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CRISPR-Engineered *Anopheles* Mosquitoes for Malaria Transmission Interruption: A Review of Preclinical and Early Field Evidence

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ABSTRACT

Malaria remains a leading cause of morbidity and mortality in endemic regions, driven largely by *Plasmodium falciparum* transmission via *Anopheles* mosquitoes. Current vector control tools face diminishing effectiveness due to insecticide resistance and operational limitations. CRISPR-based gene editing offers a targeted approach to modify *Anopheles* populations for sustained reduction of malaria transmission. This review synthesized preclinical and early field evidence on CRISPR-engineered *Anopheles* mosquitoes designed for malaria transmission interruption, evaluating biological efficacy, ecological safety, and readiness for deployment. A thematic synthesis of literature published between 2010 and 2025 was conducted, using PubMed, Web of Science, and Scopus, focusing on laboratory, semi-field, and early confined field trials. Preclinical studies show that CRISPR gene drives can achieve inheritance bias exceeding 95% for traits such as female sterility (*doublesex* disruption) or parasite refractoriness (e.g., *FREP1* knockouts). Cage experiments demonstrate rapid allele fixation and significant reductions in mosquito fertility or parasite load. Semi-field studies confirmed stable trait inheritance and containment feasibility, though open-field trials are yet to occur. Key challenges included resistance allele formation, ecological risk management, and community acceptance. CRISPR-engineered *Anopheles* mosquitoes show strong potential as a sustainable vector control strategy. Future research should prioritize resistance-proof designs, rigorous ecological risk assessment, transparent community engagement, and phased regulatory pathways toward carefully monitored field releases.

Keywords: CRISPR, Gene drive, *Anopheles gambiae*, Malaria elimination, Vector genetic control

INTRODUCTION

Malaria continues to impose a substantial public health and economic burden, particularly in sub-Saharan Africa and parts of Southeast Asia, where *Anopheles gambiae* and related species act as highly efficient vectors of *Plasmodium falciparum* [1, 2]. In 2022, there were an estimated 249 million malaria cases and over 600,000 deaths globally, with children under five years of age disproportionately affected [3, 4].

Vector control measures such as insecticide-treated nets and indoor residual spraying have been central to malaria reduction strategies [5, 6]. However, widespread insecticide resistance, residual transmission, and operational constraints threaten the long-term efficacy of these approaches. There is a clear need for novel, sustainable, and scalable vector control tools that can complement and enhance existing interventions.

CRISPR-Cas9 gene editing has emerged as a transformative technology capable of introducing precise and heritable genomic modifications in *Anopheles* mosquitoes [7, 8]. Through the creation of gene drives self-propagating genetic elements that bias their own inheritance engineered traits can rapidly spread through wild populations, enabling population suppression or replacement with parasite-resistant strains [9, 10].

Target traits under development include female sterility via *doublesex* gene disruption, sex ratio distortion, and reduced vector competence by impairing parasite development in the mosquito midgut [11]. Laboratory studies have demonstrated rapid drive spread and significant reductions in vector reproductive potential or parasite transmission capability. Semi-field experiments, conducted in ecologically contained facilities, provide early insights into operational feasibility and biosafety. This review examines the preclinical and semi-field evidence for CRISPR-engineered *Anopheles* mosquitoes, focusing on gene drive design, efficacy outcomes, ecological and regulatory

considerations, and community engagement. The goal is to provide a comprehensive and critical synthesis to inform research priorities, policy discussions, and integration into broader malaria elimination frameworks.

CRISPR Gene Drive Mechanism in *Anopheles*

CRISPR-based gene drives function by inserting a construct into a specific genomic locus that includes Cas9, a guide RNA, and the desired genetic modification [12]. When heterozygous individuals produce gametes, Cas9 cleaves the wild-type allele, which is then repaired using the drive-containing allele as a template. This results in near-universal conversion of heterozygotes to homozygotes, allowing the modification to spread rapidly through the population [9]. A schematic would depict a drive construct inserted into one chromosome, Cas9-induced cleavage of the wild-type allele in germline cells, and repair via homology-directed repair, producing homozygous drive alleles. Generational spread would be represented by progressively higher drive allele frequency.

