mouse, Immunology, Gene Expression, Animal Experiment, and Amino Acid Sequence.

Finally, the temporal evolution of the publications around the four *Plasmodium* species that appear most often in the keywords of the analyzed articles has been studied (Fig. 5). These four species are *P. falciparum* and *P. vivax*, which cause malaria in humans, and *P. berghei* and *P. yoelii*, which infect rodents and are used

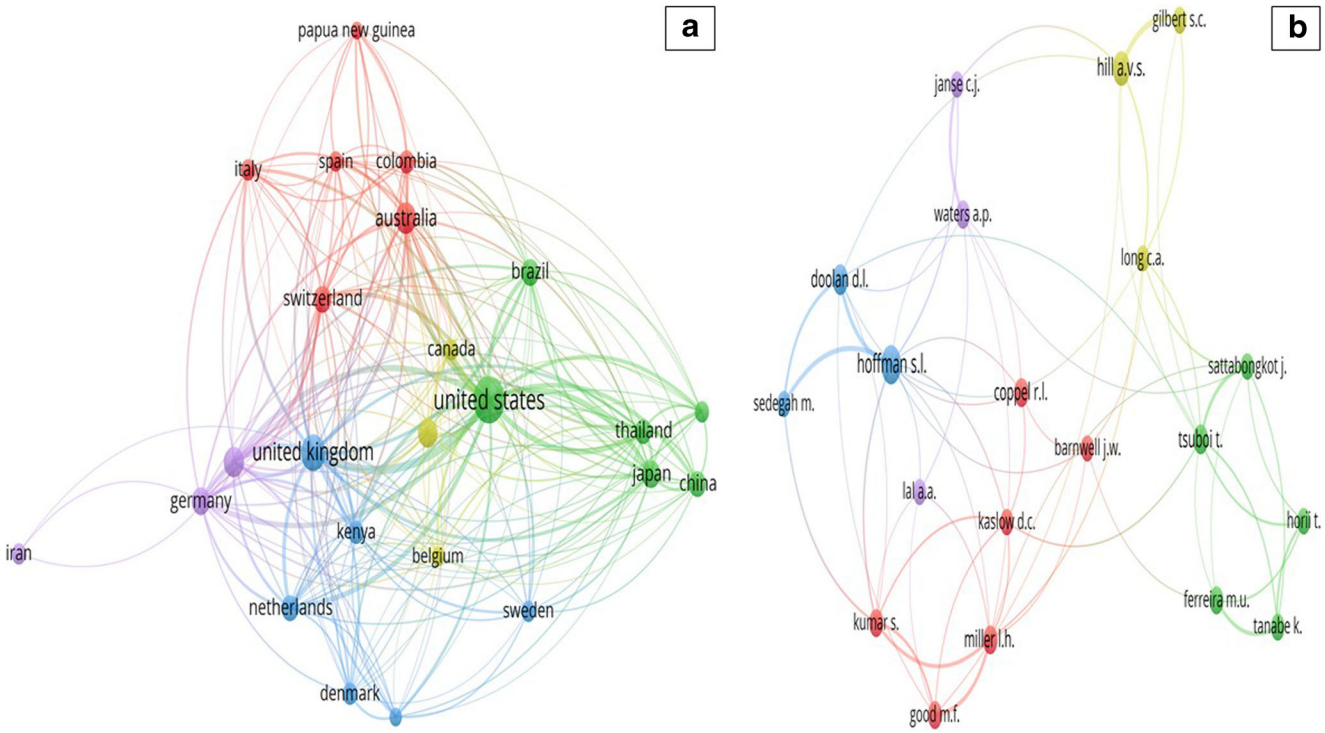

Fig. 4 Scientific communities and their associations in publications on vaccines and genes in malaria. Each cluster is represented by a color. **a** Communities of countries. **b** Communities of researchers

as model organisms and in vaccine studies. As can be seen, although *P. falciparum* has always occupied a prominent place in this ranking, since 2002, coinciding with the publication of the sequencing of its genome, publications on this parasite have soared. This analysis was also carried out considering the publications of the three countries with the greatest scientific production, the USA, the UK, and Australia, and the result was similar, indicating that the observed trend is independent of the country considered.

