

Evaluation of *Plasmodium berghei* Models in Malaria Research

Sarah Oluwatobi Otun^{1,*}, Richard Graca¹, Olalekan Onisuru¹, Ikechukwu Achilonu¹

¹Protein Structure-function Unit, Department of Molecular and Cell Biology, University of Witwatersrand, Johannesburg, South Africa

*Correspondence should be addressed to Sarah Oluwatobi Otun, sarahholabamiji@gmail.com

Received date: June 15, 2024, Accepted date: July 12, 2024

Citation: Otun SO, Graca R, Onisuru O, Achilonu I. Evaluation of *Plasmodium berghei* Models in Malaria Research. J Cell Signal. 2024;5(3):96-113.

Copyright: © 2024 Otun SO, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

As a key model organism for studying malaria, *Plasmodium berghei* provides essential information on pathophysiology, host immunological responses, and possible targets for treating this debilitating illness. This narrative review compares other *Plasmodium* species models, including *P. falciparum* and *P. vivax*, to assess the evolution, traits, and applicability of *P. berghei* models. We discuss the development of *P. berghei* research and its historical background, emphasizing its advantages in genetic modification and experimental accessibility. The difficulties and constraints these models have, as well as new methods and prospects for their development in the future, are discussed. This study highlights the potential of *P. berghei* models for translating malaria research and creating efficient control methods by combining important discoveries and comparative studies. This thorough assessment seeks to maximize the use of *P. berghei* in the battle against malaria and to direct future research endeavors.

Keywords: *Plasmodium berghei*, Cellular signalling, Malaria research, Animal models, Drug discovery, Anti-microbial compounds

Introduction

Malaria continues to be one of the biggest threats to world health, especially in areas with little access to medical treatment [1]. Millions of individuals contract malaria every year, which is primarily responsible for a significant burden of illness and mortality [2]. The disease is caused by *Plasmodium* parasites, which are spread by infected mosquito bites. *Plasmodium falciparum* and *Plasmodium vivax* are the two species of *Plasmodium* that cause malaria in humans the most frequently [3]. However, animal models are crucial to studying malaria pathophysiology, immunology, and therapy; *P. berghei* is one of the most researched species.

The single-celled parasite *P. berghei* causes rodent malaria in thicket rats in Central Africa [4]. It was one of the four *Plasmodium* species reported in African murine rodents; the other three are *P. chabaudi*, *P. vinckei*, and *P. yoelii* [5]. It is a widely used model organism in studying human malaria because of its capacity to infect rats and its simplicity of genetic modification [6]. In malaria research, animal models

are essential and indispensable because, while *in vitro* studies provide valuable insights, they often need to capture the intricate interplay between the parasite, the host immune system, and the vector. This is where animal models, particularly rodent models like *P. berghei*, come into play, offering a more comprehensive approach [7]. Thus, a thorough assessment of *P. berghei*'s models is necessary to fully harness their potential and ensure the reliability and repeatability of study findings.

P. berghei models are significant in several vital areas of malaria research. Researchers may investigate the effectiveness of possible medications and vaccinations by using these models to analyze the parasite's whole life cycle, including stages in the liver and blood. Furthermore, *P. berghei*'s genetic tractability makes it easier to investigate gene function and find new pharmaceutical targets. The knowledge gathered from *P. berghei* models has been invaluable in comprehending the intricate biology of malaria and creating effective defense mechanisms. Although *P. berghei* is a valuable model organism, there are also drawbacks; for instance, the degree to which findings may be directly applied to human malaria

may be limited by distinctions between malaria in rodents and humans, including differences in immune response and pathogenesis. It takes a sophisticated grasp of the model's advantages and disadvantages and continual work to overcome these constraints to improve its applicability to human malaria.

This review aims to provide a comprehensive overview of *P. berghei* as a model organism for malaria research. By critically evaluating existing research, we seek to identify the benefits, drawbacks, and potential avenues for further developing *P. berghei* models (Figure 1). The outcomes of this study are expected to enhance our understanding of malaria pathogenesis, expedite the discovery of new drugs, and contribute to the development of effective preventive measures against this life-threatening disease, thereby significantly advancing malaria research.

Plasmodium berghei as a Model Organism

Vincke and Lips made the first discovery and isolation of *P. berghei* in the Belgian Congo in 1940 [8]. This ground-breaking discovery established the basis for further studies using *P. berghei* as a model organism for malaria investigations. For instance, the work of Scheller *et al.* [9] is particularly noteworthy among the pioneering studies in rat malaria research. They tested the susceptibility of different strains of mice to hepatic infection with *P. berghei*, providing early proof of *P. berghei*'s potential as a model for researching malaria in rats. This study, along with others, has paved the way for the extensive use of *P. berghei* as a model organism in malaria research.

The works of Matsuoka *et al.* [10] and Jin *et al.* [11] further

cemented *P. berghei*'s status as a laboratory model organism. They reported that *P. berghei* was successfully transferred to mice by mosquito bites [10,11]. Because of these critical findings, *P. berghei* infections could be sustainably maintained in laboratory settings, leading to its widespread use as a model organism for malaria research. In addition, mice are valuable models for investigating malaria because of their biology [9], which includes the liver and blood phases of the *P. berghei* lifecycle (Figure 2). This compatibility enables thorough investigations of the infection dynamics, pathophysiology, and immune responses by allowing researchers to witness the parasite lifecycle within a single host [12]. Furthermore, the availability of well-characterized inbred strains and the ease with which mice can be genetically modified improve the repeatability and dependability of research results, allowing for in-depth analyses of the pathophysiology of malaria and the assessment of possible treatments and vaccines.

This figure illustrates the lifecycle of *P. berghei*, including stages of mosquito transmission, liver-stage development, and blood-stage replication, providing an overview of the parasite's biology.

Consequently, *P. berghei*'s research in malaria studies has evolved, starting from the early genetic characterization by determining the parasite's genetic composition [13]. Peters and others conducted studies in the 1960s that revealed several *P. berghei* strains with differing drug susceptibilities and levels of virulence [14]. This work established the framework for further studies of the genetic factors influencing parasite phenotypes. Genetic modification techniques (Figure 3) were used as the next step in the evolution of malaria research. In the 1990s, transgenic methods transformed research

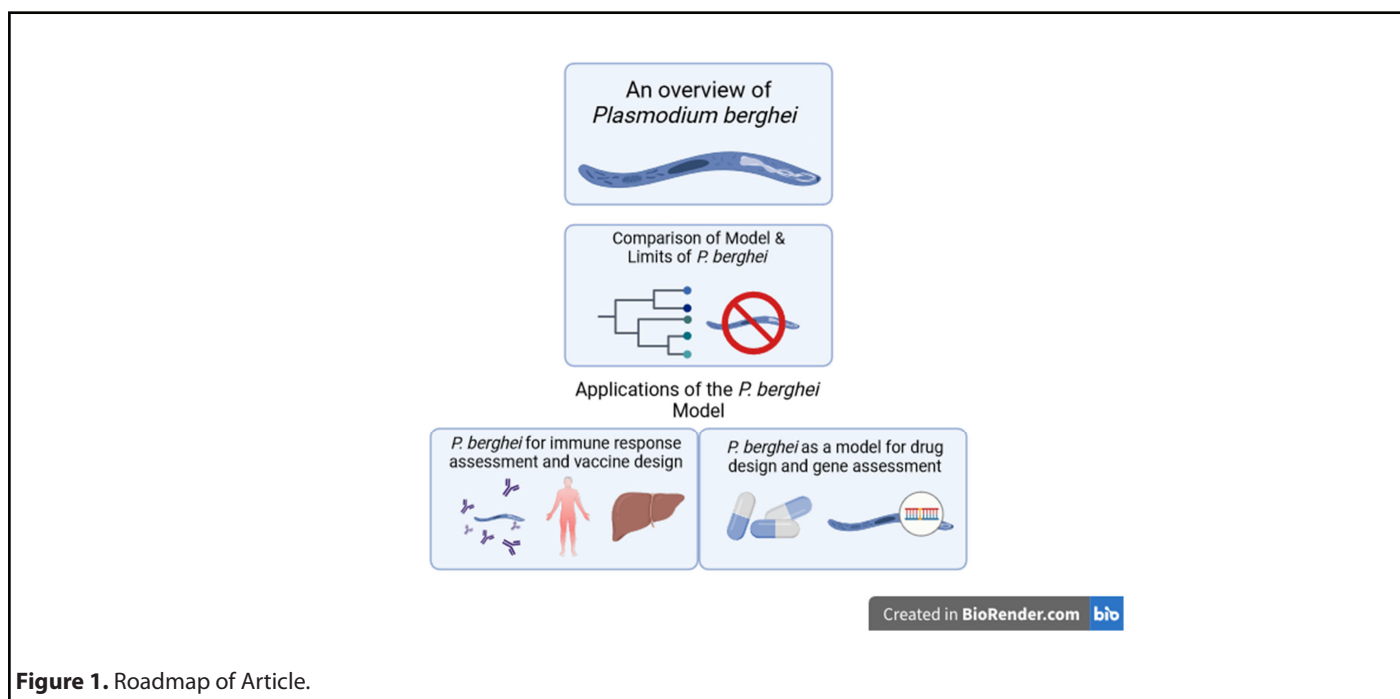


Figure 1. Roadmap of Article.

