

REVIEW ARTICLE

A Review on Novel Recombinant Chimeric Multi-Stage AdFalciVax Vaccine for *Plasmodium falciparum* Malaria



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Abstract: Malaria remains a persistent public health emergency, with *Plasmodium falciparum* responsible for the vast majority of severe morbidity and mortality worldwide. While recent approvals of pre-erythrocytic vaccines mark significant progress, the complex life cycle of the parasite necessitates next-generation tools capable of interrupting transmission dynamics more effectively. AdFalciVax, an indigenous multi-stage vaccine candidate developed by the Indian Council of Medical Research (ICMR) and its collaborative network, represents a strategic innovation in this domain. This recombinant chimeric vaccine is engineered using a food-grade *Lactococcus lactis* expression platform, distinguishing it from traditional yeast or mammalian cell systems. The construct integrates the full-length circumsporozoite protein (PfCSP) to target the pre-erythrocytic infection stage, alongside a fusion of Pfs230 and Pfs48/45 proteins designed to elicit transmission-blocking immunity in the mosquito vector. Preclinical evaluations indicate that this dual-targeting mechanism not only induces robust antibody responses but also offers significant logistical advantages, including enhanced thermostability and cost-effective scalability. AdFalciVax aligns with global malaria eradication goals by addressing both the infection of the human host and the transmission to the anopheline vector. This article critically analyzes the design, preclinical immunogenicity, production advantages, and the potential clinical positioning of AdFalciVax within the broader context of malaria elimination strategies.

Keywords: *Plasmodium falciparum*; AdFalciVax; Transmission-blocking vaccine; *Lactococcus lactis*; Recombinant chimeric antigen

1. Introduction

Malaria constitutes one of the most resilient parasitic diseases affecting humanity, disproportionately impacting tropical and subtropical regions. Despite decades of concerted control efforts involving long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and artemisinin-based combination therapies (ACTs), the global burden remains staggeringly high. The World Health Organization (WHO) reported approximately 249 million cases and over 600,000 deaths in 2023, with the African region bearing the brunt of this mortality [1]. The etiological agent *Plasmodium falciparum* is responsible for the most severe clinical presentations, including cerebral malaria and severe anemia, making it the primary target for vaccine development efforts.

The biological complexity of the *Plasmodium* life cycle presents a unique challenge for immunological interventions. The parasite transitions through distinct developmental stages in both the human host and the *Anopheles* mosquito vector, each characterized by stage-specific antigen expression and sophisticated immune evasion mechanisms. Historically, vaccine development has focused predominantly on the pre-erythrocytic stage to prevent hepatocyte infection and subsequent blood-stage disease. This strategy culminated in the development of RTS,S/AS01 and R21/Matrix-M, both of which target the circumsporozoite protein (CSP) [2]. While these vaccines have achieved WHO prequalification and demonstrate public health value, their protection is partial and wanes over time, necessitating the exploration of multi-stage candidates that can provide broader, more durable immunity.

AdFalciVax has emerged as a promising candidate in this area. It is developed through a consortium led by the ICMR-Regional Medical Research Centre (RMRC) Bhubaneswar, in partnership with the National Institute of Malaria Research (NIMR) and the National Institute of Immunology (NII), this vaccine employs a novel approach. It utilizes a *Lactococcus lactis* expression system to produce a chimeric protein combining pre-erythrocytic antigens with sexual-stage transmission-blocking antigens [3]. This review

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provides the scientific rationale, manufacturing methodology, and preclinical data supporting AdFalciVax, positioning it as a potentially critical tool for future malaria elimination campaigns.

2. Vaccine Design and Molecular Architecture

2.1. Genetic Construction and Antigen Selection

The structural efficacy of AdFalciVax lies in its chimeric design, which physically links antigens from disparate life stages of the parasite to induce a multi-pronged immune response. This approach addresses the limitation of single-antigen vaccines by targeting multiple bottlenecks in the parasite's life cycle.

2.1.1. The Pre-erythrocytic Component: PfCSP

The primary component targeting the human host is the full-length *P. falciparum* circumsporozoite protein (PfCSP), derived from the 3D7 strain. PfCSP is the dominant surface protein on sporozoites and plays a critical role in their motility and hepatocyte invasion. By incorporating the full-length sequence, the vaccine design aims to elicit high-titer antibodies that bind to sporozoites immediately after inoculation by the mosquito. This neutralization prevents the sporozoites from reaching the liver, thereby aborting the infection before the symptomatic blood stage can occur [4]. The inclusion of T-cell epitopes within the full-length protein further enhances the potential for a cellular immune response, which is vital for clearing infected hepatocytes.

