RESEARCH PAPER

Molecular Characterization and Vaccine Development Targeting Merozoite Surface Protein 1 in Plasmodium Falciparum (PfMSP1): A Critical Review

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Received: 24th April, 2026; Revised: 20th May 2026; Accepted: 30th June, 2026; Available Online: 10th July, 2026

ABSTRACT
In spite of considerable advancement in malaria elimination and eradication policies, the infectious disease, particularly that caused by the Plasmodium falciparum parasite, persist as a major public health problem. The deployment of the RTS, S/AS01 vaccine and artemisinin-based combination therapies (ACTs) is inadequate to prevent the spread of multidrug-resistant parasites. Therefore, new molecular targets must be identified. A comprehensive review was conducted using peer-reviewed literature retrieved from major biomedical databases. This review underscores the molecular characterization along with functional role of the Merozoite Surface Protein 1 of Plasmodium falciparum (PfMSP1) and its possible application as vaccine candidate. PfMSP1 plays a vital role in merozoite invasion in erythrocytes, undergoing multi-step proteolytic cleavage that produces functionally distinct fragments, including PfMSP1-19, a vaccine candidate. Evidence indicates that naturally acquired antibodies against conserved regions of PfMSP1 are associated with partial protection. However, allelic diversity of variable regions of PfMSP1 compromises vaccine efficacy. Clinical and preclinical trials of PfMSP1-based vaccines have established immunogenicity but inadequate protection. Nevertheless, these roadblocks are currently being addressed by evolving strategies including conserved epitope targeting, multi-valent vaccine constructs and structure-guided antigen design. A comprehensive understanding of host–parasite interactions is vital to enhance PfMSP1-centred interventions in integrated malaria eradication efforts.
Keywords: *Malaria, Plasmodium falciparum, Merozoite Surface Protein 1 (MSP1), vaccine development, erythrocyte invasion, antigenic polymorphism.*
How to cite this article: Chakraborty S, Chakraborty T, Bhunia S and Ghoshal S., Molecular Characterization and Vaccine Development Targeting Merozoite Surface Protein 1 in Plasmodium Falciparum (PfMSP1): A Critical Review. Int J Drug Deliv Technol. 2026;16(7): 1093-1111; DOI: 10.25258/ijddt.16.7.116
Source of support: Nil.
Conflict of interest: None

1. INTRODUCTION

Malaria continues to be a formidable global public health issue in the 21st century, even after years of sustained large-scale control efforts. (World Health Organisation, 2025). Primarily caused by *Plasmodium falciparum*, this parasitic infection exerts a disproportionate socioeconomic and clinical burden across affected areas, especially throughout sub-Saharan Africa and South East Asia (Li et al., 2025). The 2025 World Malaria Report underscores a mixed outcome: some regional successes are noticeable, yet overall global morbidity remains mostly stagnant. (World Health Organisation, 2025). The persistence of malaria as a major health challenge lies in the multifaceted interplay between biological and environmental determinants. (Dhorda et al., 2024). Though artemisinin-based combination therapies remain front-line of defences, evolving resistance in *falciparum* parasite jeopardises its therapeutic efficacy and threatens to reverse clinical advancement. (Balmer et al., 2025; Champagne et al., 2025). On the other hand, vector control srategies, such as insecticide-treated nets, are undermined by intensifying insecticide resistance in *Anopheles* mosquitoes (World Health Organisation, 2025) (Feleke et al., 2025). Various studies report that new parasite strains lacking pfhrp2/3 genes are progressively evolving, rendering them undetectable by commonly used histidine-rich protein 2 (HRP2)-based rapid diagnostic tests (RDTs) (Fuentes et al., 2025). Although the introduction of RTS, S/AS01, and R21/Matrix-M vaccine showcases a turning point in malaria elimination effort, current formulations offer modest efficacy and quite transient immunological memory. (World Health Organisation, 2025) (Florens et al., 2002). In this context, there is an urgent need to prioritise the characterisation of highly conserved

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functionally essential blood-stage antigens with minimal antigenic polymorphism (Lin et al., 2014). PfMSP1 (Merozoite Surface Protein 1), one of the most important functionally essential erythrocytic-stage antigens remains a cornerstone of blood-stage vaccine development. Despite its prominence, there is a critical requirement to map the highly conserved regions within this complex protein, specifically those domains with minimal antigenic polymorphism that have remained poorly understood so far. Targeting the conserved segments of PfMSP1, it is possible to inhibit erythrocyte invasion pathway and to overcome challenges due to immune-evasive variability. we can target the parasite's primary mechanism for erythrocyte entry while bypassing the immune-evasive variability that often hinders broader vaccine efficacy (Lin et al., 2014).

2. THE GAP IN CURRENT KNOWLEDGE

For More than four decades of rigorous study and analysis that resulted in pointing to PfMSP1 as a key candidate for vaccine development, yet no in-depth analysis connects its molecular features to recent preventive shortcomings (Harris et al., 2005). Earlier summaries tended to split the fact into two separate tracks: one exploring genetic diversification in the variable blocks of PfMSP1, while the other assessing results from clinical trials using PfMSP1 fragments such as PfMSP1-42 or PfMSP1-19 as vaccine components (Child et al., 2010). However, such efforts failed to link how the protein's physical makeup influences its exposure to immune attack (Servín-Blanco et al., 2016). In the study of existing reviews, it has been seen that two critical dimensions have remained unexplored:

- **The dynamic structural landscape:** According to the studies regarding the **r**ecent structural and biochemical data, PfMSP1 is established as a highly dynamic structure that undergoes a process that consist two steps of cleavage on the merozoite surface. The Cryo-electron microscopic (an advanced imaging microscopic tool used mostly in structural analysis in the field of biology to visualize biological molecules, viruses, proteins and viruses at near atomic resolution) findings, alongside lab-based tests, point to its role as a flexible framework rather than a fixed structure. These dynamic conformational changes are essential for activating interactions with its host protein Band 3 on the erythrocyte surface (Goel et al., 2003, 1993; Servín-Blanco et al., 2016).
- **The blocking antibody paradox:** It represents a significant challenge in the field of malarial vaccinology, regarding the fact that the protective potential of PfMSP1-19 is functionally undermined by a subset of non-neutralising antibodies. These blocking antibodies possess high affinity for specific epitopes that either overlap with or are sterically adjacent to the binding sites of protective antibodies that neutralise them (Ferreira et al., 2007). Thus, by occupying these critical sites, the blocking antibodies physically preclude the attachment of the inhibitory antibodies, thereby facilitating the process of successful merozoite invasion despite a robust host immune response.