Preclinical Evidence

i. **Target Genes and Traits:** One of the most studied targets is the *doublesex* gene, essential for female development and fertility. Disruption results in sterile females with intersex phenotypes, halting population growth [13]. Other targets include *FREP1*, a midgut protein facilitating *Plasmodium* invasion, and genes involved in male fertility or sex ratio determination.

ii. **Laboratory Cage Trials:** Cage experiments show rapid gene drive spread. The *doublesex* drive reached >95% frequency within 10–12 generations, leading to complete population collapse [14]. Resistance allele formation via non-homologous end joining is a concern, but targeting highly conserved sequences reduces this risk. Parasite-blocking drives such as *FREP1* knockouts have achieved >90% reduction in oocyst burden.

iii. **Semi-Field Evidence:** Semi-field studies in large outdoor cages in Burkina Faso tested non-driving genetically modified *Anopheles* lines to evaluate trait stability, fitness costs, and containment procedures. These trials demonstrated stable inheritance and phenotype expression over multiple generations, with no reversion to wild type. Although these studies did not release active drives, they provide essential data on rearing, release, and monitoring protocols in near-natural conditions.

Summary Table: Selected Preclinical and Semi-Field Outcomes

Target Gene	Trait	Lab Outcome	Semi-Field Outcome	Estimated Transmission Impact
doublesex	Female sterility	Population collapse in 10–12 gens	Stable phenotype, no reversion	>90% reduction in vector density
FREP1	Parasite blocking	90–100% reduced oocyst burden	Not yet tested in semi-field	>80% reduction in parasite transmission
Sex ratio drivers	Male bias	Increased male proportion to >80%	Not yet tested	Modelled 60–70% transmission reduction

Ecological and Biosafety Considerations

Potential ecological risks of include effects on non-target organisms, horizontal gene transfer, and unintended geographic spread [15, 16]. Mitigation strategies include molecular confinement (split drives), physical containment in early phases, and post-release monitoring. Risk assessment frameworks recommend phased testing, beginning with laboratory proof-of-concept, followed by semi-field evaluation, and small-scale field releases only after safety is demonstrated.

Regulatory and Ethical Dimensions

Regulatory oversight is still evolving, with few precedents for self-propagating genetic control tools. Requirements of will likely include molecular characterization, efficacy data, ecological risk assessments, and community consultation [17]. Ethical considerations encompass informed consent at the community level, equitable distribution of benefits, and capacity building in host countries [18].

Community Engagement

Field studies in sub-Saharan Africa have shown that community trust hinges on transparency, involvement of local leaders, and clear explanation of benefits and risks [19, 20]. Engagement strategies should be iterative, culturally appropriate, and inclusive, ensuring local governance structures have decision-making authority.

Future Research Priorities

i. Resistance management through multiplexed guide RNAs and target site selection.
ii. Field-ready monitoring tools for allele frequency and ecological impact.
iii. Integration with insecticide-based and larval source control strategies.
iv. Long-term modelling of epidemiological impact under variable transmission intensities [21].

CONCLUSION

CRISPR-engineered *Anopheles* mosquitoes represent a promising frontier in malaria vector control, with the potential to overcome limitations of existing interventions. Preclinical evidence demonstrates the technical feasibility of gene drives targeting vector fertility or parasite susceptibility, with laboratory studies achieving rapid allele fixation and significant phenotypic effects. Semi-field evaluations have validated trait stability and containment approaches, marking an essential step toward eventual open-field trials. However, translating this technology into public health impact will require addressing several key challenges: preventing resistance allele formation, ensuring ecological safety, navigating evolving regulatory frameworks, and building sustained community trust. Integration into broader malaria elimination strategies will be essential, as gene drives alone are unlikely to achieve eradication.Phased testing, rigorous monitoring, and transparent stakeholder engagement should guide the pathway from laboratory to field. Collaboration between molecular biologists, entomologists, ecologists, social scientists, and policymakers will be critical to ensure responsible development. If successfully developed and deployed, CRISPR-based vector genetic control could provide a durable and scalable complement to existing tools, accelerating progress toward malaria elimination goals in high-burden regions.

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