Table 1. Functional Roles of Antigenic Components in AdFalciVax

Antigen Component	Biological Origin	Parasite Life Stage	Physiological Function in Parasite	Immunological Goal in AdFalciVax
PfCSP (Full length)	<i>P. falciparum</i> (3D7 strain)	Sporozoite	Motility and hepatocyte invasion	Induce antibodies to neutralize sporozoites and prevent liver infection (Personal Protection).
Pfs230	<i>P. falciparum</i>	Gametocyte / Gamete	Gamete emergence and fertilization	Induce antibodies that are ingested by mosquitoes, binding to gametes to block fertilization (Transmission Blocking).
Pfs48/45	<i>P. falciparum</i>	Gametocyte / Gamete	Anchoring protein on gamete surface	Induce antibodies that prevent gamete fusion in the mosquito midgut (Transmission Blocking).

2.1.2. The Sexual-Stage Component: Pfs230 and Pfs48/45

To augment the protective mechanism, the vaccine design includes a fusion of two potent sexual-stage antigens: Pfs230 and Pfs48/45. These proteins are expressed on the surface of gametocytes and gametes within the mosquito midgut but are not targets of natural immunity in the human host. The fusion strategy links these two transmission-blocking candidates to ensure their co-expression and stability. When a mosquito takes a blood meal from a vaccinated individual, it ingests antibodies specific to these antigens. These antibodies then bind to the gametes in the mosquito midgut, inhibiting fertilization and subsequent oocyst development [5]. This transmission-blocking activity is crucial for reducing the overall parasite reservoir in the community.

2.2. The *Lactococcus lactis* Expression System

A distinguishing feature of AdFalciVax is its reliance on *Lactococcus lactis*, a Gram-positive, food-grade bacterium, as the expression host. This choice represents a significant departure from traditional eukaryotic systems.

2.2.1. Advantages of a Food-Grade Bacterial Platform

Lactococcus lactis is generally regarded as safe (GRAS) and has a long history of use in the food industry. Unlike *Escherichia coli* systems, which often produce endotoxins (lipopolysaccharides) that require extensive and costly purification steps to remove, *L. lactis* is endotoxin-free. This characteristic simplifies the downstream processing significantly, reducing both the cost and complexity of manufacturing. Furthermore, *L. lactis* has the ability to secrete recombinant proteins directly into the culture medium or display them on the cell surface, which can facilitate easier recovery and purification compared to intracellular expression systems [6].

2.2.2. Nisin-Inducible Expression Mechanism

The genetic construct for AdFalciVax was cloned into the pNZ8148 expression vector utilizing a nisin-inducible promoter system, known as the NICE (Nisin Controlled Expression) system. This system allows for tightly regulated gene expression. During the fermentation process, the bacterial biomass can be grown to high densities before protein production is initiated by the addition of nisin, a peptide bacteriocin. This temporal separation of growth and production phases minimizes the metabolic burden on the host cells and prevents the potential toxicity of the recombinant protein, ensuring optimal yield and stability of the chimeric antigen [7]

Table 2. Advantages of the *Lactococcus lactis* Expression Platform

Parameter	<i>Lactococcus lactis</i> (AdFalciVax)	<i>Escherichia coli</i> (Standard Bacterial)	Mammalian/Yeast Systems (Standard Eukaryotic)
Bio-Safety Status	GRAS (Generally Regarded As Safe)	Requires careful strain selection	Safe but complex
Endotoxin Presence	Endotoxin-Free	High (Requires expensive removal)	Absent
Protein Folding	Good for complex membrane proteins	Often forms inclusion bodies (refolding needed)	Excellent (Post-translational modifications)
Downstream Processing	Simplified (Secreted or Cell-surface)	Complex (Lysis + Purification)	Complex (Requires expensive media)
Cost of Production	Low	Low to Medium	High
Scalability	High (Simple fermentation)	High	Medium to High

3. Preclinical Development and Immunogenicity

3.1. Production and Purification Protocols

The manufacturing process for AdFalciVax was rigorously standardized to ensure reproducibility, high purity, and structural fidelity of the chimeric protein.