Recent structural evidence suggests that the earlier analyses may have overlooked the influence of the p42 (42 kDa C-terminal fragment of PfMSP1) peptide conformation in the framework of modulating this sequential response. The complex folding and spatial orientation of the p42 polypeptide appear to tilt the immunological balance, preferentially exposing decoy epitopes that elicit interfering antibodies over those that trigger a protective response (Child et al., 2010). This phenomenon is observed both in the context of naturally acquired immunity and in recombinant vaccine trials, where the antigen's structural integrity determines whether the resulting antibody repertoire is predominantly inhibitory or unintentionally permissive to parasitic entry (Gowda, D. C., et al., 2002). Consequently, the efficacy of MSP1-based vaccines depends not merely on high antibody titers but also on a precise epitope-specific dominance that favours neutralisation over steric interference (Child et al., 2010).

3. LITERATURE SEARCH STRATEGY

In order to develop a comprehensive overview of the conformational modification of PfMSP1 and its potential as an emerging vaccine candidate in the context of treating malaria, relevant literature was searched within the Scopus, PubMed, and Google Scholar databases. The literature search strategy was designed to retrieve high-impact research articles and recent developments in the study of PfMSP1 as a vaccine candidate. Instead of relying on the quantitative inclusion/exclusion criteria for the articles, a qualitative approach has been used for data synthesis to focus on peer-reviewed articles that have provided significant insights into the field of molecular biology and immunological impact of PfMSP1. These articles are therefore considered to be the most robust source of information on the role of PfMSP1 as a vaccine candidate. The data synthesis has been conducted according to a standardised thematic framework that aims to maintain the uniformity in reporting information on articles' characteristics, methodologies, and results. Any discrepancies were resolved based on internal consensus to develop a balanced view on the current knowledge of MSP1 of *falciparum* merozoite.

4. MOLECULAR STRUCTURE AND PROTEOLYTIC PROCESSING OF PFMSP1

The *Plasmodium falciparum* Merozoite surface protein 1 (PfMSP1) is the most abundant, complex and surface-exposed *Plasmodium falciparum* protein, which is indeed crucial not only for erythrocyte invasion, but also for host–parasite interactions and parasite survival (Beeson et al., 2016; Lin et al., 2014). Structurally, the PfMSP1 is comprised of multiple interconnected, yet functionally distinct domains (Jean et al., 2005). *Plasmodium falciparum* Merozoite surface protein 1 (PfMSP1) is synthesised initially as a 193 kDa precursor protein that, during merozoite maturation and invasion, undergoes a

series of proteolytic cleavages producing some specific peptide fragments (Child et al., 2010). The structural complexity of PfMSP1 makes it the most desirable target not only for therapeutic but also for vectored vaccine strategies (Ferreira et al., 2007). A subtilisin-like protease (PfSUB1) cleaves the PfMSP1 precursor into four major fragments of 83 kDa, 30 kDa, 38 kDa, and 42 kDa. (Blackman et al., 1990). These fragments remain non-covalently associated with the merozoite surface (Harris, P. K., et al., 2005). The 42 kDa fragment is further processed into PfMSP1-33 (33 kDa) and PfMSP1-19 (19 kDa) during invasion by a secondary cleavage event mediated by the PfSUB1. PfMSP1-19 remains attached by a glycosylphosphatidylinositol (GPI) anchor to the merozoite which enters into erythrocytes, whereas other PfMSP1 fragments are shed off (Figure_ 1). PfMSP1-19 proves to be strongly immunogenic in both human and animal infections (Tanabe et al., 1987). The molecular structure of PfMSP1 provides critical insights into its role in parasite survival and host interactions. (Beeson et al., 2016). The N-terminal domain is highly variable among the *Plasmodium* isolates, suggesting its role in immune evasion. In contrast, the C-terminal PfMSP1-19 fragment, which consists of two epidermal growth factor (EGF)-like domains that are stabilised by disulfide bonds, is remarkably conserved, which indicates its importance in parasite invasion (Bergmann-Leitner et al., 2012; Ferreira et al., 2007; Wilson et al., 2011; Withers-Martinez et al., 2002). According to the crystallographic studies, it has shown that the EGF-like domains of the PfMSP1-19 fragment are positioned to facilitate the process of binding to Band 3, a major surface protein of the erythrocyte. The fragment adopts a compact globular shape (Goel et al., 2003, 1993; Servín-Blanco et al., 2016). On the other hand, the N-terminal variable blocks of *Plasmodium falciparum* Merozoite surface protein 1, that plays a major role in antigenic diversity. Sequence analysis of *Plasmodium falciparum* field isolates from Africa, Southeast Asia, and South America has revealed an extensive allelic variation in the PfMSP1 gene, particularly within the blocks of 2, 4, 6, 16, and 17 (Ferreira et al., 2007). Due to significant genetic variation among parasite strains, a single antibody may not be able to identify the distinct protein variants, which is why the immune responses are frequently strain-specific; other variants may not always be protected (Molina-Franky et al., 2022).

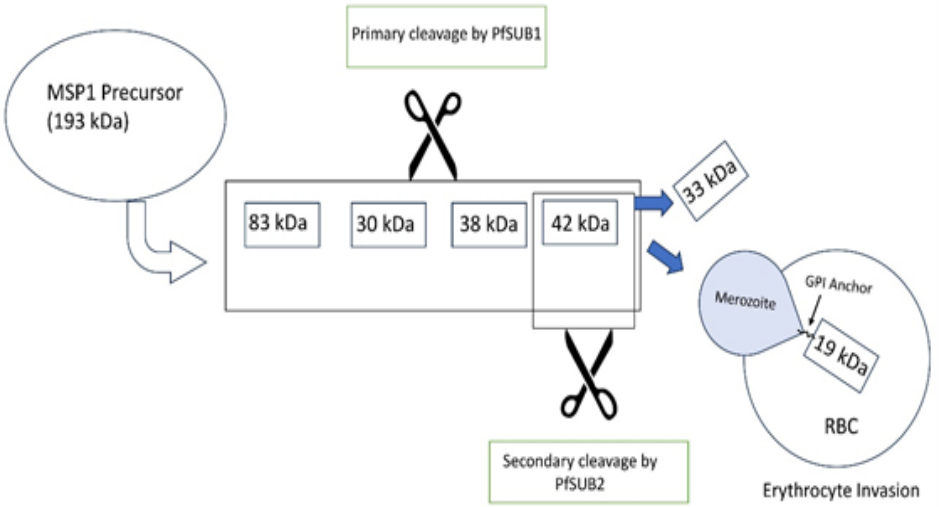

Figure_1 Sequential Proteolytic Processing of the PfMSP1 Precursor during Merozoite Maturation.