Discussion

An analysis of the evolution in the number of publications on vaccines and genes in malaria since 1979, when the first article on this subject was published, to 2017, the last year for which complete data are available, shows an increase that is not constant, but which does mark a clear trend. This trend is consistent with the great interest that malaria provokes in the international scientific community and with the growing awareness that the fight against the disease and its eradication

Table 1 Main institutions that publish on vaccines and genes related to malaria

Institution	Country	*N* of articles	H-index	*N* of cites received	*N* cites/article
National Institute of Allergy and Infectious Diseases	USA	163	55	8761	53.7
National Institutes of Health, Bethesda	USA	158	54	8550	54.1
University of Oxford	UK	108	41	8055	74.6
Walter Reed Army Institute of Research	USA	104	44	6212	59.7
London School of Hygiene & Tropical Medicine	UK	75	33	4282	57.1
Institut Pasteur, Paris	France	72	31	2268	31.5
Naval Medical Research Center	USA	66	29	6083	92.2
Monash University	Australia	57	22	1197	21.0
Imperial College London	UK	53	26	3115	58.8
Seattle Biomedical Research Institute	USA	51	27	3031	59.4
Universidade de Sao Paulo - USP	Brazil	51	22	1878	36.8

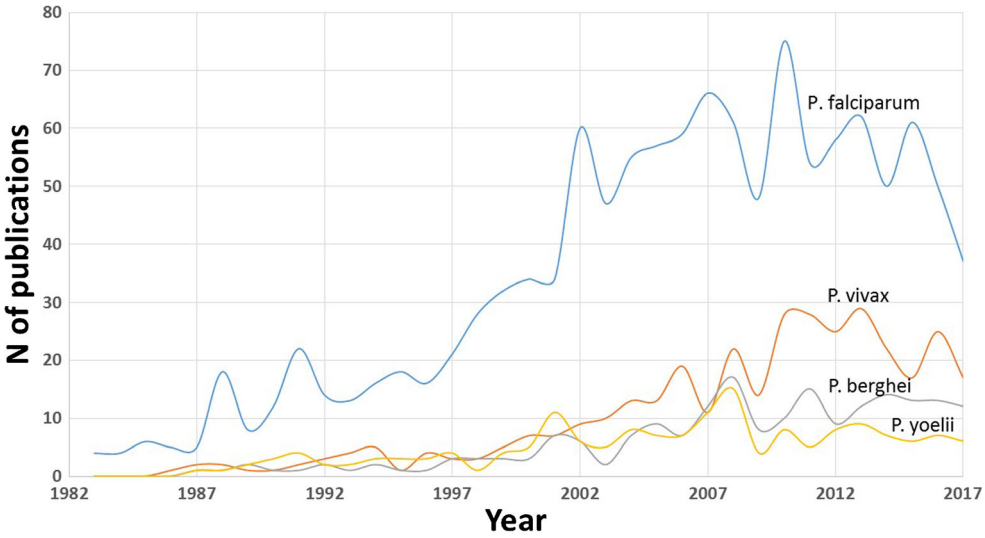

Fig. 5 Time evolution of the number of publications for the most studied *Plasmodium* species

must be aimed at a definitive vaccine. An important milestone was achieved in 2002: Sequencing of the complete genome of *P. falciparum*, the human malaria parasite that causes the most deaths each year. This was clearly reflected in the number of publications. There was an increase of 37.8% over the previous year worldwide.

In this analysis, in addition to the trend in the number of publications, other variables have been studied. Thus, 70% of the publications are research articles, 20% are reviews, and the remaining 10% are conference paper, note, editorial, etc., or that most of these articles are published in journals in the areas of immunology and microbiology, or medicine. But, above all, special attention has been paid to studying which institutions, countries, or researchers play a fundamental role in the publication of articles on vaccines and genes in malaria.

Thus, it has been possible to establish a ranking led by the National Institute of Allergy and Infectious Diseases, the National Institutes of Health, and the Walter Reed Army Institute of Research, in the USA, and by the University of Oxford and the London School of Hygiene & Tropical Medicine, in the UK, with more than 500 documents published by all of them. Among the main authors, the American SL Hoffman and the British AVS Hill stand out from the rest. His research on immunology, on vaccine development, and on the study of resistance in severe malaria highlights the direction of global research on vaccines and genes in malaria.

In summary, it can be concluded that research on vaccines and genes in malaria is of great concern to the international scientific community. The number of publications on this subject has grown enormously in the last 40 years, highlighting the increase produced by the introduction of next-generation sequencing as a fundamental tool in the molecular study of malaria. This gives us an idea of the path that research in this area will take. The analysis of the genomes of *P. falciparum* and *P. vivax* to find a vaccine to help eradicate malaria has become essential, and all efforts are now focused on the development of bioinformatics tools to help process all the information generated. However, the overall objective of achieving the vaccine that will make it possible to eradicate malaria once and for all is only possible through cooperation and joint efforts, and not only at the level of scientific parallels or economic interests, but also at the political and institutional level. For this reason, it is essential to know the relationships established between the different research groups working on these lines, to join forces and to add up knowledge.