3.1.1. Fermentation and Induction Strategies

Recombinant *L. lactis* NZ9000 strains harboring the vaccine construct were cultured in M17 broth supplemented with 0.5% glucose and chloramphenicol to maintain plasmid stability. The fermentation was conducted at 30°C under static conditions, which is optimal for *L. lactis* growth. Protein expression was induced at the mid-logarithmic growth phase (OD600 = 0.6) using 10 ng/mL of nisin. The culture was then incubated for an additional four hours to allow for maximal protein synthesis. This optimized protocol ensured a high specific yield of the recombinant fusion protein [8].

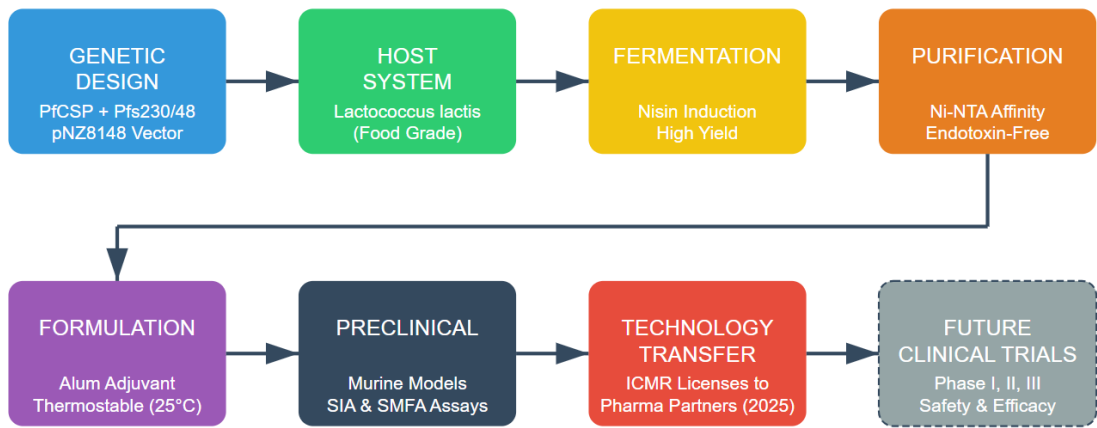


Figure 1. AdFalciVax Development & Manufacturing

(The use of the *Lactococcus lactis* (NICE) expression system, which allows for cost-effective, endotoxin-free production. The process culminates in a thermostable formulation that has recently undergone technology transfer to Indian pharmaceutical companies for scale-up and clinical testing)

3.1.2. Downstream Processing and Characterization

Following cell harvest, the protein was extracted using a lysis buffer and purified under native conditions utilizing Ni²⁺-NTA affinity chromatography. This method exploits the affinity of a histidine tag on the recombinant protein for nickel ions, allowing for efficient separation from host cell proteins. The eluted fractions were analyzed via SDS-PAGE to confirm purity and molecular weight. Western blotting using monoclonal antibodies specific to PfCSP, Pfs230, and Pfs48/45 confirmed the antigenic integrity of the fusion protein, verifying that the conformational epitopes required for antibody binding were preserved during expression and purification [9].

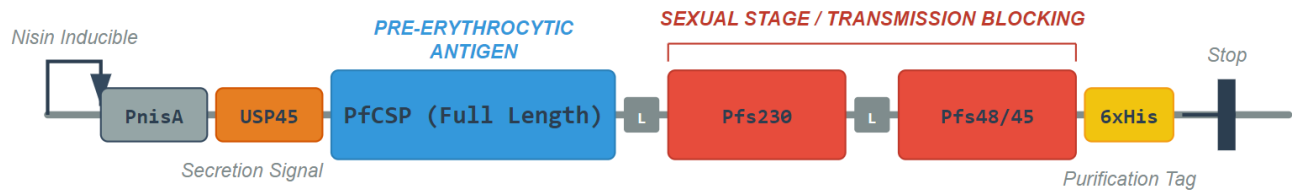


Figure 2. Genetic Composition of the Chimeric Recombinant Protein

(The AdFalciVax construct is designed under the control of the nisin-inducible promoter (PnisA). It includes the USP45 signal peptide for secretion, followed by the full-length PfCSP (targeting the human liver stage) fused via flexible linkers (L) to the sexual-stage antigens Pfs230 and Pfs48/45 (targeting the mosquito stage). A C-terminal hexahistidine (6xHis) tag is included to facilitate efficient purification via Ni-NTA chromatography.)

