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Impact of CRISPR-Based Parasite Attenuation on Recurrence Rates in Adults with *Plasmodium falciparum*: A Comparative Review

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ABSTRACT

Plasmodium falciparum malaria persists as a leading cause of morbidity and mortality in endemic regions despite widespread use of artemisinin-based combination therapies (ACTs). Recurrence due to reinfection or recrudescence remains a significant barrier to malaria elimination. Emerging genome-editing technologies, particularly clustered regularly interspaced short palindromic repeats (CRISPR), offer innovative strategies for parasite attenuation to prevent infection and reduce recurrence rates. This review examined the impact of CRISPR-based parasite attenuation on recurrence rates in adults with *P. falciparum* infection compared to standard therapies. A comparative narrative synthesis was conducted by reviewing peer-reviewed articles and preprints from 2010–2025 sourced from PubMed, Scopus, and BioRxiv databases, focusing on CRISPR-mediated attenuation of *P. falciparum* and its effects on recurrence. CRISPR-Cas9 enables targeted disruption of genes critical for parasite invasion, development, and transmission. Preclinical studies demonstrate that genetically attenuated parasites (GAPs) arrest during liver stage development, elicit robust immune responses, and confer sterile protection without recurrence in animal models. While ACTs effectively clear blood-stage parasites, recurrence remains common due to reinfection. Although direct comparative human studies are lacking, GAP-based interventions show promise for superior prophylactic efficacy. However, translational challenges such as manufacturing complexity, regulatory approval, and ethical considerations hinder immediate deployment. CRISPR-based parasite attenuation offers a novel avenue to reduce malaria recurrence and complement existing control strategies. Future research should prioritise first-in-human trials, multi-target gene editing, and ethical frameworks to enable safe and effective implementation.

Keywords: CRISPR, Plasmodium falciparum, Parasite attenuation, Recurrence, Genetically attenuated parasites.

INTRODUCTION

Plasmodium falciparum malaria remains a major global health burden, accounting for over 200 million cases and over 600,000 deaths annually, predominantly among vulnerable populations in sub-Saharan Africa and Southeast Asia [1–3]. Despite the widespread deployment of artemisinin-based combination therapies (ACTs) and vector control measures, malaria eradication remains elusive due to persistent transmission, emerging drug resistance, and frequent recurrence of infection. Recurrence may result from reinfection or recrudescence due to incomplete parasite clearance, with severe implications for individual morbidity and community-level transmission dynamics.

Recent advances in genome-editing technologies have opened novel avenues for malaria control, particularly the use of clustered regularly interspaced short palindromic repeats (CRISPR) systems to attenuate parasite virulence [4, 5]. CRISPR-based parasite attenuation involves targeted disruption of genes essential for parasite invasion, development, or immune evasion, thereby rendering the parasite incapable of causing severe disease or sustaining infection. This approach holds promise not only for vaccine development through genetically attenuated parasites but also as a transmission-blocking strategy if attenuation reduces parasite fitness within human hosts or mosquito vectors.

The comparative impact of CRISPR-based parasite attenuation on recurrence rates in adults with *P. falciparum* infection remains underexplored despite its potential to transform malaria management paradigms. This review

critically examines existing literature on CRISPR-mediated attenuation of *P. falciparum*, synthesises evidence on its efficacy in reducing recurrence compared to conventional therapeutic approaches, and evaluates the translational feasibility of integrating such genetic interventions into malaria elimination programmes. By elucidating the mechanistic, clinical, and epidemiological dimensions of CRISPR-based attenuation, the review aims to inform future translational research, policy considerations, and ethical frameworks required for deploying genetic technologies in endemic settings.

Methodology

A comparative narrative synthesis approach was utilised to review peer-reviewed articles and preprints from 2010–2025 focusing on CRISPR-based attenuation of *P. falciparum* and its reported or inferred impact on recurrence, using databases such as PubMed, Scopus, and BioRxiv.

Mechanisms of CRISPR-Based Parasite Attenuation

CRISPR-Cas9 has emerged as a powerful genome-editing tool for *P. falciparum* due to its ability to introduce precise double-stranded breaks at target loci, facilitating gene disruption or replacement [6, 7]. Parasite attenuation strategies typically target genes critical for hepatocyte invasion (e.g. *p36*, *p52*), erythrocytic development (e.g. *AMA1*, *MSP1*), or sexual stage maturation (e.g. *Pfs25*) [8]. Knockout of pre-erythrocytic stage genes such as *p36* and *p52* produces genetically attenuated parasites (GAPs) that arrest during liver stage development, preventing blood-stage infection and symptomatic disease. This approach mirrors the sterile immunity observed in irradiated sporozoite vaccines but with improved genetic stability and reproducibility.

Additionally, CRISPR-mediated deletion of sexual stage genes renders parasites incapable of completing transmission within mosquito vectors, offering community-level benefits. Recent studies have engineered dual knockout parasites targeting both pre-erythrocytic and sexual stage genes to simultaneously block infection and transmission. These mechanistic interventions reduce the parasite biomass, limit antigenic diversity, and potentially interrupt recrudescence arising from dormant or partially cleared subpopulations.

Comparative Efficacy: CRISPR-Based Attenuation Versus Standard Therapies

Current antimalarial therapies, including ACTs, effectively clear blood-stage parasites but do not eliminate pre-erythrocytic stages, leaving individuals vulnerable to reinfection [9]. Moreover, the emergence of artemisinin resistance in Southeast Asia threatens the long-term utility of ACTs. In comparison, CRISPR-based attenuation offers prophylactic and therapeutic benefits by targeting upstream stages of infection.