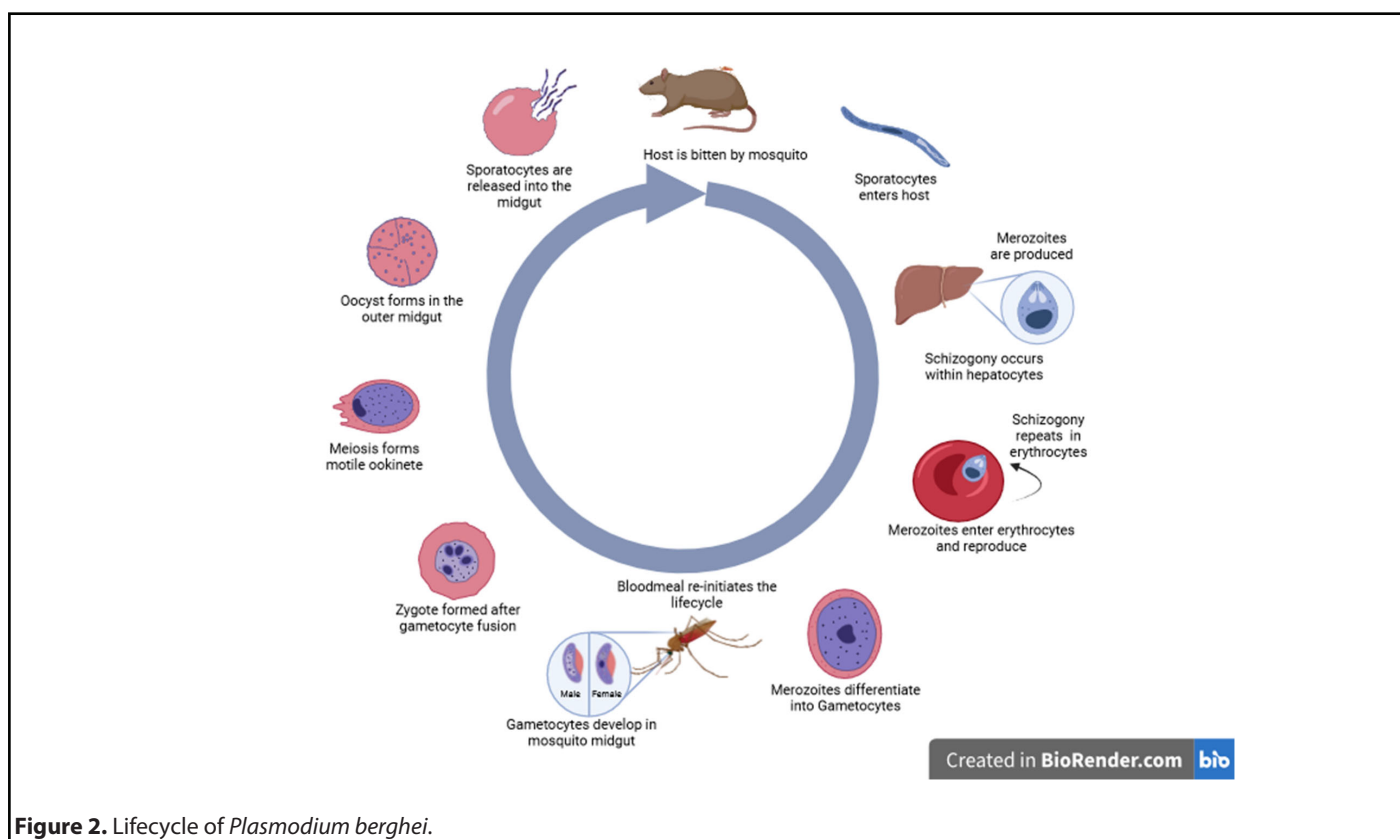


Figure 2. Lifecycle of *Plasmodium berghei*.

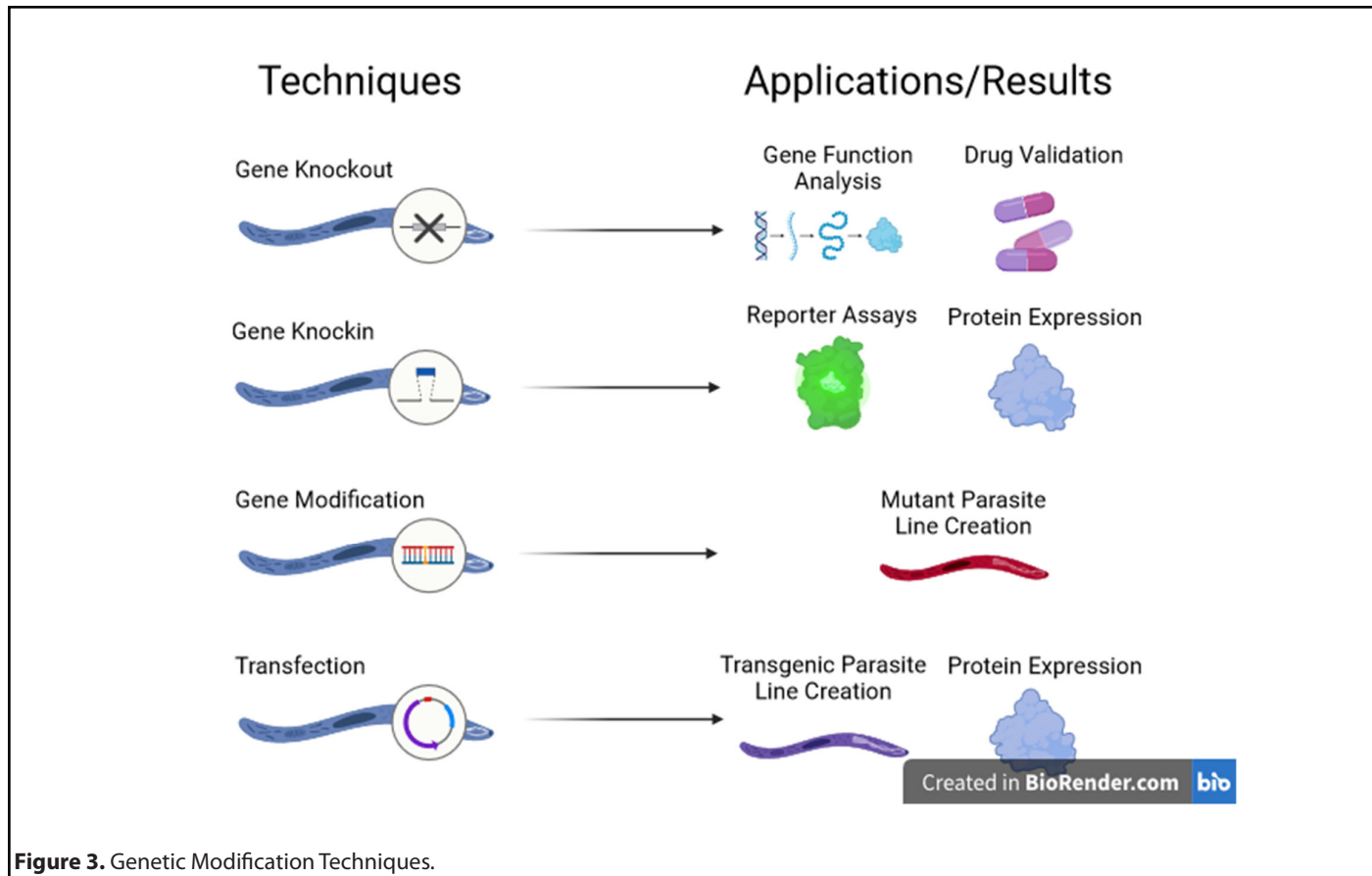


Figure 3. Genetic Modification Techniques.

on *P. berghei* by allowing for precise genetic modification of the pathogen [15]. Essential developments, such as the transfection techniques developed by Waters *et al.* [15] made the creation of genetically engineered *P. berghei* strains with changed characteristics possible [15]. These methods increased the usefulness of *P. berghei* as a model organism for researching the pathophysiology of malaria and assessing potential treatments. **Figure 2** summarizes the different genetic modifying techniques used in *P. berghei*'s malaria research.

Gene knockouts, knock-ins, alterations, and examples of their uses and results may all be shown graphically in this image, providing information on the numerous genetic modification approaches utilized in *P. berghei* models.

Furthermore, integrating *P. berghei* research with omics technologies, including transcriptomics, proteomics, and genomes, has made unprecedented insights into parasite biology and host-parasite interactions possible. Caldelari and colleagues conducted studies using transcriptomics analysis to clarify gene expression dynamics at various phases of the *P. berghei* life cycle [16]. These omics-based methods have helped identify new therapeutic targets and improved our knowledge of the pathophysiology of malaria [17]. Lastly, *P. berghei* has been an essential component of attempts to create vaccines to prevent malaria. According to research by Moita and associates, irradiated *P. berghei* sporozoites effectively generate protective immunity against recurrent malaria infections [18]. This groundbreaking research aided in the continuous quest to produce a vaccine against malaria and cleared the path for developing vaccines based entirely on parasites.

Characteristics of *Plasmodium berghei* as a Model in Malaria Research

With its ability to provide many insights into the complex dynamics of malaria pathogenesis, immunology, and medication development, *P. berghei* has become a key model organism in malaria research [19]. Its importance comes from a combination of host specificity, genetic tractability, and experimental modification amenability, which makes it a priceless tool for researching many aspects of malaria biology.

P. berghei is known for its genetic diversity, which results in various strains displaying different traits, including virulence, drug sensitivity, and transmission efficiency [20]. Different *P. berghei* strains were found in early studies by Yoeli [21], which also provided the framework for further research into these strains' genetic composition and phenotypic differences. Furthermore, advances in transgenic techniques have made a precise genetic modification of *P. berghei* possible through gene deletion, knock-in, and tagging tactics (**Table 1**). This genetic flexibility has made it easier to research the biology of parasites and to identify critical molecular pathways that underlie the pathogenesis of malaria [22].

Although *P. berghei* has genetic characteristics and host specificity, it primarily infects rodents, especially mice, making it possible to study host-parasite interactions in a mammalian host. *P. berghei* infections cause immunological solid responses in the host, making this host specificity crucial for researching the immune response dynamics [23]. Researchers have used mouse *P. berghei* infections to study immunopathogenesis, immunity mechanisms, and vaccine-induced protection [18,19,24,25].

Table 1. Summary of Genetic Manipulation Technique.

Genetic Manipulation Technique	Description	Applications
Gene Knockout	Removal or disruption of a specific gene in the parasite genome	Study of gene function, validation of drug targets
Gene Knock-in	Insertion of a foreign gene into the parasite genome	Introduction of reporter genes, expression of exogenous proteins
Gene Modification	Alteration of specific gene sequences or regulatory elements	Generation of mutant parasite lines with modified phenotypes
CRISPR-Cas9	Genome editing tool using RNA-guided nucleases to induce double-stranded breaks in DNA	Precise gene editing, multiplex targeting of multiple genes
Transfection	Introduction of foreign DNA into the parasite via electroporation or other methods	Expression of exogenous genes, creation of transgenic parasite lines

This table compiles the standard genetic manipulation methods—gene knockout, knockin, modification, CRISPR-Cas9 editing, and transfection—employed in Plasmodium berghei models.

Research on *P. berghei* is notable for its application in studying malaria pathogenesis and illness development. The typical symptoms of malaria, such as fever, anemia, and organ damage, are caused by *P. berghei*'s fast reproduction cycle within erythrocytes [26]. A helpful model for examining the pathophysiology of cerebral malaria is provided by some strains of *P. berghei*, including ANKA, which cause cerebral malaria in mice, emulating severe human malaria pathology [27]. To further support drug development efforts, *P. berghei* infections in mice provide a platform for assessing antimalarial drug pharmacokinetics, resistance mechanisms, and effectiveness [28]. Overall, the qualities of *P. berghei* as a model organism have advanced our understanding of malaria and led to important discoveries on the parasite's biology, host immunological responses, and treatment development.

Utility of *Plasmodium berghei* Models in Malaria Research

Studying malaria pathogenesis

Plasmodium berghei models, which provide a stable platform to study the complex interactions between the parasite and its host, have changed our knowledge of the pathophysiology of malaria. These models mostly use mice as hosts and are a vital resource in critical research since they closely resemble human malaria infection. First, *P. berghei* models have the crucial advantage of accurately simulating the malaria parasite's whole life cycle inside a mammalian host [7]. The ability to track infection from its initial liver stage to its later blood stage allows researchers to conduct in-depth studies of the kinetics of parasite replication, the host immune system, and tissue-specific disease [29].