Table 3. Summary of Preclinical Immunogenicity and Efficacy Data

Assay	Methodology	Target Metric	Observed Outcome with AdFalciVax
ELISA	Indirect Enzyme-Linked Immunosorbent Assay	Antibody Titers (IgG)	High-titer seroconversion against PfCSP, Pfs230, and Pfs48/45 comparable to individual antigens.
SIA	Sporozoite Inhibition Assay (in vitro)	Hepatocyte Invasion Rate	Significant reduction in sporozoite entry into hepatocytes compared to control sera.
SMFA	Standard Membrane Feeding Assay	Transmission Blocking	Substantial reduction in both oocyst prevalence (% infected mosquitoes) and intensity (oocysts per mosquito).
Stability Testing	SDS-PAGE / ELISA after storage	Structural/Antigenic Integrity	Stable at 4°C and Room Temperature (25°C) for extended periods (reported >9 months).

3.2. Immunological Evaluation in Murine Models

Preclinical immunogenicity assessments were conducted using BALB/c mice to determine the vaccine's ability to elicit specific antibody responses.

3.2.1. Immunization Regimen and Serum Analysis

The experimental protocol involved groups of mice (n=10) receiving intramuscular injections of 10 µg of the purified AdFalciVax antigen formulated with an aluminum hydroxide (Alum) adjuvant. Control groups received Alum alone or an unrelated protein. The immunization schedule comprised three doses administered on days 0, 21, and 42. Serum samples were collected two weeks after each dose to monitor the kinetics of the antibody response. This prime-boost regimen is standard for subunit vaccines to ensure the generation of high-affinity antibodies and immunological memory [10].

3.2.2. Antigen-Specific Antibody Titers

Enzyme-linked immunosorbent assays (ELISA) performed on the collected sera revealed robust seroconversion in all vaccinated animals. The chimeric protein successfully elicited high-titer IgG responses against all three component antigens PfCSP, Pfs230, and Pfs48/45. Crucially, the antibody titers against the fusion components were comparable to those generated by individual antigens, indicating that the chimeric design did not compromise the immunogenicity of the individual domains. The balanced response against both pre-erythrocytic and sexual-stage antigens is a prerequisite for the dual-protective efficacy of the vaccine.

3.3. Functional Assays

The presence of antibodies is necessary but not sufficient for protection; their functional capacity to neutralize the parasite is paramount.

3.3.1. Inhibition of Sporozoite Invasion (SLA)

The protective efficacy targeting the pre-erythrocytic stage was evaluated via Sporozoite Inhibition Assays (SIA). In this *in vitro* assay, *P. falciparum* sporozoites were incubated with hepatocytes in the presence of immune sera from vaccinated mice. The results demonstrated a significant reduction in the number of sporozoites capable of invading the hepatocytes compared to control sera. This inhibition suggests that the anti-PfCSP antibodies induced by AdFalcVax are functional and capable of neutralizing sporozoites, thereby preventing the establishment of the liver-stage infection [11].

3.3.2. Transmission-Blocking Activity (SMFA)

The transmission-blocking potential was assessed using the Standard Membrane Feeding Assay (SMFA), the gold standard for evaluating transmission-blocking vaccines. *Anopheles* mosquitoes were fed gametocyte-infected blood mixed with immune sera. The midguts of the mosquitoes were subsequently dissected and examined for the presence of oocysts. The data showed a substantial reduction in both the prevalence (percentage of infected mosquitoes) and the intensity (number of oocysts per mosquito) of infection. This confirms that the antibodies against Pfs230 and Pfs48/45 are biologically active and can effectively interrupt the parasite's development within the vector [12].

4. Strategic Advantages and Formulation Characteristics

4.1. Thermostability Profile

One of the critical barriers to vaccine deployment in low- and middle-income countries (LMICs) is the requirement for a stringent cold chain, which is often difficult to maintain in rural and remote areas.

4.1.1. Cold Chain Independence

AdFalcVax addresses this logistical challenge through its inherent formulation stability. Stability studies involving sterile-filtered vaccine formulations stored at varying temperatures indicated remarkable resilience. The vaccine retained its structural integrity and antigenicity when stored at 4°C for extended periods, aligning with standard cold chain requirements. However, its performance at higher temperatures distinguishes it from many current biologicals.