The 193 kDa MSP1 precursor undergoes primary cleavage by the protease PfSUB1(*Plasmodium falciparum* Subtilisin-like protease 1**)**, resulting in four major fragments (83, 30, 38, and 42 kDa). Subsequently, the 42 kDa C-terminal fragment is targeted for secondary cleavage by PfSUB1(*Plasmodium falciparum* Subtilisin-like protease 1**)**, yielding a 33 kDa soluble fragment and a 19 kDa membrane-bound fragment. The 19 kDa fragment remains attached to the merozoite surface via a GPI anchor during the invasion of the Red Blood Cell (RBC).

4.1 Functional significance of the GPI anchor in PfMSP1-mediated entry of merozoite in host cell

The glycosylphosphatidylinositol (GPI) anchor is a specialised glycolipid structure that attaches certain proteins to the outer surface of a cell membrane, enabling the *Plasmodium falciparum* Merozoite surface protein 1 (PfMSP1) to move laterally across the parasite surface, thereby promoting strong adhesion of this protein with its host receptor and subsequent invasion (Gowda, 2002). Mechanistically, PfMSP1 localises to lipid rafts, which may facilitate signalling events leading to the release of additional invasion-associated proteins (Child et al., 2010). Immediately prior to complete invasion, the PfMSP1 complex, except for the *Plasmodium falciparum* Merozoite surface protein 1 - 19 (PfMSP1-19) fragment, is proteolytically cleaved (Child et al., 2010). The glycosylphosphatidylinositol (GPI) anchor remains associated with the PfMSP1-19, the only fragment that is carried into the host cell (Li et al., 2008; Blackman et al.,

1991), where sequential proteolysis acts like a switch that turns on the invasion machine (Child et al., 2010; Morales et al., 2015).

4.2 Post-translational Glycosylation: An Immune Evasion Strategy

The spatial extent of the modifications that are experimentally validated in these proteins still remains a subject of debate. However, it has been seen that even minor modifications may significantly influence the folding of *Plasmodium falciparum* Merozoite surface protein 1 (Dugan et al., 2022). The post-translational Glycosylation may serve as an immune evasion mechanism, whereas surface glycans sterically hinder the binding of neutralising antibodies to critical functional regions (Dugan et al., 2022).

Beyond the mechanism of merely blocking antibodies that access through physical obstruction, these surface glycans can also be supported by manipulating the host immune signalling to the parasite's advantage (Dugan et al., 2022), which is made possible by a phenomenon called molecular mimicking that works on the mechanism of mimicking these host-like glycan structures. It explains that *Plasmodium falciparum* can exploit the mechanism of host pattern recognition receptors, such as C-type lectin receptors on dendritic cells and macrophages (Dugan et al., 2022). This interaction often undermines the mechanism of normal immune activation, biasing the host's response towards an anti-inflammatory or tolerogenic state rather than a robust, protective clearance mechanism. Accordingly, the post-translational glycosylation acts as a dual-action shield that not only creates a dynamic, physical barrier against humoral immunity but also biochemically dampens the host's innate capacity to orchestrate an effective attack (Invergo et al., 2017).

4.3 Phosphorylation of PfMSP1: a spatiotemporal regulation of erythrocyte invasion

The phosphorylation of PfMSP1 has progressively been recognised as a critical regulatory signal that governs the transition from merozoite release to host cell attachment (Gilson et al., 2009). As the extracellular lifespan of the merozoite is remarkably transient, rapid post-translational modifications remain essential to optimise the affinity of PfMSP1 for erythrocyte receptors. According to the studies, current evidence suggests that parasite-derived enzymes strategically target this surface antigen to synchronise the mechanical stages of invasion with the functional maturation of the PfMSP1 complex (Child et al., 2010).

This mechanism that includes highly coordinated enzymatic targeting is further underscored by the localised activity of parasite protein kinases, which rapidly phosphorylate specific serine and threonine residues on the PfMSP1 complex upon erythrocyte contact (Gilson et al., 2009) This localized phosphorylation event initiates the process of inducing an allosteric conformational shift, effectively by exposing hidden receptor-binding domains that are otherwise shielded during circulation (Gilson et al., 2009). Thus, by linking the structural activation of PfMSP1 directly to the precise moment of host recognition, the parasite minimises premature exposure of vulnerable epitopes to the host's immune system (Child et al., 2010). Thus, phosphorylation operates not merely as a structural toggle, but as a high-fidelity temporal regulator that aligns the short-lived viability of the merozoite with the immediate biophysical demands of erythrocyte entry (Child et al., 2010).

5. DUAL FUNCTIONALITY OF PFMSP1: ORCHESTRATING ERYTHROCYTE INTERNALIZATION AND HOST IMMUNOMODULATION

Erythrocyte invasion is a highly coordinated, multistep molecular event that comprises a series of procedural steps, which include initial merozoite attachment, apical reorientation, tight junction formation, and subsequent internalisation (Guevara Patiño et al., 1997). PfMSP1 serves as a critical mediator during the attachment and reorientation phases, which facilitates stable interfacial contact between the parasite and the host membrane (Lin et al., 2014). This ligand-receptor interplay is further evidenced by the studies that target the C-terminal PfMSP1-19 fragment by monoclonal antibodies. These research works report on the potent inhibitory effects on parasite entry (Baruch et al., 1995). As per other studies, it is reported that immunisation with MSP1-33 can induce noticeably high antibody titres, showing cross-reactivity with parasite MSP1. Even deletion of the major conserved peptide sequences within MSP1-33 shows minimal effect on its immunogenicity, signifying that these conserved determinants are not major B cell epitopes. Most notably, anti-MSP1-33 antibodies appear to cooperate with anti-MSP1-42 antibodies to prevent *falciparum* parasite growth in vitro. (Siddiqui et al., 2007). Beyond its mechanical role, PfMSP1 functions as a pivotal agent of immunomodulation. During the invasion process, proteolytic shedding of PfMSP1 fragments specifically $PfMSP1_{33}$ and $PfMSP1_{19}$ releases immunogenic peptides that prime both humoral and cellular immune responses (Holder et al., 2009). As a result, these shed decoys or remnants elicit specific antibodies capable of neutralising merozoite entry and triggering complement-mediated parasite lysis, highlighting the dual evolutionary pressure on MSP1 as both an invasion essential and an immunological target (Holder et al., 2009).

