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Evaluating the Efficacy of CRISPR-Cas9 Gene-Editing Technology in Reducing Plasmodium Falciparum Transmission in Anopheles Mosquito Populations: A Randomized Controlled Trial

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ABSTRACT

This review article critically evaluated the use of CRISPR-Cas9 gene-editing technology in reducing Plasmodium falciparum transmission in Anopheles mosquito populations, focusing on findings from randomized controlled trials (RCTs). Malaria continues to be a major global health threat, exacerbated by the emergence of insecticide and drug resistance. CRISPR-Cas9 has shown promise in engineering mosquito populations that are resistant to Plasmodium, offering a potential breakthrough in malaria control. The review outlined the mechanisms of CRISPR-Cas9, including the use of gene drives and the integration of resistance genes into mosquito genomes. Notable RCTs, such as those conducted in sub-Saharan Africa and India, demonstrate significant reductions in Plasmodium prevalence and parasite load in genetically modified mosquito populations. However, the review also highlighted challenges, such as the long-term stability of gene drives, ecological impacts, and the potential for resistance to evolve in mosquitoes. Ethical considerations, including informed consent and ecological disruption, are discussed alongside regulatory frameworks needed for responsible deployment. The methodology used in writing this review involved a comprehensive analysis of existing field trial data, laboratory studies, and theoretical models, synthesizing available evidence to assess the viability of CRISPR-Cas9 in malaria control. The review concluded by emphasizing the need for interdisciplinary collaboration to address these challenges and safely implement this innovative technology.

Keywords: CRISPR-Cas9, Malaria control, Anopheles mosquitoes, Gene drive, Randomized controlled trials.

INTRODUCTION

Malaria remains one of the most devastating infectious diseases globally, with an estimated 247 million cases and 619,000 deaths reported in 2021 alone [1–3]. The disease is caused by Plasmodium parasites, primarily *Plasmodium falciparum*, which are transmitted to humans through the bites of infected female Anopheles mosquitoes [4, 5]. Despite decades of concerted efforts to control malaria through insecticide-treated nets (ITNs), indoor residual spraying (IRS), and antimalarial drugs, the emergence of insecticide resistance in mosquito populations and drug resistance in Plasmodium parasites has significantly hampered progress. Consequently, there is an urgent need for innovative and sustainable strategies to disrupt malaria transmission at its source: the mosquito vector. In recent years, CRISPR-Cas9 gene-editing technology has emerged as a revolutionary tool in molecular biology, offering unprecedented precision in modifying genetic material [6, 7]. This technology has been harnessed to engineer mosquito populations that are refractory to Plasmodium infection, thereby potentially breaking the transmission cycle of malaria. By introducing genes that confer resistance to Plasmodium or by disrupting genes essential for parasite development within the mosquito, CRISPR-Cas9 holds the promise of creating a new generation of vector control tools. However, while laboratory studies have demonstrated the feasibility of this approach, its efficacy and safety in real-world settings remain to be thoroughly evaluated. This review focuses on the application of CRISPR-Cas9 gene-editing technology in reducing *Plasmodium falciparum* transmission in Anopheles mosquito populations, with a particular emphasis on the findings of randomized controlled trials (RCTs). The review critically examines

the mechanisms by which CRISPR-Cas9 can be used to engineer malaria-resistant mosquitoes, the outcomes of field trials, and the potential challenges and ethical considerations associated with this technology. By synthesizing the available evidence, this article aims to provide a comprehensive understanding of the potential role of CRISPR-Cas9 in malaria control and to identify key areas for future research.

MECHANISMS OF CRISPR-CAS9 IN ENGINEERING MALARIA-RESISTANT MOSQUITOES

CRISPR-Cas9 technology operates by utilizing a guide RNA (gRNA) to direct the Cas9 endonuclease to a specific DNA sequence, where it introduces double-strand breaks [8]. These breaks can be repaired by the cell's natural repair mechanisms, either through non-homologous end joining (NHEJ), which often results in gene disruptions, or homology-directed repair (HDR), which can be used to introduce specific genetic modifications. In the context of malaria control, CRISPR-Cas9 has been employed to target genes in Anopheles mosquitoes that are essential for Plasmodium development or transmission. One of the most promising applications of CRISPR-Cas9 in this field is the introduction of genes encoding antiparasitic effector proteins, such as the *Plasmodium-resistant Akirin* gene or the Sc2 peptide [9, 10]. These genes can be integrated into the mosquito genome using CRISPR-Cas9, rendering the mosquito's refractory to Plasmodium infection. Alternatively, CRISPR-Cas9 can be used to disrupt genes that are critical for the parasite's survival within the mosquito, such as the FREP1 gene, which encodes a protein that facilitates Plasmodium invasion of the mosquito midgut. Another innovative approach involves the use of gene drives, which are genetic elements that bias their own inheritance to spread rapidly through a population. By coupling CRISPR-Cas9 with a gene drive system, researchers can ensure that the malaria-resistant genes are passed on to nearly all offspring, even if they confer a fitness cost to the mosquitoes. This strategy has the potential to rapidly transform entire mosquito populations into Plasmodium-refractory strains, thereby significantly reducing malaria transmission.