Compliance with ethical standards

Accepted principles of ethical and professional conduct have been followed.

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Not necessary. The research did not involve human participants.

References

1. Barber BE, Rajahram GS, Grigg MJ, William T, Anstey NM (2017) World malaria report: time to acknowledge Plasmodium knowlesi malaria. Malar J 16:135. https://doi.org/10.1186/s12936-017-1787-y
2. Naing C, Whittaker MA, Nyunt Wai V, Mak JW (2014) Is *Plasmodium vivax* malaria a severe malaria?: A systematic review and meta-analysis. PLoS Negl Trop Dis 8:e3071. https://doi.org/10.1371/journal.pntd.0003071
3. Mendis K, Sina BJ, Marchesini P, Carter R (2001) The neglected burden of Plasmodium vivax malaria. Am J Trop Med Hyg 64:97–106. https://doi.org/10.1179/2047772413Z.000000000179

4. Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J et al (2017) malERA: an updated research agenda for malaria elimination and eradication. PLoS Med. 14:e1002456. https://doi.org/10.1371/journal.pmed.1002456
5. Rieckmann KH, Beaudoin RL, Cassells JS, Sell KW (1979) Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria. Bull World Health Organ 57:261–265
6. Baird JK, Baird JK (1998) Age-dependent characteristics of protection v. susceptibility to Plasmodium falciparum. Ann Trop Med Parasitol 92:367–390
7. Gupta S, Snow RW, Donnelly CA, Marsh K, Newbold C (1999) Immunity to non-cerebral severe malaria is acquired after one or two infections. Nat Med 5:340–343. https://doi.org/10.1038/6560
8. Webster D, Hill AVS (2003) Progress with new malaria vaccines. Bull World Health Organ 81:902–909. https://doi.org/10.1590/S0042-96862003001200009
9. Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P et al (1991) Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg 45:297–308
10. Conway DJ (2015) Paths to a malaria vaccine illuminated by parasite genomics. Trends Genet 31:97–107. https://doi.org/10.1016/j.tig.2014.12.005
11. Osier FH, Mackinnon MJ, Crosnier C, Fegan G, Kamuyu G, Wanaguru M et al (2014) New antigens for a multicomponent blood-stage malaria vaccine. Sci Transl Med 6:247ra102. https://doi.org/10.1126/scitranslmed.3008705
12. Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F et al (2011) A research Agenda to underpin malaria eradication. PLoS Med. 8. https://doi.org/10.1371/journal.pmed.1000406
13. Hoffman SL, Subramanian GM, Collins FH, Venter JC (2002) Plasmodium, human and Anopheles genomics and malaria. Nature 415:702–709. https://doi.org/10.1038/415702a
14. Ames SK, Hysom DA, Gardner SN, Lloyd GS, Gokhale MB, Allen JE (2013) Scalable metagenomic taxonomy classification using a reference genome database. Bioinformatics 29:2253–2260. https://doi.org/10.1093/bioinformatics/btt389
15. Rutgers T, Gordon D, Gathoye AM, Hollingdale M, Hockmeyer W, Rosenberg M et al (1988) Hepatitis B surface antigen as carrier matrix for the repetitive epitope of the circumsporozoite protein of Plasmodium falciparum. Bio/Technology 6:1065–1070. https://doi.org/10.1038/nbt0988-1065
16. Gosling R, von Seidlein L (2016) The future of the RTS,S/AS01 malaria vaccine: an alternative development plan. PLoS Med 13:e1001994. https://doi.org/10.1371/journal.pmed.1001994
17. Phillips MA, Burrows JN, Manyando C, Van Huijsduijnen RH, Van Voorhis WC, Wells TNC (2017) Malaria. Nat Rev Dis Prim 3:17050. https://doi.org/10.1038/nrdp.2017.50
18. Draper SJ, Higgins MK (2018) A new site of attack for a malaria vaccine. Nat Med 24:382–383. https://doi.org/10.1038/nm.4533
19. D'Alessandro HTU, Sorgho H, Valea I (2015) Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet 386:31–45. https://doi.org/10.1016/S0140-6736(15)60721-8
20. Sack B, Kappe SHI, Sather DN (2017) Towards functional antibody-based vaccines to prevent pre-erythrocytic malaria infection. Expert Rev Vaccines 16:403–414. https://doi.org/10.1080/14760584.2017.1295853