This dual evolutionary pressure manifests in the precise structural kinetics that dictate the fate of each processing fragment, especially during the physical transition into the host cell (Baruch et al., 1995). While the synergistic action of anti-PfMSP1-33 and anti-PfMSP1-42 antibodies can disrupt the structural integrity of the complex in vitro, on the other hand, the timing of fragment shedding under natural physiological conditions dictates the protective efficacy of the host's response. As the merozoite achieves apical reorientation and thereby anchors itself via the tight junction, the massive PfMSP1-42 precursor complex is cleaved at a very specific site (Baruch et al., 1995). This cleaving event releases the soluble PfMSP1-33 fragment

into the surrounding bloodstream, while the minimal PfMSP1-19 remains covalently tethered to the parasite membrane via its glycosylphosphatidylinositol (GPI) anchor, entering the erythrocyte interior intact (Lin et al., 2014). Consequently, the host immune system is presented with two radically different target profiles: a highly visible, abundant pool of shed PfMSP1-33 "remnants" acting as an extracellular distraction, and a tightly shielded, rapidly internalised PfMSP1-19 domain that offers only a fleeting window for neutralising antibody engagement (Siddiqui et al., 2007).

Furthermore, the immune-modulatory role of these shed peptides extends beyond simple antibody sequestration; it actively shapes the cellular microenvironment at the interface of infection (Siddiqui et al., 2007). The concentration of the shed PfMSP1-33 and processing byproducts which are localized can engage the pattern recognition receptors on patrolling monocytes and dendritic cells, inducing a hyper-inflammatory cytokine profile characterised by elevated levels of TNF and interleukin-6 (IL-6). While this robust immune priming can drive complement activation and subsequent complement-mediated lysis of free merozoites, it simultaneously acts as a double-edged sword (Lin et al., 2014). The localised inflammatory cascade can cause transient alterations in bystander erythrocyte membranes, indirectly facilitating downstream invasion clusters by destabilising localised host cell clustering. Thus, the deliberate shedding of PfMSP1 fragments serves as a complex metabolic regulatory system—allowing the parasite to fine-tune its mechanical machinery for host entry while systematically manipulating host humoral and cellular defences to sustain overall parasitemia within the high-transmission zone (Lin et al., 2014).

6. GENETIC DIVERSITY AND EVOLUTION OF PFMSP1

In the landscape of malaria immunology, *Plasmodium falciparum* Merozoite Surface Protein 1 remains a primary focal point because of its subtle immunogenicity and essential role in erythrocyte commitment (Angov et al., 2003). As a leading blood-stage vaccine candidate, PfMSP1, particularly the C-terminal PfMSP1-19 fragment, has demonstrated a robust capacity to elicit antibodies which as a result prevent merozoite entry and limit clinical parasitemia (Blank et al., 2020; Burghaus et al., 1994). According to comparative proteomics revealation the C-terminal domain topology is evolutionarily conserved across *Plasmodium* orthologs (e.g., *P. vivax, P. knowlesi*), and significant allelic heterogeneity in the N-terminal region suggests a sophisticated adaptation to host immune pressure. The primary obstacle to a globally effective PfMSP1 vaccine still remains the extensive allelic polymorphism concentrated within the N-terminal blocks. PfMSP1 sequence isolates are traditionally categorized into three major families, namely MAD20, K1, and RO33, that are defined by amino acid substitutions and repetitive motifs that facilitate strain-specific immune escape (Tukwasibwe et al., 2023). This diversity suggests that the antibodies that are generated against a single recombinant variant may lack sufficient cross-reactivity in the high transmission zone, where multiple genotypes coexist (Narain and Nath, 2025). Furthermore, the parasite employs dynamic survival strategies beyond the genetic variation. Not only the continuous proteolytic processing but also the shedding of PfMSP1 during the invasion sequence act as a 'molecular sink' or antigenic decoy, potentially sequestering neutralizing antibodies away from the invading merozoite (Alves et al., 2022; Adepoju et al., 2024). Understanding the interplay between these conserved EGF-like domains and the conformational polymorphism exhibited by the N-terminus is critical for the rational design of next-generation, pan-species malaria vaccines (Alves, K. C. S., et al., 2022; Adepoju, O. J., et al., 2024).

The complex interplay between both the rigid structure and the highly variant nature serves as an indication of the highly advanced evolutionary compromise aimed at capitalizing on the humoral immune response of the host (Angov et al., 2003). Polymorphic N-terminal sequences, such as MAD20, K1, and RO33, play an immunological role by exposing various immunodominant loops and repeats, thereby causing a condition known as “clonotypic restriction” or immunodominance based on strain specificity (Narain and Nath, 2025). The host immune system, in return, repeatedly exhausts its clonal repertoire, generating antibodies tailored to these hyper-variable regions, which become functionally obsolete upon subsequent exposure to a different parasite strain. Meanwhile, the critical erythrocyte-binding operations remain safely mediated by the downstream, structurally sequestered C-terminal PfMSP1-19 fragment (Angov et al., 2003). However, even if the host manages to generate antibodies against this conserved C-terminus, the parasite employs a kinetic evasion strategy: the rapid, sequential cleavage by parasite-derived proteases (such as subtilisin-like protease 1, or SUB1) results in the wholesale shedding of the large N-terminal fragments (Narain and Nath, 2025). This shedding event forms a localised, transient density of non-functional antigens—essentially an antigenic cloud—that physically traps and neutralises surrounding antibodies before they can access the tight junction of the invading merozoite.

In order to bypass this organised deception strategy, modern-day structural vaccinology has moved on to adopt a more "epitope-centric" approach to vaccine formulation, concentrating particularly on the two EGF motifs present within PfMSP1-19 (Narain and Nath, 2025). Because this C-terminal domain relies on a hyper-stabilised, rigid framework of precisely mapped intramolecular disulfide bonds, it is highly sensitive to conformational corruption during recombinant engineering (Angov et al., 2003). Recent structural mapping has revealed that minor misfolding or improper disulfide pairing in synthetic antigens exposes cryptic, non-protective loops. When the immune system targets these distorted configurations, it generates high-affinity "blocking" or "interfering"

antibodies that do not inhibit invasion, but instead sterically block functional neutralising antibodies from binding to the authentic, native epitope. Consequently, resolving these translational bottlenecks demands the execution of precise protein engineering strategies, such as introducing artificial disulfide stapling or mutating flanking residues, to lock the recombinant PfMSP1-19 into its native, biologically active conformation. Thus, by selectively silencing these decoys and suppressing the presentation of non-neutralising surfaces, the study aim to optimise vaccine formulations capable of driving strain-transcending, pan-species protection across highly diverse endemic populations (Angov et al., 2003).