Moreover, *P. berghei* infections in mice elicit strong immunological responses, making them an excellent model organism for researching host-parasite relationships and immune evasion techniques [30]. Researchers can unravel the processes behind immunopathology, vaccine-induced protection, and protective immunity by analyzing the dynamics of innate and adaptive immune responses during malaria infection. This information is essential for creating cutting-edge treatments to fight malaria, such as vaccinations and immunomodulatory medications [31]. Ultimately, by accurately simulating essential components of natural malaria in laboratory settings, *P. berghei* models have made substantial progress towards our knowledge of malaria pathophysiology.

Investigating host immune responses

The complex interactions between the malaria parasite and the human immune system have been primarily clarified because of *P. berghei* models [32]. These models have also provided vital insights into immunopathology, protective immunity, and the processes behind vaccine development. In particular, they use mice as hosts and provide a controlled

setting for researching the dynamic immunological responses triggered by malaria infection [7,32]. This is one of their main benefits since *P. berghei* models may elicit strong and consistent immune responses in mice that closely resemble those seen in human malaria infections [33]. Research has indicated that infections with *P. berghei* elicit innate and adaptive immunological responses typified by activating T and B lymphocytes, natural killer cells, dendritic cells, and macrophages [33-37]. The immune cells coordinate a complex response to prevent parasite reproduction, eliminate contaminated red blood cells, and create durable resistance to future infections [38].

Researchers have analyzed the kinetics of innate immune responses during malaria infection using *P. berghei* models [39]. Gamma interferon (IFN- γ) is essential for controlling the immune response to *P. berghei*, while mice lacking IFN- γ showed heightened susceptibility to infection and decreased parasite clearance [40]. Subsequent studies have demonstrated that the innate immune response to malaria parasites is shaped by Toll-like receptors (TLRs), inflammasomes, and cytokine signaling pathways [41,42].

Models of *P. berghei* infection have shed light on the dynamics of adaptive immune responses and innate immunity against malaria infection [43]. Studies have shown that antigen-specific T and B cells are activated and proliferate during infection with *P. berghei*, producing effector T cell responses and antibodies specific to the parasite [44]. These adaptive immune responses make controlling parasite multiplication and averting severe disease symptoms possible. Additionally, *P. berghei* models are valuable tools for analyzing immune responses elicited by vaccinations and determining the effectiveness of potential malaria vaccines [45,46]. Researchers can examine vaccine-induced immunity's immunogenicity, protective efficacy, and persistence using genetically engineered parasite strains expressing vaccine antigens or immunomodulatory substances in preclinical settings [47]. This strategy has made the creation of innovative vaccine formulations that target several phases of the malaria parasite life cycle more accessible [48]. **Table 2** summarizes the related genes and factors involved in the immune response induced by malaria parasites.

Furthermore, using *P. berghei* models makes assessing vaccine-induced cellular immune responses, including T cell responses and cytokine production, possible [53]. After vaccination, researchers can evaluate the growth and activation of antigen-specific T cells using methods such as intracellular cytokine staining (ICS) and enzyme-linked immunospot (ELISpot) tests [52,54]. These investigations shed light on how potential vaccines induce cellular immunity and how they function to mediate protective immunity against malaria parasites [50,55]. Lastly, *P. berghei* models make it easier to conduct long-term research to evaluate how long-lasting immune responses elicited by vaccination are [55,56].

Table 2. Related genes and factors involved in the immune response induced by malaria parasites.

Gene/Factor	Function	References
IFN- γ (Interferon-gamma)	Activates macrophages and enhances antigen presentation	[40]
TNF- α (Tumor Necrosis Factor-alpha)	Mediates inflammation and immune response	[47]
IL-10 (Interleukin-10)	Regulates immune response, anti-inflammatory effects	[43]
IL-12 (Interleukin-12)	Promotes differentiation of T cells into Th1 cells	[46]
CD8+ T cells	Directly kill infected cells and secrete cytokines	[37]
CD4+ T cells	Help B cells and CD8+ T cells secrete cytokines	[36]
TGF- β (Transforming Growth Factor-beta)	Regulates immune response and promotes tolerance	[49]
NK cells (Natural Killer cells)	Lyse infected cells and secrete IFN- γ	[50]
TLR (Toll-like Receptors)	Recognize pathogen-associated molecular patterns and activate innate immunity.	[51]
FOXP3 (Forkhead box P3)	Key marker for regulatory T cells (Tregs)	[52]

Researchers can determine if booster shots are necessary to maintain long-term protective immunity and measure the durability of antigen-specific immune responses in vaccinated mice by tracking them at different intervals after vaccination [50,51]. This research influences the adjustment of vaccination tactics and dose schedules to improve vaccine durability and efficacy [57].

Hence, our knowledge of the host immune responses during malaria infection has greatly benefited from using *P. berghei* models. These models may help scientists better understand the interplay between innate and adaptive immunity, pinpoint immunological correlates of protection, and hasten the creation of potent malaria vaccines and immunotherapies.

Exploring parasite biology and drug targets

Plasmodium berghei models are crucial for better understanding malaria parasites' complex biology and pinpointing possible therapeutic targets. These models have allowed scientists to significantly advance their knowledge of the molecular pathways necessary for parasite survival, virulence, cell biology, and metabolism. Because *P. berghei* models are genetically manipulable, researchers may study the role of specific genes and pathways in parasite biology, one of the models' main benefits [58]. Using gene knockdown, knock-in, and tagging techniques, researchers may clarify the function of specific genes in parasite growth, interactions with hosts, and medication resistance mechanisms [59].

Research employing *P. berghei* models has clarified numerous facets of parasite biology, including metabolism, cell cycle control, and host cell invasion. Studies examining

the function of vital enzymes in the parasite's metabolic processes, including glycolysis, the tricarboxylic acid cycle, and nucleotide biosynthesis, have revealed possible targets for medication intervention [60]. Additionally, *P. berghei* models have helped identify and validate new malaria therapy medication candidates [61]. Researchers can assess potential drugs' pharmacokinetics, safety, and effectiveness *in vivo* using high-throughput screening experiments conducted on *P. berghei*-infected mice. By evaluating parasitemia levels, survival rates, and histopathological changes in infected animals treated with prospective medicines, researchers can find interesting leads for additional preclinical and clinical development [62].

Figure 4 displays several drug screening tests in *P. berghei* models, methods, and findings from high-throughput screening platforms, *in vitro* assays, and *in vivo* effectiveness investigations.

Furthermore, *P. berghei* models facilitate the investigation of drug resistance mechanisms and the creation of resistance-busting tactics [63]. Researchers can determine the genetic determinants of drug resistance by using combination medicines to stop or postpone the development of resistance, tracking the formation of resistance mutations, and subjecting parasites to suboptimal drug concentrations [64]. Finally, models of *P. berghei* offer a potent platform for investigating parasite biology and discovering new therapeutic targets for malaria treatment. By utilizing genetic editing methods and high-throughput screening tests, researchers may better understand the intricate relationships between parasites and hosts, identify novel therapeutic options, and advance efforts to eradicate malaria worldwide.

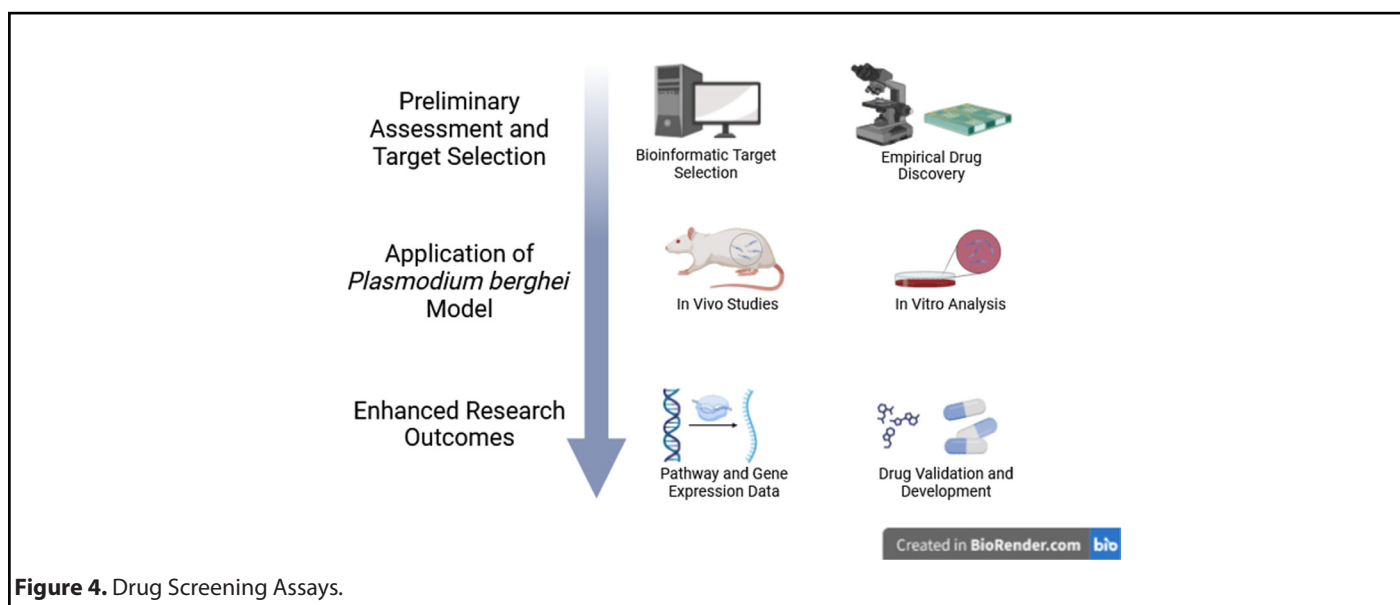


Figure 4. Drug Screening Assays.

Plasmodium berghei as a tool for vaccine development

The rodent malaria pathogen *P. berghei* helps find new drugs to treat malaria. Its genetic tractability, simplicity of maintenance, and resemblance to human malaria parasites make studying therapeutic effectiveness and mechanisms of action easier [65]. Utilizing *P. berghei* models, scientists may find lead compounds, carry out systematic screens, and progress the creation of innovative antimalarial treatments. Notwithstanding obstacles, *P. berghei* continues to play a crucial role in advancing drug research initiatives to combat the enduring menace of malaria [66].

P. berghei models are essential tools for assessing the effectiveness of malaria vaccinations. They offer vital information on immune responses triggered by vaccines and protective immunity [67]. These models enable researchers

to do thorough preclinical evaluations of vaccine candidates, allowing them to make well-informed judgements about moving such candidates forward into clinical trials and ultimately deploying them in malaria-endemic areas. Testing the effectiveness of vaccines is a crucial use of *P. berghei* models in vaccine research. The degree of protection provided by candidate vaccines is evaluated in mice by exposing them to live *P. berghei* parasites after vaccination. The vaccine's effectiveness in preventing malaria infection and illness development may be assessed by researchers using this challenge model by evaluating several factors, such as parasitemia levels, disease severity, and survival rates [68].

Figure 5 shows vaccination regimens, challenge experiments, and results from investigations on the effectiveness of vaccine candidates in protecting against *P. berghei* models.