21. Tan J, Sack BK, Oyen D, Zenklusen I, Piccoli L, Barbieri S et al (2018) A public antibody lineage that potently inhibits malaria infection through dual binding to the circumsporozoite protein. Nat Med 24:401–407. https://doi.org/10.1038/nm.4513
22. Kisalu NK, Idris AH, Weidle C, Flores-Garcia Y, Flynn BJ, Sack BK et al (2018) A human monoclonal antibody prevents malaria infection by targeting a new site of vulnerability on the parasite. Nat Med 24:408–416. https://doi.org/10.1038/nm.4512
23. Kariu T, Ishino T, Yano K, Chinzei Y, Yuda M (2006) CelTOS, a novel malarial protein that mediates transmission to mosquito and vertebrate hosts. Mol Microbiol 59:1369–1379. https://doi.org/10.1111/j.1365-2958.2005.05024.x
24. Bauza K, Malinauskas T, Pfander C, Anar B, Jones EY, Billker O et al (2014) Efficacy of a Plasmodium vivax malaria vaccine using ChAd63 and modified vaccinia Ankara expressing thrombospondin-related anonymous protein as assessed with transgenic Plasmodium berghei parasites. Infect Immun 82:1277–1286. https://doi.org/10.1128/IAI.01187-13
25. O'Hara GA, Duncan CJA, Ewer KJ, Collins KA, Elias SC, Halstead FD et al (2012) Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. J Infect Dis 205:772–781. https://doi.org/10.1093/infdis/jir850
26. Ewer KJ, O'Hara GA, Duncan CJA, Collins KA, Sheehy SH, Reyes-Sandoval A et al (2013) Protective CD8+ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. Nat Commun 4:2836. https://doi.org/10.1038/ncomms3836
27. Vasconcelos JR, Dominguez MR, Araújo AF, Ersching J, Tararam CA, Bruna-Romero O et al (2012) Relevance of long-lived CD8(+) T effector memory cells for protective immunity elicited by heterologous prime-boost vaccination. Front Immunol 3:358. https://doi.org/10.3389/fimmu.2012.00358
28. Chuang I, Sedegah M, Cicatelli S, Spring M, Polhemus M, Tamminga C et al (2013) DNA prime/adenovirus boost malaria vaccine encoding P. falciparum CSP and AMA1 induces sterile protection associated with cell-mediated immunity. PLoS One 8:e55571. https://doi.org/10.1371/journal.pone.0055571
29. de Camargo TM, de Freitas EO, Gimenez AM, Lima LC, de Almeida Caramico K, Françoso KS et al (2018) Prime-boost vaccination with recombinant protein and adenovirus-vector expressing Plasmodium vivax circumsporozoite protein (CSP) partially protects mice against Pb/Pv sporozoite challenge. Sci Rep 8:1118. https://doi.org/10.1038/s41598-017-19063-6
30. Rampling T, Ewer KJ, Bowyer G, Bliss CM, Edwards NJ, Wright D et al (2016) Safety and high level efficacy of the combination malaria vaccine regimen of RTS,S/AS01 $_B$ with chimpanzee adenovirus 63 and modified vaccinia Ankara vectored vaccines expressing ME-TRAP. J Infect Dis 214:772–781. https://doi.org/10.1093/infdis/jiw244
31. Epstein JE, Paolino KM, Richie TL, Sedegah M, Singer A, Ruben AJ et al (2017) Protection against Plasmodium falciparum malaria by PfSPZ vaccine. JCI Insight 2:e89154. https://doi.org/10.1172/jci.insight.89154
32. Clyde DF, McCarthy VC, Miller RM, Hornick RB (1973) Specificity of protection of man immunized against sporozoite-induced falciparum malaria. Am J Med Sci 266:398–403
33. Lyke KE, Ishizuka AS, Berry AA, Chakravarty S, DeZure A, Enama ME et al (2017) Attenuated PfSPZ vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. Proc Natl Acad Sci U S A 114:2711–2716. https://doi.org/10.1073/pnas.1615324114
34. Mordmüller B, Surat G, Lagler H, Chakravarty S, Ishizuka AS, Lalremruata A et al (2017) Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. Nature 542:445–449. https://doi.org/10.1038/nature21060
35. Mikolajczak SA, Lakshmanan V, Fishbaugher M, Camargo N, Harupa A, Kaushansky A et al (2014) A next-generation genetically attenuated Plasmodium falciparum parasite created by triple gene deletion. Mol Ther 22:1707–1715. https://doi.org/10.1038/mt.2014.85
36. Coelho CH, Yai J, Doritchamou A, Zaidi I, Duffy PE (2017) Advances in malaria vaccine development: report from the 2017

malaria vaccine symposium. npj Vaccines 2:34. https://doi.org/10.1038/s41541-017-0035-3