7. HOST IMMUNE RESPONSE TO PFMSP1

PfMSP1 is recognised by the immune system during the asexual blood stage of malaria infection, when merozoites are released from infected erythrocytes and exposed to circulating immune components (Lee et al., 2001), both humoral and cell-mediated immune responses are generated against PfMSP1 (Dent et al., 2016). The conserved C-terminal fragment, PfMSP1-19, elicits antibodies that constitute the predominant component of the humoral response and exhibit inhibitory, opsonising, and complement-fixing activities (Dharanivasan et al., 2016). According to the longitudinal studies that were conducted in endemic settings a positive correlation between levels of naturally acquired anti-PfMSP1-19 antibodies and protection against clinical malaria was found (Dharanivasan et al., 2016).

The mechanisms of antibody-mediated immunity associated with PfMSP1 include:

(i) The process of functional inhibition of erythrocyte invasion by blocking interactions between parasite ligands and host cell receptors (Healer et al., 2015).

(ii) Opsonization of merozoites in order to promote phagocytosis (Boyle et al., 2015)

(iii) Activation of the classical complement pathway, resulting in parasite lysis (Gadi et al., 2019)

(iv) Among these, the inhibitory antibody responses directed against conformational epitopes on the PfMSP1-19 fragment show the strongest association with protective immunity.

In the context of humoral immune responses, PfMSP1 also elicits the T-cell responses, particularly involving $CD4^+$ T helper cells, which is responsible for promoting antibody production and cytokine secretion (Parra et al., 2000). Several overlapping epitopes within PfMSP1-33 and PfMSP1-42 have been identified as strong T-cell activators, inducing Th1 and Th2 cytokine responses that enhance B-cell activation (Takala-Harrison et al., 2015). The identification of correlating immune protection is a persistent challenge in PfMSP1 vaccine development (Kaslow et al., 2024). Although increased antibody titers against PfMSP1-19 are associated with enhanced protection, not all antibodies confer protective immunity (Crowley and Ackerman, 2023). Protective immunity is further influenced by antibody functionality, including affinity, isotype distribution, and complement-activating capacity (Gunn et.al., 2016). These studies have shown that cytophilic IgG1 and IgG3 isotypes are the most effective in mediating opsonic phagocytosis and complement activation (Hinke et al., 2024). In addition, inter-individual variability in antibody responses, influenced by HLA haplotypes and immune polymorphisms, can further complicate the evaluation of vaccine efficacy. Additionally, naturally acquired immunity is often short-lived, necessitating repeated exposure to maintain antibody levels. Therefore, the inclusion of T-cell epitopes that promote the generation of long-lived plasma cells and memory B cells is critical (Takala-Harrison et al., 2015). Multi-stage vaccine approaches that combine pre-erythrocytic and blood-stage antigens (e.g., CSP and PfMSP1) are being investigated to enhance the breadth of protection (Langowski et al., 2025).

8. PFMSP1-BASED VACCINE DEVELOPMENT

Multiple vaccine candidates that are based on PfMSP1 and its fragments have undergone extensive preclinical and clinical evaluation (Adepoju, O. J., et al., 2024). Early vaccine strategies focused on using intact PfMSP1-42 and C-terminal (CT) fragments to induce antibodies capable of inhibiting parasite invasion (Parra et al., 2000). FMP1/AS02A vaccine includes a recombinant PfMSP1-42 antigen, which is formulated with the AS02A adjuvant, and it has been tested in clinical trials in endemic populations (Thera et al., 2011). Though the vaccine elicited high antibody titers, it showed limited and strain-specific protection (Healer et al., 2015).

The recent formulations aim to improve the immunogenicity by replacing the larger PfMSP1-42 construct with the smaller PfMSP1-19 fragment (Jain et al., 2014), because this candidate preserves the key epitopes that are necessary for generating protective antibodies while reducing the antigenic variability. Recombinant $PfMSP1_{19}$ has been expressed in bacterial, yeast, and mammalian systems and has been shown to induce strong immune responses in malaria animal models (Perraut et al., 2015). In murine models, immunisation with recombinant PfMSP1-19 formulated with adjuvants such as AS01 or Montanide ISA720 elicited strong antibody responses and provided partial protection against challenge with *Plasmodium yoelii* (Lee et al., 2001).

Recent advances in epitope mapping and structural vaccinology have improved the design of PfMSP1-based vaccine strategies (Table 1). The mapping of conserved conformational epitopes within the EGF-like domains of PfMSP1-19 has enabled the development of peptide-based vaccines that can induce the strain-transcending neutralising antibodies against *Plasmodium falciparum*. The recent studies indicate that achieving high vaccine efficacy depends not only on the quantity of antibody response but also on its functional quality, specifically on its ability to target conserved regions and avoid

interference from non-protective immune responses (Tang et al., 2023). Additionally, the multi-epitope chimeric constructs incorporating PfMSP1 along with PfAMA1 and PfEBA175 have shown enhanced potential for protective immunity. Recent research highlights a paradigm shift toward chimeric, multi-stage vaccines that combine epitopes from multiple parasite proteins to produce broader and more durable immune protection (Mazumdar et al., 2010) (Figure_2) (Table 2).

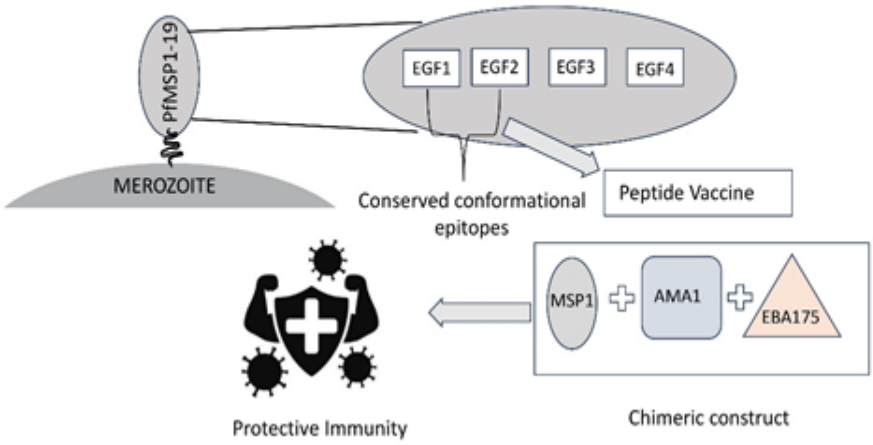

Figure_2. Strategic development of a chimeric vaccine construct targeting *Plasmodium falciparum* invasion antigens.