4.1.2. Stability at Elevated Temperatures

Crucially, the vaccine formulation demonstrated stability at room temperature (25°C) for reportedly up to nine months. Structural analysis via SDS-PAGE and antigenicity testing via ELISA confirmed that the protein did not degrade or lose its immunological potency under these conditions [13]. This thermostability could drastically reduce vaccine wastage due to cold chain excursions and lower the logistical costs associated with transport and storage, making it particularly suitable for deployment in sub-Saharan Africa and India.

4.2. Economic and Operational Viability

The economic viability of a malaria vaccine is a determinant of its large-scale adoption and sustainability.

4.2.1. Scalability of the *L. lactis* System

The *L. lactis* expression system offers a scalable manufacturing route. The fermentation process is relatively simple, requiring less complex media and equipment compared to mammalian cell culture. The high-yield expression of the soluble fusion protein further enhances the efficiency of the process. This scalability is essential for meeting the massive demand required for a malaria vaccine intended for widespread use in endemic regions.

4.2.2. Cost-Effectiveness for Low-Resource Settings

The combination of a high-yield bacterial expression system, simplified purification due to the lack of endotoxins, and reduced cold chain dependence contributes to a lower cost of goods sold (COGS). Furthermore, the multi-stage design potentially negates the need for co-administering multiple distinct vaccines to achieve both personal protection and transmission reduction. This efficiency aligns with the operational mandates of international health organizations, which prioritize interventions that deliver high impact at sustainable costs [14].

5. Clinical Applications

5.1. Industrial Partnership

Following successful preclinical validation, the progression of AdFalcVax has moved toward clinical development and industrial partnership.

5.1.1. Technology Transfer and Licensing

The ICMR has actively sought collaboration with pharmaceutical entities to facilitate technology transfer and industrial scale-up. In late 2025, non-exclusive licenses were awarded to major Indian biotechnology firms, including Panacea Biotec, Biological E Limited, and Indian Immunologicals Limited. This strategic move ensures that multiple manufacturers can produce the vaccine, thereby securing the supply chain and fostering competitive pricing [15]

Table 4. Commercialization and Licensing

Stakeholder	Role in Development	Status/Contribution
ICMR - RMRC Bhubaneswar	Primary Developer	Concept, design, and lead research institute.
ICMR - NIMR & NII	Collaborative Partners	Antigen selection, preclinical validation, and immunological assays.
Panacea Biotec	Industrial Licensee	Non-exclusive license holder for manufacturing scale-up and clinical trials.
Biological E. Limited	Industrial Licensee	Non-exclusive license holder for manufacturing scale-up.
Indian Immunologicals Ltd	Industrial Licensee	Non-exclusive license holder for manufacturing scale-up.

5.1.2. Proposed Clinical Trial Phases

The immediate roadmap involves the initiation of Phase I clinical trials. These studies will primarily assess the safety, tolerability, and immunogenicity of AdFalcVax in healthy adult volunteers. Subsequent Phase II trials will focus on optimizing dosing regimens, evaluating different adjuvant formulations, and expanding the study population to include age groups most at risk, such as children. Phase III trials will eventually be required to demonstrate field efficacy in protecting against clinical malaria and reducing transmission in endemic communities.

5.2. Impact on Global Malaria Elimination

The introduction of AdFalcVax could necessitate a shift in malaria control paradigms, moving from control to elimination.

5.2.1. Interruption of Transmission Dynamics

Current vaccines like RTS,S and R21 are primarily tools for reducing childhood morbidity and mortality. In contrast, AdFalcVax, with its transmission-blocking components, functions as an elimination tool. By reducing the reservoir of infectious parasites in the human population and preventing their development in mosquitoes, it contributes to herd immunity. This "altruistic" component of the vaccine protects the community by breaking the cycle of transmission

5.2.2. Integration with Existing Control Measures

Mathematical modeling suggests that transmission-blocking vaccines are most effective when integrated with vector control measures (like bed nets) and mass drug administration. AdFalcVax is envisioned not as a standalone solution but as a powerful

component of an integrated malaria elimination strategy. Its deployment could accelerate the timeline for malaria eradication in regions that have stagnated using current control tools alone [16].