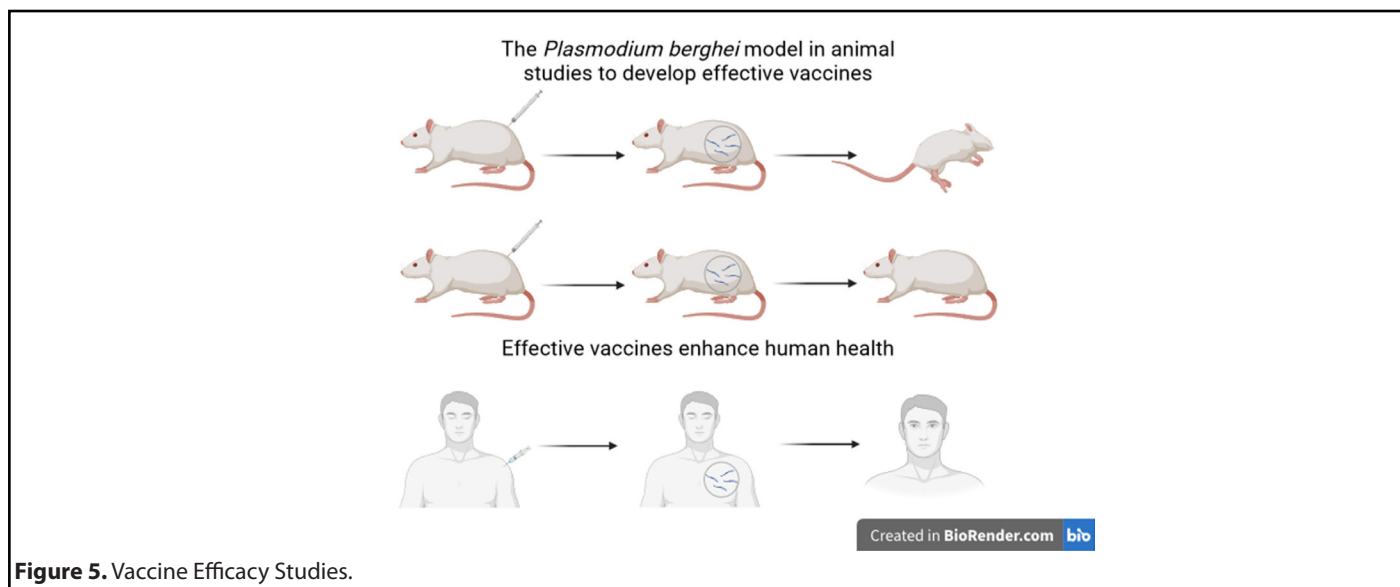


Figure 5. Vaccine Efficacy Studies.

P. berghei models also make assessing immunological responses elicited by vaccinations easier, offering mechanistic insights into vaccine effectiveness. By examining vaccine-induced immune responses, researchers can determine the relationship between protection against malaria infection and specific immunological indicators, such as antibody titers, T cell responses, and cytokine profiles [69]. These investigations inform the optimization of vaccination formulations and advance our knowledge of vaccine-induced protective mechanisms.

Furthermore, vaccination effectiveness against various phases of the malaria parasite life cycle may be evaluated using *P. berghei* models [70]. Vaccines directed against pre-erythrocytic stages seek to avoid infection by preventing the growth of parasites in the liver [71]. On the other hand, blood-stage vaccinations are designed to stop the spread of the disease and stop parasite reproduction after erythrocyte invasion [53]. Researchers can assess the effectiveness of vaccinations targeting particular parasite antigens and phases of the life cycle by using *P. berghei* animals that simulate diverse stages of malaria infection [69,71].

Vaccine candidates tested in *Plasmodium berghei* models

Plasmodium berghei models were used for testing to evaluate the effectiveness and potential for future development of specific vaccine candidates. Designed to improve their immunogenicity and protective qualities, these candidates are tailored to target specific antigens. An immune response directed against the merozoite stage is elicited by the protein

subunit vaccination MSP-1 (Merozoite Surface Protein-1), which may prevent parasite multiplication [72]. Due to its significant genetic heterogeneity, adjuvants could be necessary to increase immunogenicity, which might result in strain-specific immunity. Preclinical research is still being done on MSP-1-based vaccinations.

The recombinant protein vaccine AMA-1 (Apical Membrane Antigen-1) demonstrates strong immunogenicity and partial protection. It is now undergoing optimization efforts in the preclinical stage despite its notable polymorphism impacting the breadth of the immune response and manufacturing issues [73]. The viral vector vaccine CSP (Circumsporozoite Protein) has the potential to generate potent immune responses and defense against sporozoite assault [74]. Pre-existing immunity to the viral vector and worries regarding long-term safety are obstacles. Human studies are presently being conducted on CSP-based vaccinations. Lastly, a DNA vaccination called RAP1 (Rhoptry-Associated Protein-1) is intended to stimulate both humoral and cellular immunity [75]. Despite being usually less immunogenic and may need several doses or adjuvants, it is still in the preclinical stage, and research is being done to increase its efficacy. These vaccine candidates demonstrate the variety of approaches and continuous work being done to create potent malaria vaccinations using *P. berghei* models.

Table 3 summarizes the antigen targets, vaccine formulations, protective effectiveness data (such as survival rate and parasitemia decrease), and pertinent references of vaccine candidates tested in *P. berghei* models.

Table 3. Summary of vaccine candidate tested in *Plasmodium berghei* models.

Vaccine Candidate	Antigen Target	Vaccine Formulation	Current Status	Advantages	Disadvantages	References
MSP-1	Merozoite surface protein-1	Protein subunit vaccine	Preclinical	Induces immune response targeting merozoite stage	High genetic variability may require adjuvants	[72]
AMA-1	Apical membrane antigen-1	Recombinant protein vaccine	Preclinical	Strong immunogenicity, partial protection	Significant polymorphism, production and scalability challenges	[73]
CSP	Circumsporozoite protein	Viral vector vaccine	Human trials	Robust immune responses, protection against sporozoite challenge	Pre-existing immunity to viral vector, long-term safety concerns	[74]
RAP1	Rhoptry-associated protein-1	DNA vaccine	Preclinical	Easy to produce, induces both humoral and cellular immunity	Lower immunogenicity may require multiple doses or adjuvants	[75]

Therefore, *P. berghei* models are essential for assessing the effectiveness of vaccinations against malaria because they offer a flexible and manageable framework for preclinical assessment. By utilizing these models, researchers may evaluate the immune responses elicited by vaccinations, ascertain the effectiveness of preventative measures against malaria infections, and progress the creation of vaccines to prevent and manage this grave illness [68,71].

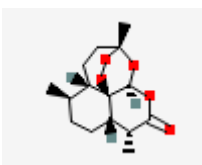
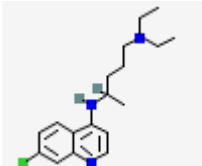
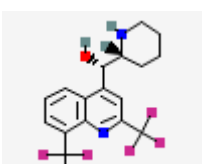
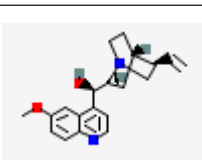
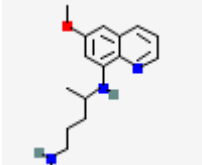
Screening for antimalarial compounds

The rodent malaria pathogen *P. berghei* helps find new drugs to combat malaria. Its short life span, genetic manipulation capacity, and simplicity of maintenance in lab environments make it a perfect model organism for researching the pathophysiology of malaria and assessing the effectiveness

of prospective antimalarial drugs [76]. One of *P. berghei*'s primary benefits for drug development is its adaptability for high-throughput screening (HTS) of chemical libraries. Using *P. berghei*-infected mice, HTS experiments enable the quick assessment of many compounds for their antimalarial potential [77]. To determine if test compounds are effective in lowering parasite burden, these experiments usually entail giving test compounds to infected mice and tracking their levels of parasitemia over time [78]. Interestingly, lead compounds with vigorous antimalarial activity have been found through these screens.

P. berghei models have been used to assess several antimalarial drugs, such as new chemical scaffolds, artemisinin derivatives, and chloroquine analogues. For example, a great deal of research has been done in mice infected with

Table 4. Summary of Anti-malaria drug screening.

Compound Name	Chemical Structure (PubChem)	Mechanism of Action	References
Artemisinin		Inhibition of heme detoxification	[22,85]
Chloroquine		Inhibition of heme polymerization	[14,86]
Mefloquine		Disruption of parasite membrane	[87]
Quinine		Inhibition of heme detoxification	[88]
Primaquine		Clearance of liver-stage hypnozoites	[89]

This table provides an overview of the antimalarial drugs studied in *Plasmodium berghei* models, along with information on their chemical compositions, modes of action, effectiveness metrics like IC₅₀ (half-maximal inhibitory concentration) and ED₅₀ (median effective dosage), and pertinent references.

P. berghei on the pharmacokinetics, mechanism of action, and potential for combination therapy of artemisinin and its derivatives, which are well-known for their quick clearance of parasites and effectiveness against strains that are resistant to multiple drugs [79,80]. Analogues of chloroquine, formerly thought to be first-line antimalarials, have also been evaluated in *P. berghei* models to find analogues with better safety profiles and action against parasites resistant to drugs [81]. Similarly, *P. berghei* models have proven useful in assessing new therapeutic candidates, such as chemicals originating from plants, natural products, synthetic compounds with various chemical structures, and traditional small-molecule medications [82]. These screens frequently involve giving test compounds to infected mice, tracking parasitemia levels, estimating survival rates, and performing histological analyses to evaluate therapeutic effectiveness, safety, and mechanism of action [83]. This research has found new chemical entities with vigorous antimalarial activity and development potential.

Additionally, *P. berghei* models have been used in target-based drug discovery methods, identifying and evaluating specific molecular targets inside the parasite as possible drug targets [84]. Using genetic manipulation techniques such as gene knockout and knockdown, researchers may determine if target genes are necessary and how gene disruption affects parasite survival and virulence [13,59]. By combining target-based screening with phenotypic tests in *P. berghei* models, Researchers can identify and evaluate new drug targets to develop antimalarial drugs.

Therefore, *P. berghei* helps speed up drug development efforts to screen antimalarial drugs. It's a model organism of great value for research on medication effectiveness, mechanism of action, and resistance because of its genetic modification capacity, appropriateness for HTS experiments, and relevance to malaria pathophysiology [87,88]. Researchers continue to find and evaluate new antimalarial compounds using *P. berghei* models, which opens the door for creating next-generation malaria treatments.

Pharmacokinetic and pharmacodynamic studies

The development of antimalarial medications heavily relies on pharmacokinetic (PK) and pharmacodynamic (PD) research, which offer valuable information on therapeutic effectiveness and toxicity profiles as well as drug absorption, distribution, metabolism, and excretion [90]. These investigations are crucial for determining the pharmacokinetic characteristics of potential drugs, comprehending how they interact with the parasite, and maximizing the effectiveness of dosage schedules in *P. berghei* models.