The membrane-anchored PfMSP1-19 fragment contains two Epidermal Growth Factor-like (EGF) domains. The conserved conformational epitopes within these domains serve as the basis for peptide vaccine design. In order to enhance protective immunity, these MSP1 components are integrated with other key invasion ligands, such as AMA1 (Apical Membrane Antigen 1) and EBA175 (Erythrocyte Binding Antigen 175), to form a multivalent chimeric construct.

The immunogenicity of PfMSP1-based vaccines is strongly influenced by the choice of adjuvant and delivery systems (Takala-Harrison et al., 2015), whereas traditional adjuvants (e.g., alum) promote strong antibody responses but induce limited cell-mediated immunity (Kool et al., 2012). The newer adjuvant and delivery systems (e.g., AS01, AS02, and CpG oligodeoxynucleotides) can induce both humoral and cellular immune responses by activating the innate immune system (Pulendran, B. et al., 2021). Additionally, Toll-like receptor (TLR) agonists, such as monophosphoryl lipid A (MPLA) and saponin QS-21, can promote more balanced Th1/Th2 immune responses (Burghaus and Holder, 1994)). In recent research it has been highlighted that vaccine efficacy depends not only on the antigen but also on the adjuvant, which functions as an immune activation signal (Foley et al., 2016; Yadagiri et al., 2026).

Recent advances in recombinant vector systems and nanotechnology have expanded the possibilities for antigen delivery (Ludwig et al., 2024). For example, virus-like particles (VLPs), liposomes, and biodegradable nanoparticles have been explored as delivery platforms for PfMSP1 antigens in murine models, that play its essential role in enhancing antigen stability and immunogenicity (Memvanga et.al., 2021). Conjugation of PfMSP1-19 to hepatitis B surface antigen-based VLPs has resulted in generating strong antibody responses with enhanced avidity compared to soluble protein formulations (McConkey et al., 2003; Pusic et al., 2013). The other vector platforms, such as adenoviral vectors and modified vaccinia Ankara (MVA) expressing the PfMSP1 fragments, have resulted in the demonstration of enhanced immunogenicity not only by boosting cytotoxic T-cell responses but also by stimulating antibody production (Supplementary table 1). Advances in the fields of computational biology, structural modelling, and immune-informatics have transformed the landscape of PfMSP1 vaccine development (Lee et al., 2022; Chackerian et al., 2020) by the predictive algorithms that are capable of screening and evaluating the binding affinity of conserved epitopes across common HLA alleles (Kahatano et al., 2025; Pendyala et al., 2023). Molecular dynamics simulations provide insights into conformational flexibility and epitope accessibility (Patel et al., 2023), whereas reverse vaccinology and AI-based approaches have significantly enhanced the identification of novel epitope combinations capable of inducing broad-spectrum protection (Koné et al., 2025). The integration of computational tools with experimental immunology has enabled the development of next-generation PfMSP1-based vaccines that are cross-protective and structurally stabilised. Techniques such as epitope grafting enable the presentation of critical motifs within heterologous scaffolds while preserving native-like conformation and immunogenicity (Salinas et al., 2023; Syeda et al., 2024). Multi-epitope nanoparticle formulations incorporating conserved PfMSP1 epitopes in repetitive arrays have demonstrated enhanced antibody avidity and breadth (Xu et al., 2024; Poland et al., 2004). Alongside immune recognition, the vaccine development targeting PfMSP1

represents a complex interplay among parasite diversity, host immunity, and technological innovation played a major role (Aung et al., 2024; Bhatt and Sharma, 2023). Antigenic polymorphism and immune evasion remain significant obstacles; however, recent advances in the field of molecular design, adjuvant technology, and computational vaccinology have brought the development of an effective PfMSP1 vaccine closer to realisation (Larbi et al., 2025) (Table 3).

8.1 Early vaccine development approaches using PfMSP1:

Early vaccine development has delivered its efforts utilising recombinant forms of PfMSP1 fragments to induce antibody-mediated immunity (Darko et al., 2005). Preclinical trials using recombinant PfMSP1-19 and PfMSP1-42 proteins in rodent and non-human primate models demonstrated partial protection, including reduced parasitemia and delayed onset of infection (Alves, K. C. S., et al., 2022). The development of early vaccines has delivered its efforts by utilising the recombinant forms of PfMSP1 fragments to induce antibody-mediated immunity (Darkoet al., 2005). The preclinical trials with the usage of recombinant PfMSP1-19 and PfMSP1-42 proteins in rodent and non-human primate models demonstrated partial protection, which includes reduced parasitemia and delayed onset of infection (Alves, K. C. S., et al., 2022). FMP1/AS02A, which is considered to be one of the first human malaria vaccines, consisted of a recombinant PfMSP1-42 antigen that was formulated with the AS02A adjuvant (Ogutu, B. R., et al., 2009). A limited efficacy was noticed, which was likely due to strain-specific antigenic variation and the short-lived nature of the antibody response (Kahatanoet al., 2025). Thus, with the help of subsequent studies that focused on improving antigen presentation and enhancing the durability of immune responses (You et al., 2024). The chimeric constructs, which were done by combining PfMSP1 fragments with other malaria antigens, such as Apical Membrane Antigen 1 (AMA1), Erythrocyte Binding Antigen 175 (EBA175), and MSP3, have been developed (Cavanagh et al., 2011). These approaches' main aim is to achieve innovation in two areas: to broaden the immune response and to overcome strain-specific variability. For example, fusion constructs such as PfMSP1-19-AMA1 have obtained both stronger antibody responses and improved inhibition of merozoite invasion compared to single-antigen vaccines (Stump et al., 2024). The advances made in recombinant protein expression systems, including yeast (*Pichia pastoris*), insect-cell (baculovirus), and mammalian platforms, have facilitated the production of properly folded and structurally stable PfMSP1 proteins with native disulfide bonds, which is critical for preserving conformational epitopes (Stump, N. et al., 2024). Modern adjuvants, such as AS01, GLA-SE, and Matrix-M, have been employed to enhance balanced Th1/Th2 immune responses and prolong antibody persistence (Beeson et al., 2016).

8.2 Multi-antigen and multi-stage vaccine development strategies incorporating PfMSP1:

Due to the high genetic variability of the parasite, recent studies have focused on multi-stage vaccine strategies targeting different phases of the parasite life cycle (Khan et al., 2024). The combination of PfMSP1 with pre-erythrocytic antigens, such as circumsporozoite protein (CSP) or thrombospondin-related anonymous protein (TRAP), might result in enhancement of protective immunity by eliciting responses at multiple stages of infection (Angov et al., 2003). Alongside that, the innovative delivery platforms, including viral vectors and mRNA-based vaccines encoding PfMSP1, have shown promising results in early studies by inducing robust T-cell and antibody responses. The incorporation of PfMSP1 epitopes into virus-like particles (VLPs) or lipid nanoparticles represents an advanced approach to vaccine delivery (Beeson et al., 2016). These delivery systems not only result in enhancing the antigen stability but also facilitate the process of cellular uptake and promote the sustained immune responses (Beeson et al., 2016). The preliminary studies indicate that the mRNA-based PfMSP1 vaccines elicit immune responses that are comparable to protein-based formulations, with the added advantage of rapid adaptability and design flexibility (MacMillen et al., 2024) (Supplementary Table 2).