Table 5. Comparison of Leading Malaria Vaccine Candidates

Feature	RTS,S/AS01 (Mosquirix)	R21/Matrix-M	AdFalcivax (Candidate)
Developer	GSK	University of Oxford / Serum Institute of India	ICMR - RMRC Bhubaneswar
Primary Target	<i>Plasmodium falciparum</i>	<i>Plasmodium falciparum</i>	<i>Plasmodium falciparum</i>
Life Cycle Stage	Pre-erythrocytic (Sporozoite)	Pre-erythrocytic (Sporozoite)	Multi-Stage (Pre-erythrocytic + Sexual/Transmission)
Key Antigens	Truncated CSP (C-terminus)	Fusion CSP	Full-length PfCSP + Pfs230 + Pfs48/45
Expression Platform	Yeast (<i>Saccharomyces cerevisiae</i>)	Yeast (<i>Pichia pastoris</i>)	Bacteria (<i>Lactococcus lactis</i>)
Primary Mechanism	Prevent Liver Infection	Prevent Liver Infection	Prevent Liver Infection + Block Transmission to Mosquitoes
Current Status	WHO Prequalified	WHO Prequalified	Preclinical / Tech Transfer Phase

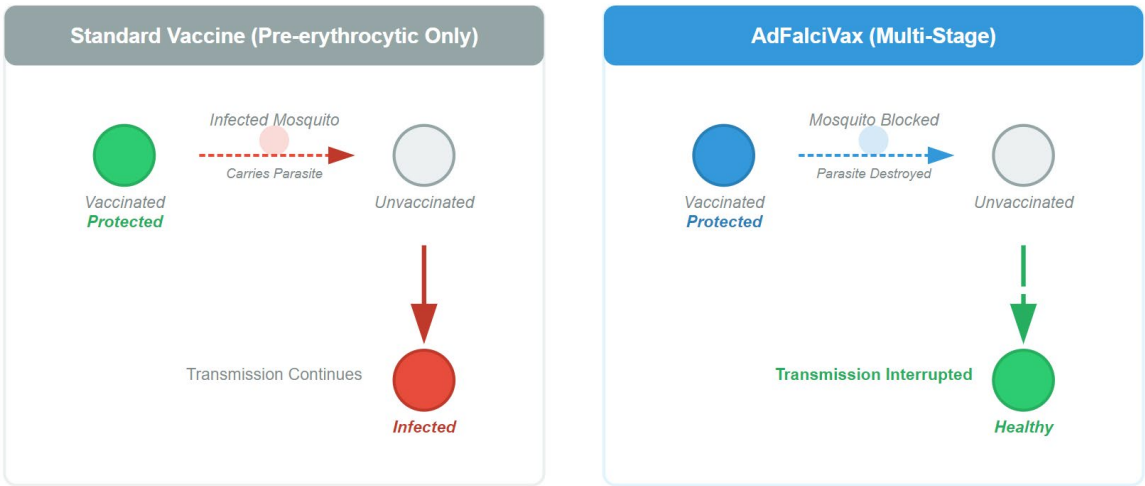


Figure 3. Interruption of Transmission Cycle & Herd Effect

(Left) With standard pre-erythrocytic vaccines, a vaccinated individual is protected, but parasites can still develop in mosquitoes biting adjacent infected individuals, continuing the cycle to unvaccinated populations. (Right) **AdFalcivax** induces antibodies that are ingested by the mosquito, killing the parasite within the vector. This prevents the mosquito from transmitting the disease to subsequent victims (even if they are unvaccinated), creating a "herd immunity" effect essential for elimination.

6. Conclusion

AdFalcivax represents a paradigm shift in malaria vaccinology, moving from single-stage protection to a comprehensive multi-stage defense strategy. It addresses the dual imperatives of protecting the individual and protecting the community by utilizing the robust *Lactococcus lactis* platform to express a chimera of PfCSP, Pfs230, and Pfs48/45. The preclinical data demonstrate not only high immunogenicity but also functional efficacy in blocking both sporozoite invasion and mosquito transmission. Furthermore, its favorable thermal stability profile and cost-effective production metrics address key implementation bottlenecks in low-resource settings. While rigorous clinical testing is required to validate these findings in human populations, AdFalcivax stands as a testament to the growing capability of indigenous research to contribute vital solutions to global health challenges. If successful, it could become a cornerstone of the final push toward malaria eradication.

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