For instance, analytical methods like liquid chromatography-mass spectrometry (LC-MS) or high-performance liquid chromatography (HPLC) are used in PK studies on *P. berghei*-infected mice to measure the concentrations of

test compounds in blood, plasma, or tissues over time [91]. Test compounds are administered via oral, intravenous, or subcutaneous routes to help with dosage selection and formulation development; these investigations offer vital data on drug bioavailability, half-life, clearance, and tissue distribution [92]. PK studies also evaluate the effects of variables on drug absorption and disposition, including meal consumption, the co-administration of other medications, and illness conditions [84].

Meanwhile, in *P. berghei*-infected mice, PD investigations assess the connection between medication exposure and pharmacological response. These investigations usually determine survival rates, parasitemia levels, and parasite development suppression after various medication dosages and regimens. Drug potency, effectiveness, and dose frequency needs are evaluated by calculating PD parameters, such as the minimum inhibitory concentration (MIC), maximum parasitocidal concentration (MPC), and duration above MIC (T>MIC) [93]. Additionally, PD research clarifies the pharmacodynamic pathways that underlie the effect of drugs, including host immune response regulation, disruption of parasite metabolism, and growth inhibition [94].

Integrating PK and PD data establishes pharmacokinetic-pharmacodynamic (PK-PD) correlations. These interactions guide the best antimalarial medication dosages and therapeutic outcomes. By correlating medication exposure levels with parasite-killing kinetics and treatment results, researchers can minimize the likelihood of drug resistance development and establish target drug concentrations associated with desired efficacy [95]. Moreover, using preclinical data from *P. berghei* models, PK-PD modelling helps with regimen selection, dosage optimization, and clinical outcome prediction in humans.

Ultimately, the pharmacological characteristics of antimalarial medications, the optimization of dosage schedules, and the forecasting of treatment results depend on pharmacokinetic and pharmacodynamic investigations in *P. berghei* models [96]. By amalgamating PK, PD, and PK-PD modelling methodologies, Scholars may expedite drug development endeavors, pinpoint lead compounds with ideal effectiveness and safety profiles, and eventually enhance therapeutic treatments for malaria.

Translational potential and clinical relevance

An essential part of malaria research is the possibility of translating results from *P. berghei* models to clinical settings. Although *P. berghei* is a rodent parasite that does not directly infect humans, research conducted on this model organism has essential ramifications for creating new treatments, vaccinations, and diagnostic aids for malaria in humans [45,95]. A significant advantage of *P. berghei* models is their genetic manipulation capacity, which enables researchers

to investigate the roles of specific genes related to parasite biology, host-parasite interactions, and medication resistance mechanisms [97]. Also, *P. berghei* shares genes with human malaria parasites like *P. falciparum* and *P. vivax*, which may make them suitable targets for drugs or vaccines. Genetic research on *P. berghei* can provide valuable insights into human malaria research by discovering orthologous genes and confirming their significance in human illness [98].

Additionally, preclinical data on the safety, effectiveness, and pharmacokinetic characteristics of possible antimalarial drugs are obtained using drug screening tests in mice infected with *P. berghei* [99]. Evaluating promising compounds in *P. berghei* models in human clinical trials can accelerate drug development and facilitate the search for novel malaria therapy alternatives. Furthermore, *P. berghei* models have proven helpful in unlocking the secrets of drug resistance pathways and discovering new ways to combine drugs to combat resistance [100]. Candidates for vaccines that elicit strong immune responses and protect against parasite challenge in mice infected with *P. berghei* may advance to human clinical trials, where their safety and effectiveness may be assessed in areas where malaria is prevalent.

Additionally, translational research endeavors seek to cross the divide between fundamental scientific findings in *P. berghei* models and clinical implementations in communities endemic to malaria [76]. Collaborative efforts among university researchers, pharmaceutical corporations, and public health organizations facilitate the translation of preclinical discoveries into practical therapies for malaria control and eradication [1,63]. By utilizing the advantages of *P. berghei* models and incorporating interdisciplinary methods, scientists might expedite the creation of novel instruments and tactics to counter malaria and enhance worldwide health consequences. Hence, models of *P. berghei* have important clinical and translational implications for malaria research. Studies employing this model organism yield insights that help develop new treatments, vaccines, and diagnostic techniques for human malaria, eventually improving attempts to lessen the disease's horrific global impact.

Comparative Analysis with Other *Plasmodium* Species Models

A comparative examination of *Plasmodium* species models is necessary to comprehend the advantages and disadvantages of various model organisms used in malaria research. Although *P. berghei* is the most employed species because it can be genetically modified and causes an intense infection in rats, other species, such as *P. falciparum* and *P. yoelii*, have particular benefits [32,34]. The deadliest human malaria parasite, *P. falciparum*, illuminates elements of malaria pathophysiology and immune responses unique to humans [101]. Another rodent parasite useful for researching liver-stage malaria and developing vaccines is *P. yoelii*.

Comparing *P. berghei* to other *Plasmodium* species models, such as *P. falciparum* and *P. vivax*, reveals certain advantages and disadvantages. Comprehending these comparative characteristics is crucial for choosing suitable model animals and creating customized experiments to address specific research inquiries in malaria biology and medication development. *P. berghei*'s genetic manipulation capacity is one of its main advantages, made possible by reliable transfection techniques and a comprehensive gene editing toolset. Because of this genetic tractability, scientists may generate mutant parasite lines with particular gene knockouts, knock-ins, or alterations, facilitating the study of gene function and the confirmation of potential therapeutic targets [19,59]. Furthermore, mouse infections with *P. berghei* provide a regulated and manageable experimental framework for investigating several facets of malaria pathogenesis, immunological responses, and medication effectiveness [56].

The host specificity of *P. berghei* for rats limits its applicability to human malaria infections, which is one drawback of utilizing it as a model organism. *P. berghei* differs from *P. falciparum*, the deadliest human malaria parasite, in that it does not usually infect people and presents distinct clinical symptoms, host-parasite interactions, and immune responses. Therefore, it may only sometimes be possible to immediately apply the results of investigations conducted using *P. berghei* models to human malaria infections. Instead, suitable humanised or non-human primate models must be used for validation [26]. In contrast, using *P. falciparum* as a model to investigate malaria infections in humans has several benefits. For instance, the capacity to cultivate *P. falciparum* *in vitro* makes high-throughput screening of antimalarial drugs and controlled studies of parasite biology possible. However, *P. falciparum*'s broad usage as a model organism must be improved due to difficulties in maintaining its cultures, genetic modification, and low transfection effectiveness [102].

Another important human malaria parasite, *P. vivax*, offers particular difficulties and opportunities for malaria research. In contrast to *P. falciparum*, *P. vivax* produces latent liver-stage hypnozoites, which can complicate treatment and removal attempts and cause relapses [103]. Though few *in vivo* models of *P. vivax* infection exist, recent developments in humanized mouse models and *in vitro* culture techniques have made it easier to research liver-stage malaria and find new drugs [104]. Hence, every *Plasmodium* species model, *P. berghei*, *P. falciparum*, and *P. vivax*, offer uniqueness when studying malaria (Table 5). Even though *P. berghei* offers a manageable system for genetic modification and experimental research in rodents; its lack of applicability to human malaria infections means that additional research in other model organisms, like *P. falciparum* and *P. vivax*, is necessary to advance our knowledge of malaria biology and create successful countermeasures against this threat to global health.

Table 5. Comparison of *Plasmodium* Species Models.

Aspect	<i>Plasmodium berghei</i>	<i>Plasmodium falciparum</i>	<i>Plasmodium vivax</i>
Genetic Manipulation	Amenable to genetic manipulation	Limited efficiency and challenges in genetic editing	Limited tools and models for genetic manipulation
Host Specificity	Rodent host, not infective to humans	Infective to humans, primarily responsible for severe malaria	Infective to humans, it causes relapsing malaria
Relevance to Human Infections	Limited relevance due to species differences	Direct relevance to human malaria infections	Direct relevance to human malaria infections
Research Applications	Study of malaria pathogenesis, immunity, and drug efficacy in rodents	Study of human-specific aspects of malaria, drug screening, and immune responses	Study of relapsing malaria, liver-stage biology, and drug discovery
Experimental Manipulation	High efficiency in creating mutant parasite lines	Limited efficiency in genetic manipulation	Limited tools for genetic manipulation and culture
Model Limitations	Lack of direct relevance to human infections	Ethical considerations in working with human subjects	Limited <i>in vivo</i> models and culture systems
Advantages	The controlled experimental system, tractable for research	Direct relevance to human malaria, <i>in vitro</i> culture capabilities	Insights into relapsing malaria, potential for drug discovery
Current Research Focus	Understanding parasite biology and drug discovery	Drug resistance, vaccine development, and pathogenesis	Liver-stage Biology, relapse mechanisms, and drug discovery
References	[8,17,105]	[3,91,102]	[67,98,103,106]

This table-based style compares the strengths, shortcomings, and research applications of *P. berghei* and other *Plasmodium* species models.

However, to ensure that study findings are repeatable and generalizable, results must be experimentally validated across several *Plasmodium* species models. This entails doing parallel tests in many model organisms to determine species-specific changes and evaluate the consistency of results. To confirm results and guide translation to clinical settings, research examining medication effectiveness, immunological responses, or vaccine-induced protection, for instance, frequently assesses potential treatments in rodent and human malaria models [107].

Cross-species studies provide experimental validation and avenues to clarify species-specific variations in malaria pathophysiology, medication susceptibility, and immune evasion tactics. By analyzing how distinct *Plasmodium* species react to experimental interventions or environmental stimuli, researchers can discern vulnerabilities and adaptive mechanisms unique to each species. This information can be used to understand the factors influencing variations in malaria epidemiology and clinical outcomes between species [32]. Cross-species validation of results is essential to improving our comprehension of malaria biology and turning scientific insights into practical therapies. Researchers can

verify results, pinpoint conserved biological processes, and clarify species-specific distinctions by utilizing various model organisms and complementary experimental techniques. Ultimately, this helps generate new approaches for controlling and eradicating malaria.

Challenges and Future Directions

Although research on malaria and the production of vaccines have significantly advanced, thanks to *P. berghei* models, some limits and problems need to be considered. These variables may affect how results from preclinical research are applied in clinical settings and may also point to areas where malaria research needs to be improved. These issues must be resolved for these models to be as helpful as possible and for our knowledge of malaria biology and treatment to advance.

First, the limited applicability of *P. berghei* to human malaria infections is a barrier posed by its species' uniqueness. Enhancing non-human primate models or creating humanized mouse models should be the main goals of future research to mimic the immunological responses and mechanism of human malaria more accurately [108]. Secondly, advances in imaging methods, including bioluminescence imaging and intravital microscopy, provide novel insights into parasite-host interactions and medication effectiveness *in vivo* [109]. Integrating omics technologies, such as transcriptomics,

proteomics, and genomes, makes finding new therapeutic targets and vaccine candidates easier and allows for thorough characterization of parasite biology and host responses [110].