9. CHALLENGES AND LIMITATIONS

Despite recent advances, several challenges remain before PfMSP1 targeted strategies that is blocking its function to be translated into clinical practice (Angov et al., 2003). The extensive genetic polymorphism in the PfMSP1 gene, particularly within the block 2 region, represents a major obstacle to the development of an effective vaccine (Polley et al., 2003). This variability results in strain-specific immune responses and limits cross-protective vaccine efficacy. Antigenic variation further complicates the development of broadly protective vaccine formulations (Tobin et al., 2008). Naturally acquired immune responses against PfMSP1 are often short-lived and non-sterilising, necessitating repeated booster doses or the use of potent adjuvant systems to sustain protective immunity (Angov et al., 2003). Challenges related to large-scale production and maintaining the conformational stability of recombinant PfMSP1 proteins remain significant, particularly in resource-limited settings (Burghaus and Holder, 1994)).

Thus, by compounding these structural and immunological hurdles, the intricate spatial and temporal window is required for neutralising the antibodies in order to effectively intercept the protein. This is for the fact that *Plasmodium falciparum* Merozoite Surface Protein 1 (PfMSP1) undergoes a complex cascade of proteolytic processing events immediately before and during invasion, a vaccine must elicit antibodies that not only recognise the polymorphic full-length antigen but also structurally conserved, transiently exposed cleavage fragments like MSP1-19 (Angov et al., 2003). If the immune response fails to target these precise conformational states with high affinity, the parasite can rapidly shed the heavily targeted

portions of the complex, rendering the antibody response ineffective (Burghaus and Holder, 1994). Therefore, addressing the problem of bringing efficacy to clinical reality requires that we move toward multiple antigens/alleles that would be able not only to consider geographic diversity but also to keep pace with the speed of infection and remain chemically stable at cold temperatures (Tobin et al., 2008).

10. FUTURE PERSPECTIVES

The recent advancement in the field of computational vaccinology, particularly in reverse vaccinology and multi-epitope design, has opened new and essential avenues to address challenges in malaria vaccine development (Ahlawat et al., 2024). Immunoinformatics-based identification of conserved B-cell and T-cell epitopes within PfMSP1 protein sequence has enabled the design of peptide-based vaccines capable of providing cross-strain protection (Rodríguez et.al., 2023). Incorporation of PfMSP1 epitopes into nanoparticle or viral vector platforms may have the chances of enhancing immunogenicity and reducing the required antigen dose (Mukhiya et al., 2024). Furthermore, with the success of mRNA-based vaccines during the COVID-19 pandemic highlights their potential applicability to malaria (Matarazzo and Bettencourt, 2023), which has resulted in the rapid production and scalability of mRNA platforms enabling the inclusion of multiple PfMSP1 variants within a single formulation, thereby promoting broad strain coverage (Lee et al., 2022).

In addition to the increased coverage within the area of variants, these new-generation platforms help in optimising the structural presentation of PfMSP1 epitopes (Lee et al., 2022). By utilising these machine-learning algorithms that predict not only the structural stability but also the MHC-binding affinities, researchers can engineer multi-epitope constructs that bypass the highly polymorphic, non-protective regions of the antigen while focusing the host immune response on highly conserved, functional domains (Ahlawat et al., 2024). When this is delivered via self-assembling nanoparticle matrices or lipid nanoparticle (LNP)-encapsulated mRNA, these computationally optimised epitopes closely mimic the dense, repetitive geometric arrays typical of native viral or bacterial surfaces (Rodríguez et.al., 2023). This architectural arrangement triggers the robust B-cell receptor cross-linking and potent T-follicular helper cell activation, ultimately generating long-lived plasma cells and high-titer neutralizing antibodies (Lee et al., 2022). Consequently, the convergence of immunoinformatics and advanced delivery systems provides a scalable blueprint to systematically overcome the historical bottlenecks of antigenic variation and poor immunogenicity that have long hindered PfMSP1-based vaccine candidates (Lee et al., 2022).

11. CONCLUSION

Malaria still endured as a major global health challenge, with *Plasmodium falciparum* being the most virulent human-infecting species. Despite advances in the area of molecular parasitology, the parasite's genomic plasticity and antigenic diversity continue to impede effective control strategies. Among all the candidate targets, *Plasmodium falciparum* Merozoite Surface Protein 1 (PfMSP1) remains one of the most extensively studied due to its essential role in erythrocyte invasion and its strong immunogenic properties. However, extensive polymorphism, particularly within the N-terminal regions, limits the development of broadly protective vaccines, while naturally acquired immunity remains short-lived and strain-specific. The emergence of resistance to the frontline antimalarials, which are driven by mutations in key genes such as pfcrt, pfmdr1, and kelch13, this results in complicated disease management. As per the suggestions of current evidence, the future strategies should prioritise multi-antigen and multi-stage vaccine designs, which include the integration of PfMSP1, not only with conserved targets such as AMA1, EBA175, or RH5, but also with the involvement of advanced delivery platforms and adjuvant systems. In addition, the improved characterisation of immune correlates of protection and functional antibody responses remains critical. Emerging technologies, including structural vaccinology, immune-informatics, and genome editing, offer promising tools for rational antigen design and functional validation.

The successful translation of the PfMSP1-based interventions will depend on critical points such as interdisciplinary research, standardised clinical evaluation, and sustained global collaboration. Addressing antigenic diversity and optimising immune durability is crucial to exploring PfMSP1 as a viable target in malaria control and eradication efforts.

CRediT authorship contribution statement

Soumyanil Chakraborty: Writing & editing original draft, **Tamalika Chakraborty:** Editing original draft, **Sobhanjan Bhunia:** Editing original draft, **Sharmistha Ghoshal:** Editing original draft, conceptualization, supervision.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

No data was used for the research described in the article.

ACKNOWLEDGEMENTS
Authors would like to express sincere gratitude to Prof. Dr. Sanghamitra Sengupta, Department of Biochemistry, University of Calcutta, and all the faculty members of Guru Nanak Institute of Pharmaceutical Science and Technology for their invaluable support for the preparation of this review.