Preclinical trials in humanised mouse models demonstrate that *P. falciparum* GAPs engineered via CRISPR elicit robust CD8+ T cell responses, conferring sterile protection upon sporozoite challenge without detectable breakthrough infection or recurrence over six-month follow-up [10, 11]. Although human challenge trials are limited, first-in-human studies with GAP-based whole-sporozoite vaccines indicate durable protective efficacy of 50–80% against homologous challenge, with no reported recrudescence. In contrast, ACT-treated individuals show varying rates of recurrence (up to 20% in high-transmission settings), often due to reinfection or partial drug efficacy.

However, direct comparative studies of recurrence rates between CRISPR-based attenuation and standard therapy remain lacking due to the experimental status of genetic attenuation in humans. Extrapolations from animal studies suggest superior prophylactic efficacy, but large-scale trials are needed to validate these findings in endemic adult populations with semi-immunity and repeated exposure.

Factors Influencing Recurrence Rates After Attenuation

i. **Genetic Stability and Reversion Risk:** One critical determinant of recurrence is the genetic stability of attenuated parasites. While CRISPR ensures precise gene knockout, off-target effects or incomplete editing could permit reversion to virulence, resulting in breakthrough infections [12]. Current studies report negligible off-target mutations in *P. falciparum* due to careful guide RNA design and selection. Nonetheless, rigorous preclinical validation is essential to ensure stable attenuation prior to human use.

ii. **Immune Priming and Host Factors:** The immune responses elicited by GAPs are a major factor in preventing recurrence. Attenuated parasites present the full complement of pre-erythrocytic antigens, inducing broad cellular immunity, which contrasts with the narrow antigenic targets of subunit vaccines [13]. However, host factors such as HLA genotype, prior malaria exposure, and immune senescence in older adults influence the magnitude and durability of protective immunity. Hence, efficacy against recurrence may vary across endemic populations.

iii. **Transmission Intensity and Reinfection:** In high-transmission settings, reinfection rather than recrudescence accounts for most recurrences. While GAPs prevent blood-stage infection from initial sporozoite inoculation, they do not confer sterilising immunity against heterologous strains indefinitely [14]. Therefore, integration with vector control and chemoprevention remains necessary to reduce

reinfection risk. Strategies to engineer multi-strain GAPs or combine genetic attenuation with CSP-based vaccines are under investigation to enhance cross-strain protection.

Translational Challenges and Ethical Considerations

Despite promising preclinical data, several translational barriers hinder the deployment of CRISPR-based attenuation in malaria-endemic settings:

i. **Manufacturing Complexity**: Production of GAPs requires high-containment facilities, stringent quality control, and validation of genetic edits, raising logistical and financial constraints compared to small-molecule therapies.

ii. **Regulatory Pathways**: Genetically modified organisms (GMOs) intended for human administration face rigorous regulatory scrutiny [15, 16]. Current frameworks in malaria-endemic countries are often underdeveloped for such interventions, delaying approval timelines.

iii. **Community Acceptability**: Deployment of genetically modified parasites raises ethical and sociocultural concerns, including perceptions of deliberate infection, long-term safety, and ecological risks [17, 18]. Community engagement and transparent communication are critical to foster acceptance.

iv. **Risk of Parasite Escape**: Although GAPs are designed to be non-replicative, theoretical risks of transmission to mosquitoes or reversion necessitate stringent containment and monitoring protocols.

Future Directions

To advance CRISPR-based parasite attenuation towards clinical and public health implementation, several research priorities are evident:

i. **First-in-Human Efficacy Trials**: Controlled human malaria infection (CHMI) studies are needed to establish safety, immunogenicity, and efficacy in diverse adult populations, including semi-immune individuals in endemic regions [19, 20].

ii. **Multi-Target Editing**: Engineering parasites with multiple gene knockouts may enhance attenuation robustness and transmission-blocking potential while minimising reversion risk.

iii. **Combination Strategies**: Integrating CRISPR-based GAPs with existing interventions such as ACTs, bed nets, and RTS,S/AS01 vaccines may provide synergistic effects against recurrence [21].

iv. **Longitudinal Recurrence Studies**: Extended follow-up in endemic populations will elucidate the durability of protection, patterns of reinfection, and potential for reducing transmission reservoirs.

v. **Ethical Frameworks and Governance**: Developing international guidelines on the ethical use, regulation, and community consultation for genetically engineered parasites is essential to ensure equitable and responsible deployment.

CONCLUSION

CRISPR-based parasite attenuation represents a frontier in malaria control, offering the prospect of durable prophylactic protection and reduced recurrence rates compared to conventional therapies. Mechanistic studies demonstrate that targeted gene disruptions arrest parasite development, elicit robust immune responses, and potentially block transmission. While animal model data suggest significant advantages over ACTs in preventing recrudescence, human efficacy trials remain limited, and direct comparative recurrence data are unavailable. Key challenges include ensuring genetic stability, navigating regulatory hurdles, manufacturing at scale, and addressing ethical concerns regarding the deliberate use of genetically modified parasites in humans. Nonetheless, the integration of CRISPR-based attenuation within broader malaria elimination strategies could transform disease dynamics, particularly in high-transmission settings where recurrent infections perpetuate morbidity and hinder eradication goals. Future research should prioritise translational trials, multi-target editing approaches, and community engagement frameworks to advance this promising technology towards safe, effective, and ethically grounded implementation in endemic populations.

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