Secondly, Since *P. berghei* only infects rodents rather than humans, one of the main problems with *P. berghei* models is their species-specificity. Although the lifecycle and pathogenesis of *P. berghei* and human malaria parasites are similar, host-parasite interactions, immunological responses, and clinical symptoms differ significantly. Therefore, directly applying research findings from *P. berghei* models to human malaria infections may only sometimes be possible. Instead, suitable humanized or non-human primate models must be used for validation [111].

Thirdly, researching parasite biology and developing new drugs are complicated by the genetic differences between *P. berghei* and human malaria parasites. The differences in drug targets, metabolic pathways, and gene expression between *P. berghei* and human malaria parasites may limit the utility of findings for developing interventions targeting human malaria species. Despite this, *P. berghei* models facilitate genetic manipulation and investigation of parasite biology [112]. Complementary research employing human malaria parasites, such as *P. falciparum* or *P. vivax*, is crucial to validate results and further drug discovery efforts.

Additionally, interpreting data from *P. berghei* models is challenging due to the intricacy of host reactions and malaria immunology. The results of immunogenicity and effectiveness studies may be impacted by the distinctions in immune regulation, antigenicity, and immune evasion tactics between rats and humans, even though these models facilitate the analysis of vaccine-induced immune responses and protective immunity. Consequently, to guarantee the applicability and transferability of vaccine candidates discovered using *P. berghei* models, meticulous interpretation and validation of results in human research are required [113].

Furthermore, *P. berghei* models have drawbacks and limitations, even though they provide insightful information on malaria's pathophysiology, vaccine creation, and drug discovery. Complementary research in appropriate humanized or non-human primate models and validation in human populations is necessary to address these limitations, advance our understanding of malaria, and translate findings into practical interventions to control and eradicate this devastating disease [114].

Lastly, advances in genome editing techniques, including base editing, CRISPR-Cas9, and CRISPR-Cas12a, should improve the genetic tractability of *P. berghei* models and speed up functional genomics research [115]. Furthermore, the development of humanized mouse models with reconstituted human immune systems provides possibilities for investigating human-specific elements of malaria pathogenesis and

immunity *in vivo* [116]. Hence, even though *P. berghei* models have proven helpful in the study of malaria; it is critical to overcome their drawbacks and adopt new technologies to spur innovation and further our knowledge of the disease's biology. By successfully resolving these obstacles and using the potential for model enhancement, scientists may expedite the creation of innovative countermeasures against malaria and enhance worldwide health results.

A clear roadmap is essential to address the challenges in utilizing *P. berghei* models for malaria research because they focus on enhancing model applicability, leveraging advanced imaging methods, and integrating omics technologies. First, enhancing the applicability of *P. berghei* models to human malaria involves developing humanized mouse models and utilizing non-human primates. These models can replicate human immune responses and disease mechanisms more accurately, providing a more relevant platform for testing vaccines and therapeutics [5,114]. Future research should aim to refine these models to improve their fidelity to human malaria. Secondly, advances in imaging techniques such as bioluminescence and intravital microscopy offer novel insights into parasite-host interactions and medication effectiveness *in vivo*. These methods enable real-time visualization of parasite development and disease progression, although they come with high costs and technical complexity [108,117]. Their adoption can significantly enhance our understanding of malaria pathogenesis and treatment responses. Lastly, integrating omics technologies, including transcriptomics, proteomics, and genomics, can revolutionize malaria research [110]. These approaches facilitate the identification of new therapeutic targets and vaccine candidates by providing a comprehensive characterization of parasite biology and host responses. Despite the need for advanced bioinformatics tools and expertise, omics technologies offer unprecedented opportunities for in-depth analysis and discovery. Implementing this roadmap involves fostering collaborations, developing standardized protocols, and investing in training and infrastructure to support these advanced research methodologies. By addressing these challenges, the accuracy and efficiency of malaria research using *P. berghei* models can be significantly improved, accelerating the development of effective interventions.

Implications for malaria research and control strategies

The knowledge gained from research employing *P. berghei* models significantly impacts malaria prevention and treatment methods. By clarifying parasite biology, host immunity, and drug resistance processes, these models assist in creating new malaria therapies such as medications, vaccines, and vector control strategies [3,118]. Furthermore, new developments in genome editing tools, omics technologies, and imaging techniques present excellent prospects for speeding up the development of creative treatments, preventative measures, and eradication plans for malaria and deepening our

understanding of the disease's biology. Therefore, *P. berghei* models are critical in advancing malaria research. However, to spur innovation and enhance global health outcomes in the battle against malaria, efforts must be made to overcome their shortcomings and use cutting-edge technology.

Conclusion

This review has revealed the critical contributions of *P. berghei* models to the study of malaria and the issues that still need to be resolved to make further progress in this area. The biology of malaria has been studied in detail using *P. berghei* models, including immune responses, medication development, parasite pathology, and vaccine development. The genetic tractability and simplicity of manipulating *P. berghei* have made significant discoveries and progress in our comprehension of malaria. However, restrictions such as species specificity and variations in host-parasite interactions highlight the necessity of supplementary research in additional model organisms, including *P. vivax* and *P. falciparum*, to guarantee the applicability and relevance of study findings.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgement

Dr Otun, the University of Witwatersrand's Research Office, for her post-doctoral fellowship funding.

References

1. Monroe A, Williams NA, Ogoma S, Karema C, Okumu F. Reflections on the 2021 World Malaria Report and the future of malaria control. *Malar J*. 2022 May 27;21(1):154.
2. Kogan F. Malaria Burden. In: Kogan F (Ed). *Remote Sensing for Malaria: Monitoring and Predicting Malaria from Operational Satellites*. Germany: Springer International Publishing; 2020. pp.15-41.
3. Otun S, Achilonu I. The prevalence of *Plasmodium vivax* and *Plasmodium falciparum* in Southern Africa. *Curr Trends Microbiol*. 2022; 16:75-86.
4. Killick-Kendrick R. Parasitic protozoa of the blood of rodents: a revision of *Plasmodium berghei*. *Parasitology*. 1974 Oct;69(2):225-37.
5. Carter R, Diggs CL. Plasmodia of rodents. *Parasitic Protozoa*. 1977;3:359-465.
6. Jiménez-Díaz MB, Viera S, Fernández-Alvaro E, Angulo-Barturen I. Animal models of efficacy to accelerate drug discovery in malaria. *Parasitology*. 2014 Jan;141(1):93-103.
7. Khoury DS, Aogo R, Randriafanomezantsoa-Radohery G, McCaw JM, Simpson JA, McCarthy JS, et al. Within-host modeling of blood-stage malaria. *Immunol Rev*. 2018 Sep;285(1):168-93.
8. Vincke IH, Lips M. *Plasmodium berghei*, a New *Plasmodium* of a Wild Rodent in the Belgian Congo. *Ann Soc Belg Med Trop, Societe Belge de Medecine Tropicale*. 1948;97-104.
9. Scheller LF, Wirtz RA, Azad AF. Susceptibility of different strains of mice to hepatic infection with *Plasmodium berghei*. *Infect Immun*. 1994 Nov;62(11):4844-7.
10. Matsuoka H, Yoshida S, Hirai M, Ishii A. A rodent malaria, *Plasmodium berghei*, is experimentally transmitted to mice by merely probing of infective mosquito, *Anopheles stephensi*. *Parasitol Int*. 2002 Mar;51(1):17-23.
11. Jin Y, Kebaier C, Vanderberg J. Direct microscopic quantification of dynamics of *Plasmodium berghei* sporozoite transmission from mosquitoes to mice. *Infect Immun*. 2007 Nov;75(11):5532-9.
12. Vigetti L, Tardieux I. Fostering innovation to solve the biomechanics of microbe-host interactions: Focus on the adhesive forces underlying Apicomplexa parasite biology. *Biol Cell*. 2023 Oct;115(10):e202300016.
13. Carter R. On the genetics of malaria parasites, a memoir: Part I, rodent malaria parasites and the first genetic crosses. *Parasitol Int*. 2022 Dec;91:102635.
14. Peters W. Drug resistance in *Plasmodium berghei* Vincke and Lips, 1948. I. Chloroquine resistance. *Exp Parasitol*. 1965 Aug;17(1):80-9.
15. Waters AP, Thomas AW, van Dijk MR, Janse CJ. Transfection of malaria parasites. *Methods*. 1997 Oct;13(2):134-47.
16. Caldelari R, Dogga S, Schmid MW, Franke-Fayard B, Janse CJ, Soldati-Favre D, Heussler V. Transcriptome analysis of *Plasmodium berghei* during exo-erythrocytic development. *Malar J*. 2019 Sep 24;18(1):330.
17. Xu X, Dong Y, Abraham EG, Kocan A, Srinivasan P, Ghosh AK, Sinden RE, Ribeiro JM, Jacobs-Lorena M, Kafatos FC, Dimopoulos G. Transcriptome analysis of *Anopheles stephensi*-*Plasmodium berghei* interactions. *Mol Biochem Parasitol*. 2005 Jul;142(1):76-87.
18. Moita D, Nunes-Cabaço H, Rôla C, Franke-Fayard B, Janse CJ, Mendes AM, Prudêncio M. Variable long-term protection by radiation-, chemo-, and genetically-attenuated *Plasmodium berghei* sporozoite vaccines. *Vaccine*. 2023 Dec 12;41(51):7618-25.
19. Torre S, Langlais D, Gros P. Genetic analysis of cerebral malaria in the mouse model infected with *Plasmodium berghei*. *Mamm Genome*. 2018 Aug;29(7-8):488-506.
20. Lefevre T, Ohm J, Dabiré KR, Cohuet A, Choisy M, Thomas MB, et al. Transmission traits of malaria parasites within the mosquito: Genetic variation, phenotypic plasticity, and consequences for control. *Evol Appl*. 2017 Dec 16;11(4):456-69.
21. Yoeli M. Studies on *Plasmodium berghei* in nature and under experimental conditions. *Trans R Soc Trop Med Hyg*. 1965 May;59:255-71.