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Table 1: Summary of PfMSP1-Based Vaccine Candidates

Type of Study	Country / System	Sample / Model	Methodology	Antigen / Vaccine Type	Conclusion	Ref
Clinical Trial	Endemic regions	Human populations	FMP1 formulated with AS02A adjuvant	Intact PfMSP1-42	Induced high antibody titers but demonstrated limited and strain-specific protection.	Thera et al. (2011)
Preclinical	Bacterial, yeast, and mammalian systems	Animal models	Recombinant expression and immunisation	PfMSP1-19 fragment	Induced robust immune responses; smaller fragment retains key epitopes while reducing variability.	Thera et al. (2011)
Preclinical	Murine model	Mice (Plasmodium yoelii challenge)	Immunisation with AS01 or Montanide ISA720 adjuvants	Recombinant PfMSP1-19	Generated strong antibody responses and demonstrated partial protection against parasite challenge.	Lee et al. (2001)
Review / Analysis	General	Various vaccine candidates	Extensive preclinical and clinical evaluation	PfMSP1 and fragments	Multiple candidates have undergone evaluation; recent focus is on epitope mapping and structural vaccinology.	Adepoju et al. (2024); Parra et al. (2000)

Table 2: Advanced vaccine strategies and epitope mapping of $PfMSP1_{19}$.

Research Category	Target Protein/Site	Study Model	Analysis Method	Vaccine Construct	Key Findings	Ref
Epitope Mapping	*P. falciparum*	Conserved EGF-like domains	Conformational mapping	Peptide-based PfMSP1-19	Mapping enabled the development of vaccines that induce strain-transcending neutralising antibodies.	Tang et al. (2023)
Immune Profiling	Molecular Level	Antibody quality assessment	Functional vs. Quantitative analysis	PfMSP1-19 (Conserved regions)	Efficacy depends on targeting conserved regions and avoiding interference from non-protective responses.	Tang et al. (2023)
Vaccine Engineering	Multi-stage	Multi-epitope chimeric constructs	Antigenic fusion (AMA1/EBA175)	Chimeric (PfMSP1, AMA1, EBA175)	Demonstrated enhanced potential for protective immunity compared to single-antigen models.	Mazumdar et al. (2010)
Strategic Review	Global Research	Systematic review	Multi-stage vaccine engineering	Chimeric, multi-stage vaccines	Highlighted a paradigm shift toward combining epitopes from multiple proteins for broader protection.	Mazumdar et al. (2010)

Table 3: Computational modeling and structural vaccinology in PfMSP1 development.

Methodology / Approach	Target Metric	Application	Study Model	Vaccine Design	Conclusion	Ref
Immunomics	HLA binding affinity	Predictive algorithms	In silico screening	Conserved epitopes	Algorithms identify epitopes across common HLA alleles for broad-spectrum protection.	Minassian et al. (2021); Pendyala et al. (2023)
Structural Modelling	Conformational flexibility	Molecular dynamics	Simulation-based	PfMSP1 Fragments	Simulations provide critical insights into epitope accessibility and structural stability.	Lee et al. (2022)
Reverse Vaccinology	Novel combinations	AI-based design	AI-integrated immunology	Multi-epitope vaccines	Integration of AI enhances the identification of combinations that induce cross-protection.	Koné et al. (2025)
Protein Engineering	Native conformation	Epitope grafting	Heterologous scaffolds	Structurally stabilized PfMSP1	Grafting preserves critical motifs within scaffolds to maintain native-like immunogenicity.	Salinas et al. (2023); Syeda et al. (2024)
Nanotechnology	Antibody avidity	Repetitive arrays	Nanoparticle formulation	Multi-epitope nanoparticles	Presentation of epitopes in repetitive arrays enhances the breadth and strength of the immune response.	Xu et al. (2024)
Strategic Integration	Immune evasion	Comprehensive review	Experimental immunology	Next-generation PfMSP1	Advances in molecular design and adjuvants are overcoming antigenic polymorphism obstacles.	Larbi et al. (2025); Aung et al. (2024)

Supplementary table 1

Impact of adjuvants and delivery systems on PfMSP1-based vaccine immunogenicity.

Study Category	Delivery System / Platform	Immune Target	Methodology / Adjuvant	Antigen Form	Conclusion	Ref
Adjuvant Analysis	Traditional vs. Modern	Humoral & Cellular immunity	Alum vs. AS01, AS02, CpG	PfMSP1-based	Modern adjuvants activate innate immunity, inducing both antibody and cell-mediated responses.	Harrison et al. (2024); Pulendran et al. (2022)
Immune Modulation	TLR Agonists	Th1/Th2 balance	MPLA and Saponin QS-21	PfMSP1-based	TLR agonists promote a more balanced immune response; efficacy depends on adjuvant signalling.	Pulendran et al. (2022
Nanotechnology	VLPs & Nanoparticles	Antigen stability/Avidity	Hepatitis B surface antigen VLP	PfMSP1-19 (Conjugated)	VLPs and nanoparticles enhance stability and generate higher antibody avidity than soluble proteins.	Memvanga (2021); McConkey et al. (2003)

Vector Platforms	Recombinant Vectors	Cytotoxic T-cell response	Adenoviral & MVA vectors	PfMSP1 fragments	Viral vectors boost T-cell responses while simultaneously stimulating high antibody production.	Ludwig et al. (2024)

Supplementary table 2

Multi-stage strategies and innovative delivery platforms for PfMSP1 vaccines**.**

Research Strategy	Target Phase	Study Model	Delivery Platform	Antigen Combination	Key Findings	Ref
Multi-stage Vaccine	Pre-erythrocytic & Erythrocytic	Parasite Life Cycle	Combined formulations	PfMSP1 + CSP / TRAP	Targeting multiple stages enhances protective immunity by eliciting responses across the infection cycle.	Khan et al. (2024); Angov et al. (2003)
Next-Gen Delivery	Cellular Uptake	Murine/Early Studies	mRNA-based vaccines	mRNA-encoded PfMSP1	Elicits robust T-cell and antibody responses with advantages in design flexibility and rapid adaptability.	MacMillen et al. (2024)
Advanced Scaffolding	Antigen Stability	Early Studies	VLPs / Lipid Nanoparticles	PfMSP1 Epitopes	These platforms facilitate cellular uptake and promote sustained, high-titer immune responses.	Beeson et al. (2016)
Platform Comparison	Comparative Immunology	Preliminary trials	mRNA vs. Protein-based	PfMSP1 fragments	mRNA-based formulations elicit immune responses comparable to those elicited by traditional protein-based vaccines.	MacMillen et al. (2024)