37. Cohen S, McGregor IA, Carrington S (1961) Gamma-globulin and acquired immunity to human malaria. Nature 192:733–737. https://doi.org/10.1038/192733a0
38. Satchwell TJ (2016) Erythrocyte invasion receptors for Plasmodium falciparum: new and old. Transfus Med 26:77–88. https://doi.org/10.1111/tme.12280
39. Douglas AD, Williams AR, Illingworth JJ, Kamuyu G, Biswas S, Goodman AL et al (2011) The blood-stage malaria antigen PfRH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. Nat Commun 2:601. https://doi.org/10.1038/ncomms1615
40. Douglas AD, Baldeviano GC, Lucas CM, Lugo-Roman LA, Crosnier C, Bartholdson SJ et al (2015) A PfRH5-based vaccine is efficacious against heterologous strain blood-stage Plasmodium falciparum infection in aotus monkeys. Cell Host Microbe 17:130–139. https://doi.org/10.1016/j.chom.2014.11.017
41. Payne RO, Silk SE, Elias SC, Miura K, Diouf A, Galaway F et al (2017) Human vaccination against RH5 induces neutralizing antimalarial antibodies that inhibit RH5 invasion complex interactions. JCI Insight 2:96381
42. Wright KE, Hjerrild KA, Bartlett J, Douglas AD, Jin J, Brown RE et al (2014) Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies. Nature 515:427–430. https://doi.org/10.1038/nature13715
43. Srinivasan P, Christian Baldeviano G, Miura K, Diouf A, Ventocilla JA, Leiva KP et al (2017) A malaria vaccine protects aotus monkeys against virulent Plasmodium falciparum infection. npj Vaccines 2:14. https://doi.org/10.1038/s41541-017-0015-7
44. Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK et al (2011) A field trial to assess a blood-stage malaria vaccine. N Engl J Med 365:1004–1013. https://doi.org/10.1056/NEJMoa1008115
45. Raj DK, Nixon CP, Nixon CE, Dvorin JD, DiPetrillo CG, Pond-Tor S et al (2014) Antibodies to PfSEA-1 block parasite egress from RBCs and protect against malaria infection. Science 344:871–877. https://doi.org/10.1126/science.1254417
46. Healer J, Cowman AF, Kaslow DC, Birkett AJ (2017) Vaccines to accelerate malaria elimination and eventual eradication. Cold Spring Harb Perspect Med 7:a025627. https://doi.org/10.1101/cshperspect.a025627
47. Radtke AJ, Anderson CF, Riteau N, Rausch K, Scaria P, Kelnhofer ER et al (2016) Adjuvant and carrier protein- dependent T-cell priming promotes a robust antibody response against the *Plasmodium falciparum* Pfs25 vaccine candidate. https://doi.org/10.1038/srep40312
48. MacDonald NJ, Nguyen V, Shimp R, Reiter K, Herrera R, Burkhardt M et al (2016) Structural and immunological characterization of recombinant 6-cysteine domains of the Plasmodium falciparum sexual stage protein Pfs230. J Biol Chem 291:19913–19922. https://doi.org/10.1074/jbc.M116.732305
49. Molina-Cruz A, Garver LS, Alabaster A, Bangiolo L, Haile A, Winikor J et al (2013) The human malaria parasite Pfs47 gene mediates evasion of the mosquito immune system. Science 340(80):984–987. https://doi.org/10.1126/science.1235264
50. Molina-Cruz A, Canepa GE, Kamath N, Pavlovic NV, Mu J, Ramphul UN et al (2015) *Plasmodium* evasion of mosquito immunity and global malaria transmission: the lock-and-key theory. Proc Natl Acad Sci 112:15178–15183. https://doi.org/10.1073/pnas.1520426112
51. Caignard G, Eva MM, Van Bruggen R, Eveleigh R, Bourque G, Malo D et al (2014) Mouse ENU mutagenesis to understand immunity to infection: methods, selected examples, and perspectives. Genes (Basel) 5:887–925. https://doi.org/10.3390/genes5040887
52. Montoya FG, Baños R, Meroño JE, Manzano-Agugliaro F (2016) The research of water use in Spain. J Clean Prod 112:4719–4732. https://doi.org/10.1016/j.jclepro.2015.06.042
53. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW et al (2002) Genome sequence of the human malaria parasite Plasmodium falciparum. Nature 419:498–511. https://doi.org/10.1038/nature01097
54. Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD et al (2002) A proteomic view of the Plasmodium falciparum life cycle. Nature 419:520–526. https://doi.org/10.1038/nature01107