22. Zhu L, Tripathi J, Rocamora FM, Miotto O, van der Pluijm R, Voss TS, et al. The origins of malaria artemisinin resistance defined by a genetic and transcriptomic background. *Nat Commun*. 2018 Dec 4;9(1):5158.
23. Frevert U, Engelmann S, Zougbedé S, Stange J, Ng B, Matuschewski K, et al. Intravital observation of *Plasmodium berghei* sporozoite infection of the liver. *PLoS Biol*. 2005 Jun;3(6):e192.
24. Goodman AL, Forbes EK, Williams AR, Douglas AD, de Cassan SC, Bauza K, et al. The utility of *Plasmodium berghei* as a rodent model for anti-merozoite malaria vaccine assessment. *Sci Rep*. 2013;3:1706.
25. Yoshida S, Nagumo H, Yokomine T, Araki H, Suzuki A, Matsuoka H. *Plasmodium berghei* circumvents immune responses induced by merozoite surface protein 1- and apical membrane antigen 1-based vaccines. *PLoS One*. 2010 Oct 28;5(10):e13727.
26. Albohiri HH, Alzanbagi NA. Systemic review: The pathogenicity of *Plasmodium berghei* in the liver and spleen of the experimental mice. *Journal of Pharmaceutical Research International*. 2021;33(47B):515-29.
27. Das N, Prabhu P. Emerging avenues for the management of cerebral malaria. *J Pharm Pharmacol*. 2022 Jun 9;74(6):800-11.
28. Burgert L, Rottmann M, Wittlin S, Gobeau N, Krause A, Dingemans J, et al. Ensemble modeling highlights importance of understanding parasite-host behavior in preclinical antimalarial drug development. *Sci Rep*. 2020 Mar 10;10(1):4410.
29. Loiseau C, Cooper MM, Doolan DL. Deciphering host immunity to malaria using systems immunology. *Immunol Rev*. 2020 Jan;293(1):115-43.
30. Tedla MG, Every AL, Scheerlinck JY. Investigating immune responses to parasites using transgenesis. *Parasit Vectors*. 2019 Jun 15;12(1):303.
31. Moxon CA, Gibbins MP, McGuinness D, Milner DA Jr, Marti M. New Insights into Malaria Pathogenesis. *Annu Rev Pathol*. 2020 Jan 24;15:315-43.
32. Simwela N V, Waters AP. Current status of experimental models for the study of malaria. *Parasitology*. 2022;149 (6):729-50.
33. Leitner WW, Bergmann-Leitner ES, Angov E. Comparison of *Plasmodium berghei* challenge models for the evaluation of pre-erythrocytic malaria vaccines and their effect on perceived vaccine efficacy. *Malar J*. 2010 May 27;9:145.
34. Lamb TJ, Brown DE, Potocnik AJ, Langhorne J. Insights into the immunopathogenesis of malaria using mouse models. *Expert Rev Mol Med*. 2006 Mar 23;8(6):1-22.
35. Lakkavaram A, Lundie RJ, Do H, Ward AC, de Koning-Ward TF. Acute *Plasmodium berghei* Mouse Infection Elicits Perturbed Erythropoiesis With Features That Overlap With Anemia of Chronic Disease. *Front Microbiol*. 2020 Apr 16;11:702.
36. Weiss WR, Sedegah M, Berzofsky JA, Hoffman SL. The role of CD4+ T cells in immunity to malaria sporozoites. *J Immunol*. 1993 Sep 1;151(5):2690-8.
37. Morrot A, Zavala F. Effector and memory CD8+ T cells as seen in immunity to malaria. *Immunol Rev*. 2004 Oct;201:291-303.
38. Riley EM, Wahl S, Perkins DJ, Schofield L. Regulating immunity to malaria. *Parasite Immunol*. 2006 Jan-Feb;28(1-2):35-49.
39. Kochin BF, Yates AJ, de Roode JC, Antia R. On the control of acute rodent malaria infections by innate immunity. *PLoS One*. 2010 May 6;5(5):e10444.
40. Yoneto T, Yoshimoto T, Wang CR, Takahama Y, Tsuji M, Waki S, et al. Gamma interferon production is critical for protective immunity to infection with blood-stage *Plasmodium berghei* XAT but neither NO production nor NK cell activation is critical. *Infect Immun*. 1999 May;67(5):2349-56.
41. Wu J, Tian L, Yu X, Pattaradilokrat S, Li J, Wang M, et al. Strain-specific innate immune signaling pathways determine malaria parasitemia dynamics and host mortality. *Proc Natl Acad Sci U S A*. 2014 Jan 28;111(4):E511-20.
42. Gowda DC, Wu X. Parasite Recognition and Signaling Mechanisms in Innate Immune Responses to Malaria. *Front Immunol*. 2018 Dec 19;9:3006.
43. Zhang G, Manaca MN, McNamara-Smith M, Mayor A, Nhabomba A, Berthoud TK, et al. Interleukin-10 (IL-10) polymorphisms are associated with IL-10 production and clinical malaria in young children. *Infect Immun*. 2012 Jul;80(7):2316-22.
44. Ntita M, Inoue SI, Jian JY, Bayarsaikhan G, Kimura K, Kimura D, et al. Type I interferon production elicits differential CD4+ T-cell responses in mice infected with *Plasmodium berghei* ANKA and P. chabaudi. *Int Immunol*. 2022 Jan 1;34(1):21-33.
45. Mendes AM, Machado M, Gonçalves-Rosa N, Reuling IJ, Foquet L, Marques C, et al. A *Plasmodium berghei* sporozoite-based vaccination platform against human malaria. *NPJ Vaccines*. 2018 Aug 24;3:33.
46. Torre D. Early production of gamma-interferon in clinical malaria: role of interleukin-18 and interleukin-12. *Clin Infect Dis*. 2009 May 15;48(10):1481-2.
47. Leão L, Puty B, Dolabela MF, Povoá MM, Né YGS, Eiró LG, et al. Association of cerebral malaria and TNF- α levels: a systematic review. *BMC Infect Dis*. 2020 Jun 23;20(1):442.
48. Rajneesh, Tiwari R, Singh VK, Kumar A, Gupta RP, Singh AK, et al. Advancements and Challenges in Developing Malaria Vaccines: Targeting Multiple Stages of the Parasite Life Cycle. *ACS Infect Dis*. 2023 Oct 13;9(10):1795-814.
49. Wu S, Nie Q, Tan S, Liao G, Lv Y, Lv C, et al. The immunity modulation of transforming growth factor- β in malaria and other pathological process. *Int Immunopharmacol*. 2023 Sep;122:110658.
50. Burrack KS, Hart GT, Hamilton SE. Contributions of natural killer

- cells to the immune response against *Plasmodium*. *Malar J*. 2019 Sep 18;18(1):321.
51. Mockenhaupt FP, Cramer JP, Hamann L, Stegemann MS, Eckert J, Oh NR, et al. Toll-like receptor (TLR) polymorphisms in African children: Common TLR-4 variants predispose to severe malaria. *Proc Natl Acad Sci U S A*. 2006 Jan 3;103(1):177-82.
52. Pereira LMS, Gomes STM, Ishak R, Vallinoto ACR. Regulatory T Cell and Forkhead Box Protein 3 as Modulators of Immune Homeostasis. *Front Immunol*. 2017 May 26;8:605.
53. Richards JS, Beeson JG. The future for blood-stage vaccines against malaria. *Immunol Cell Biol*. 2009 Jul;87(5):377-90.
54. Flaxman A, Ewer KJ. Methods for Measuring T-Cell Memory to Vaccination: From Mouse to Man. *Vaccines (Basel)*. 2018 Jul 21;6(3):43.
55. Good MF. Vaccine-induced immunity to malaria parasites and the need for novel strategies. *Trends Parasitol*. 2005 Jan;21(1):29-34.
56. Douradinha B, van Dijk MR, Ataide R, van Gemert GJ, Thompson J, Franetich JF, et al. Genetically attenuated P36p-deficient *Plasmodium berghei* sporozoites confer long-lasting and partial cross-species protection. *Int J Parasitol*. 2007 Nov;37(13):1511-9.
57. Yusuf Y, Yoshii T, Iyori M, Yoshida K, Mizukami H, Fukumoto S, et al. Adeno-Associated Virus as an Effective Malaria Booster Vaccine Following Adenovirus Priming. *Front Immunol*. 2019 Apr 5;10:730.
58. Janse CJ, Franke-Fayard B, Mair GR, Ramesar J, Thiel C, Engelmann S, et al. High efficiency transfection of *Plasmodium berghei* facilitates novel selection procedures. *Mol Biochem Parasitol*. 2006 Jan;145(1):60-70.
59. de Koning-Ward TF, Janse CJ, Waters AP. The development of genetic tools for dissecting the biology of malaria parasites. *Annu Rev Microbiol*. 2000;54:157-85.
60. Ranjan P, Dubey VK. Krebs cycle enzymes for targeted therapeutics and immunotherapy for anti-leishmanial drug development using: Pathways, potential targets, and future perspectives. *Life Sci*. 2023 Jun 1;322:121314.
61. Fernández E, Castellote MI, Chaparro MJ, Díaz B, Fernández J, Gordo M, et al. A decade of malaria phenotypic screenings: Key lessons on the discovery and development of new antimalarial drugs. *Annual Reports in Medicinal Chemistry*. 2019;53:1-23.
62. Kaur R, Gorki V, Katare OP, Dhingra N, Chauhan M, Kaur R, et al. Improved biopharmaceutical attributes of lumefantrine using choline mimicking drug delivery system: preclinical investigation on NK-65 *P. berghei* murine model. *Expert Opin Drug Deliv*. 2021 Oct;18(10):1533-52.
63. O'Neill G. Malaria Special Report: Aiming for a World without Malaria. *Australian Life Scientist*. 2010 May;7(3).
64. Duffey M, Shafer RW, Timm J, Burrows JN, Fotouhi N, Cockett M, et al. Combating antimicrobial resistance in malaria, HIV and tuberculosis. *Nat Rev Drug Discov*. 2024 Jun;23(6):461-79.
65. Brown JR, Drewry D, Gamo FJ, Garcia-Bustos JF. Kinase inhibitors among hits from malaria cellular screens. *Protein Phosphorylation in Parasites*. 2013 Oct 8:261-91.
66. Khera HK, Srivastava AK, Singh S. Antimalarial Drug Discovery and Development: From Bench to Bedside. In: Rajput VS, Runthala A, (eds). *Drugs and a Methodological Compendium*. Singapore: Springer; 2023.
67. Moita D, Maia TG, Duarte M, Andrade CM, Albuquerque IS, Dwivedi A, et al. A genetically modified *Plasmodium berghei* parasite as a surrogate for whole-sporozoite vaccination against *P. vivax* malaria. *NPJ Vaccines*. 2022 Dec 16;7(1):163.
68. Hamilton A, Haghpanah F, Hasso-Agopsowicz M, Frost I, Lin G, Schueller E, et al. Modeling of malaria vaccine effectiveness on disease burden and drug resistance in 42 African countries. *Commun Med (Lond)*. 2023 Oct 13;3(1):144.
69. Moita D, Nunes-Cabaço H, Mendes AM, Prudêncio M. A guide to investigating immune responses elicited by whole-sporozoite pre-erythrocytic vaccines against malaria. *FEBS J*. 2022 Jun;289(12):3335-59.
70. Mendes AM, Reuling IJ, Andrade CM, Otto TD, Machado M, Teixeira F, et al. Pre-clinical evaluation of a *P. berghei*-based whole-sporozoite malaria vaccine candidate. *NPJ Vaccines*. 2018 Nov 27;3:54.
71. Bettencourt P. Current Challenges in the Identification of Pre-Erythrocytic Malaria Vaccine Candidate Antigens. *Front Immunol*. 2020 Feb 21;11:190.
72. Ferreira MU, Ribeiro WL, Tonon AP, Kawamoto F, Rich SM. Sequence diversity and evolution of the malaria vaccine candidate merozoite surface protein-1 (MSP-1) of *Plasmodium falciparum*. *Gene*. 2003 Jan 30;304:65-75.
73. Spring MD, Cummings JF, Ockenhouse CF, Dutta S, Reidler R, Angov E, et al. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. *PLoS One*. 2009;4(4):e5254.
74. Cohen J, Nussenzweig V, Nussenzweig R, Vekemans J, Leach A. From the circumsporozoite protein to the RTS, S/AS candidate vaccine. *Hum Vaccin*. 2010 Jan;6(1):90-6.
75. Perez-Leal O, Mongui A, Cortes J, Yepes G, Leiton J, Patarroyo MA. The *Plasmodium vivax* rhoptry-associated protein 1. *Biochem Biophys Res Commun*. 2006 Mar 24;341(4):1053-8.
76. World Health Organization. The biology of malaria parasites: report of a WHO scientific group [meeting held in Washington, DC, from 24 to 28 September 1984]. World Health Organization; 1987.
77. Okombo J, Chibale K. Recent updates in the discovery and development of novel antimalarial drug candidates. *Medchemcomm*. 2018 Feb 2;9(3):437-53.