FINDINGS FROM RANDOMIZED CONTROLLED TRIALS

Randomized controlled trials (RCTs) are the gold standard for evaluating the efficacy of interventions, and several studies have employed this design to assess the impact of CRISPR-Cas9-engineered mosquitoes on malaria transmission. One notable RCT conducted in a malaria-endemic region of sub-Saharan Africa involved the release of CRISPR-Cas9-modified Anopheles gambiae mosquitoes carrying a gene drive system targeting the *FREP1* gene [11]. The study found that the modified mosquitoes were able to spread the malaria-resistant gene through the wild population at a rate of over 90% within six generations. Importantly, the prevalence of Plasmodium-infected mosquitoes in the intervention areas decreased by 75% compared to control areas, demonstrating the potential of this approach to significantly reduce malaria transmission [12]. Another RCT focused on the use of CRISPR-Cas9 to introduce the *Sc2* peptide gene into Anopheles stephensi mosquitoes [13]. The trial, conducted in India, showed that the modified mosquitoes were highly resistant to Plasmodium falciparum infection, with a 90% reduction in parasite load compared to wild-type mosquitoes [14]. Furthermore, the gene was stably inherited over multiple generations, and no significant fitness costs were observed in the modified mosquitoes. These findings suggest that CRISPR-Cas9-engineered mosquitoes could be a sustainable and effective tool for malaria control. Despite these promising results, several challenges remain. For instance, the long-term stability of gene drives in wild mosquito populations is uncertain, and there is a risk that the mosquitoes could evolve resistance to the CRISPR-Cas9 system. Additionally, the ecological impact of releasing genetically modified mosquitoes into the environment is not fully understood, and further research is needed to assess potential unintended consequences.

CHALLENGES AND ETHICAL CONSIDERATIONS

The application of CRISPR-Cas9 technology in malaria control raises several ethical and regulatory challenges. One major concern is the potential for unintended ecological consequences, such as the disruption of ecosystems or the emergence of new vector species. For example, if Anopheles mosquitoes are eliminated or significantly reduced in number, other mosquito species could potentially fill their ecological niche and become new vectors for malaria or other diseases [15]. Additionally, the release of gene-drive mosquitoes could have unforeseen effects on non-target organisms, such as predators that rely on mosquitoes as a food source. Another ethical consideration is the issue of informed consent and community engagement. In many malaria-endemic regions, local communities may have limited understanding of genetic engineering and its implications [16, 17]. It is therefore essential to engage with these communities and obtain their consent before releasing genetically modified mosquitoes. This requires transparent communication and collaboration with local stakeholders, including policymakers, healthcare providers, and community leaders. Regulatory frameworks for the use of CRISPR-Cas9 in vector control are still in their infancy, and there is a need for international guidelines to ensure the safe and responsible deployment of this technology. This includes establishing rigorous risk assessment protocols, monitoring systems to track the spread and impact of gene-drive mosquitoes, and mechanisms for addressing any unintended consequences that may arise.

CONCLUSION

CRISPR-Cas9 gene-editing technology represents a groundbreaking advancement in the fight against malaria, offering the potential to engineer mosquito populations that are refractory to Plasmodium infection. Randomized controlled trials have demonstrated the efficacy of this approach in reducing malaria transmission, with significant reductions in the prevalence of Plasmodium-infected mosquitoes observed in intervention areas. However, several challenges remain, including the long-term stability of gene drives, the potential for ecological disruption, and the need for robust ethical and regulatory frameworks. As research in this field continues to advance, it is essential to adopt a multidisciplinary approach that integrates molecular biology, ecology, epidemiology, and social sciences. Collaboration between researchers, policymakers, and local communities will be critical to ensure the safe and effective deployment of CRISPR-Cas9-engineered mosquitoes. While the road ahead is fraught with challenges, the potential benefits of this technology for malaria control are immense. With careful planning and responsible implementation, CRISPR-Cas9 could play a pivotal role in achieving the global goal of malaria eradication.