78. Jiménez-Díaz MB, Viera S, Ibáñez J, Mulet T, Magán-Marchal N, Garuti H, et al. A new in vivo screening paradigm to accelerate antimalarial drug discovery. *PLoS One*. 2013 Jun 25;8(6):e66967.
79. Umumararungu T, Nkuranga JB, Habarurema G, Nyandwi JB, Mukazayire MJ, Mukiza J, et al. Recent developments in antimalarial drug discovery. *Bioorg Med Chem*. 2023 Jun 6;88-89:117339.
80. Mukherjee S, Ray G, Saha B, Kar SK. Oral Therapy Using a Combination of Nanotized Antimalarials and Immunomodulatory Molecules Reduces Inflammation and Prevents Parasite Induced Pathology in the Brain and Spleen of *P. berghei* ANKA Infected C57BL/6 Mice. *Front Immunol*. 2022 Jan 13;12:819469.
81. Uche FI, Guo X, Okokon J, Ullah I, Horrocks P, Boateng J, et al. In Vivo Efficacy and Metabolism of the Antimalarial Cycleanine and Improved In Vitro Antiplasmodial Activity of Semisynthetic Analogues. *Antimicrob Agents Chemother*. 2021 Jan 20;65(2):e01995-20.
82. Ungogo MA, Ebiloma GU, Ichoron N, Igoli JO, de Koning HP, Balogun EO. A Review of the Antimalarial, Antitrypanosomal, and Antileishmanial Activities of Natural Compounds Isolated From Nigerian Flora. *Front Chem*. 2020 Dec 23;8:617448.
83. Tarkang PA, Appiah-Opong R, Ofori MF, Ayong LS, Nyarko AK. Application of multi-target phytotherapeutic concept in malaria drug discovery: a systems biology approach in biomarker identification. *Biomark Res*. 2016 Dec 13;4:25.
84. Uddin A, Gupta S, Mohammad T, Shahi D, Hussain A, Alajmi MF, et al. Target-Based Virtual Screening of Natural Compounds Identifies a Potent Antimalarial With Selective Falcipain-2 Inhibitory Activity. *Front Pharmacol*. 2022 Apr 6;13:850176.
85. Dietrich JA, Yoshikuni Y, Fisher KJ, Woolard FX, Ockey D, McPhee DJ, et al. A novel semi-biosynthetic route for artemisinin production using engineered substrate-promiscuous P450(BM3). *ACS Chem Biol*. 2009 Apr 17;4(4):261-7.
86. Roux AT, Maharaj L, Oyegoke O, Akoniyan OP, Adeleke MA, Maharaj R, et al. Chloroquine and Sulfadoxine-Pyrimethamine Resistance in Sub-Saharan Africa-A Review. *Front Genet*. 2021 Jun 25;12:668574.
87. Croft A, Garner P. Mefloquine to prevent malaria: a systematic review of trials. *BMJ*. 1997 Nov 29;315(7120):1412-6.
88. Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, et al. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malar J*. 2011 May 24;10:144.
89. Flannery EL, Kangwanransan N, Chuenchob V, Roobsoong W, Fishbaugher M, Zhou K, et al. *Plasmodium vivax* latent liver infection is characterized by persistent hypnozoites, hypnozoite-derived schizonts, and time-dependent efficacy of primaquine. *Mol Ther Methods Clin Dev*. 2022 Aug 2;26:427-40.
90. White NJ. Pharmacokinetic and pharmacodynamic considerations in antimalarial dose optimization. *Antimicrob Agents Chemother*. 2013 Dec;57(12):5792-807.
91. Khemawoot P, Saunders D, Rasameesoraj M, Melendez V, Imerbsin R, Ohrt C, et al. Absolute bioavailability of cis-mirincomycin and trans-mirincomycin in healthy rhesus monkeys and ex vivo antimalarial activity against *Plasmodium falciparum*. *Antimicrob Agents Chemother*. 2011 Dec;55(12):5881-6.
92. Mathias NR, Hussain MA. Non-invasive systemic drug delivery: developability considerations for alternate routes of administration. *J Pharm Sci*. 2010 Jan;99(1):1-20.
93. Rajakumar G, Rahuman AA, Chung IM, Kirthi AV, Marimuthu S, Anbarasan K. Antiplasmodial activity of eco-friendly synthesized palladium nanoparticles using *Eclipta prostrata* extract against *Plasmodium berghei* in Swiss albino mice. *Parasitol Res*. 2015 Apr;114(4):1397-406.
94. Burrows JN, Duparc S, Gutteridge WE, Hooft van Huijsduijnen R, Kaszubska W, Macintyre F, et al. New developments in anti-malarial target candidate and product profiles. *Malar J*. 2017 Jan 13;16(1):26.
95. Hughes E, Wallender E, Mohamed Ali A, Jagannathan P, Savic RM. Malaria PK/PD and the Role Pharmacometrics Can Play in the Global Health Arena: Malaria Treatment Regimens for Vulnerable Populations. *Clin Pharmacol Ther*. 2021 Oct;110(4):926-40.
96. Burgert L, Zaloumis S, Dini S, Marquart L, Cao P, Cherkaoui M, et al. Parasite-Host Dynamics throughout Antimalarial Drug Development Stages Complicate the Translation of Parasite Clearance. *Antimicrob Agents Chemother*. 2021 Mar 18;65(4):e01539-20.
97. Kirchner S, Power BJ, Waters AP. Recent advances in malaria genomics and epigenomics. *Genome Med*. 2016 Sep 7;8(1):92.
98. Cao Y, Hart RJ, Bansal GP, Kumar N. Functional Conservation of P48/45 Proteins in the Transmission Stages of *Plasmodium vivax* (Human Malaria Parasite) and *P. berghei* (Murine Malaria Parasite). *mBio*. 2018 Sep 4;9(5):e01627-18.
99. Le Bihan, de Kanter R, Angulo-Barturen I, Binkert C, Boss C, Brun R, et al. Characterization of Novel Antimalarial Compound ACT-451840: Preclinical Assessment of Activity and Dose-Efficacy Modeling. *PLoS Med*. 2016 Oct 4;13(10):e1002138.
100. Kumpornsin K, Kochakarn T, Chookajorn T. The resistome and genomic reconnaissance in the age of malaria elimination. *Dis Model Mech*. 2019 Dec 19;12(12):dmm040717.
101. Crompton PD, Moebius J, Portugal S, Waisberg M, Hart G, Garver LS, et al. Malaria immunity in man and mosquito: insights into unsolved mysteries of a deadly infectious disease. *Annu Rev Immunol*. 2014;32:157-87.
102. Nasamu AS, Falla A, Pasaje CFA, Wall BA, Wagner JC, Ganesan SM, et al. An integrated platform for genome engineering and gene expression perturbation in *Plasmodium falciparum*. *Sci Rep*. 2021 Jan 11;11(1):342.
103. Habtamu K, Petros B, Yan G. *Plasmodium vivax*: the potential obstacles it presents to malaria elimination and eradication. *Trop Dis Travel Med Vaccines*. 2022 Dec 15;8(1):27.

104. Valenciano AL, Gomez-Lorenzo MG, Vega-Rodríguez J, Adams JH, Roth A. In vitro models for human malaria: targeting the liver stage. *Trends Parasitol*. 2022 Sep;38(9):758-74.
105. Kim MJ, Chu KB, Lee SH, Kang HJ, Yoon KW, Ahmed MA, et al. Recombinant Vaccinia Virus Expressing *Plasmodium berghei* Apical Membrane Antigen 1 or Microneme Protein Enhances Protection against *P. berghei* Infection in Mice. *Trop Med Infect Dis*. 2022 Nov 4;7(11):350.
106. de Camargo TM, de Freitas EO, Gimenez AM, Lima LC, de Almeida Caramico K, Françoso KS, et al. 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*. 2018 Jan 18;8(1):1118.
107. Stanczyk NM, Brugman VA, Austin V, Sanchez-Roman Teran F, Gezan SA, Emery M, et al. Species-specific alterations in Anopheles mosquito olfactory responses caused by *Plasmodium* infection. *Sci Rep*. 2019 Mar 4;9(1):3396.
108. Ferrer M, Martin-Jaular L, De Niz M, Khan SM, Janse CJ, Calvo M, et al. Imaging of the spleen in malaria. *Parasitol Int*. 2014 Feb;63(1):195-205.
109. Affi MA. The Parasites Caught In-Action: Imaging at the Host-Parasite Interface. *J Microsc Ultrastruct*. 2020 May 8;9(1):1-6.
110. Cowell AN, Winzeler EA. Advances in omics-based methods to identify novel targets for malaria and other parasitic protozoan infections. *Genome Med*. 2019 Oct 22;11(1):63.
111. Banerjee P, Sanyal S. Disease Models in Malarial Research. In: Shegokar R, Pathak Y, (eds). *Infectious Diseases Drug Delivery Systems*. Springer, Cham; 2023: pp. 169-92.
112. Sinha S, Medhi B, Sehgal R. Challenges of drug-resistant malaria. *Parasite*. 2014;21:61.
113. Hetzel C, Anderson RM. The within-host cellular dynamics of bloodstage malaria: theoretical and experimental studies. *Parasitology*. 1996 Jul;113 (Pt 1):25-38.
114. De Niz M, Heussler VT. Rodent malaria models: insights into human disease and parasite biology. *Curr Opin Microbiol*. 2018 Dec;46:93-101.
115. Lee MCS, Lindner SE, Lopez-Rubio JJ, Llinás M. Cutting back malaria: CRISPR/Cas9 genome editing of *Plasmodium*. *Brief Funct Genomics*. 2019 Sep 24;18(5):281-9.
116. Ye W, Chen Q. Potential Applications and Perspectives of Humanized Mouse Models. *Annu Rev Anim Biosci*. 2022 Feb 15;10:395-417.
117. Wu AM. Protein Engineering for Molecular Imaging. In: Ross BD, Gambhir SS. *Molecular Imaging*. 2nd Edition. Academic Press; 2021. pp. 753-70.
118. Kotepui M, Kotepui KU, Milanez GD, Masangkay FR. Global prevalence and mortality of severe *Plasmodium malariae* infection: a systematic review and meta-analysis. *Malar J*. 2020 Jul 31;19(1):274.