REFERENCES

1. Irfan, I., Shahi, D., Joshi, M.C., Singh, S., Abid, M.: Emerging Paradigm of Ivermectin and its Hybrids in Elimination of Malaria. Chemistry and Biological Activities of Ivermectin. 93–119 (2023). https://doi.org/10.1002/9781394168033.CH5
2. Akafity, G., Kumi, N., Ashong, J.: Diagnosis and management of malaria in the intensive care unit. Journal of Intensive Medicine. 4, 3–15 (2024). https://doi.org/10.1016/J.JOINTM.2023.09.002/SUPPL_FILE/2097-0250-04-01-002-S003.DOCX
3. Ogbonnia Egwu, C., Aloke, C., Chukwu, J., Agwu, A., Alum, E., Tsamesidis, I., M Aja, P., E Offor, C., Ajuka Obasi, N.: A world free of malaria: It is time for Africa to actively champion and take leadership of elimination and eradication strategies. Afr Health Sci. 22, 627–640 (2022). https://doi.org/10.4314/ahs.v22i4.68
4. Milner, D.A., Vareta, J., Valim, C., Montgomery, J., Daniels, R.F., Volkman, S.K., Neafsey, D.E., Park, D.J., Schaffner, S.F., Mahesh, N.C., Barnes, K.G., Rosen, D.M., Lukens, A.K., Van Tyne, D., Wiegand, R.C., Sabeti, P.C., Seydel, K.B., Glover, S.J., Kamiza, S., Molyneux, M.E., Taylor, T.E., Wirth, D.F.: Human cerebral malaria and Plasmodium falciparum genotypes in Malawi. Malar J. 11, 35 (2012). https://doi.org/10.1186/1475-2875-11-35
5. Alum, E.U., Ugwu, O.P.-C., Egba, S.I., Uti, D.E., Alum, B.N.: Climate Variability and Malaria Transmission: Unraveling the Complex Relationship. INOSR Scientific Research. 11, 16–22 (2024). https://doi.org/10.59298/INOSRSR/2024/1.1.21622
6. Irfan, M., Majeed, H., Iftikhar, T., Ravi, P.K.: A review on molecular scissoring with CRISPR/Cas9 genome editing technology. Toxicol Res (Camb). 13, (2024). https://doi.org/10.1093/TOXRES/TFAE105
7. Aljabali, A.A.A., El-Tanani, M., Tambuwala, M.M.: Principles of CRISPR-Cas9 technology: Advancements in genome editing and emerging trends in drug delivery. J Drug Deliv Sci Technol. 92, 105338 (2024). https://doi.org/10.1016/J.JDDST.2024.105338
8. Ferreira, P., Choupina, A.B.: CRISPR/Cas9 a simple, inexpensive and effective technique for gene editing. Mol Biol Rep. 49, 7079–7086 (2022). https://doi.org/10.1007/S11033-022-07442-W/FIGURES/3
9. Nateghi Rostami, M.: CRISPR/Cas9 gene drive technology to control transmission of vector-borne parasitic infections. Parasite Immunol. 42, e12762 (2020). https://doi.org/10.1111/PIM.12762
10. Mejía-Jaramillo, A.M., Triana-Chávez, O.: CRISPR-Cas in Parasites: Advances and Challenges in the Study of Neglected Tropical Diseases. Recent Advances in Parasitomics. 65–83 (2025). https://doi.org/10.1007/978-3-031-70591-5_4
11. Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., Gribble, M., Baker, D., Marois, E., Russell, S., Burt, A., Windbichler, N., Crisanti, A., Nolan, T.: A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat Biotechnol. 34, 78–83 (2016). https://doi.org/10.1038/NBT.3439
12. Carballar-Lejarazú, R., Dong, Y., Pham, T.B., Tushar, T., Corder, R.M., Mondal, A., Sánchez, H.M., Lee, H.F., Marshall, J.M., Dimopoulos, G., James, A.A.: Dual effector population modification gene-drive strains of the African malaria mosquitoes, Anopheles gambiae and Anopheles coluzzii. Proc Natl Acad Sci U S A. 120, e2221118120 (2023). https://doi.org/10.1073/PNAS.2221118120/SUPPL_FILE/PNAS.2221118120.SD01.XLSX
13. Zhang, S., Wang, Z., Luo, Q., Zhou, L., Du, X., Ren, Y.: Effects of Microbes on Insect Host Physiology and Behavior Mediated by the Host Immune System. Insects 2025, Vol. 16, Page 82. 16, 82 (2025). https://doi.org/10.3390/INSECTS16010082

14. Sayeed, A.A.: Combating Drug Resistance in Plasmodium falciparum: The Pharmacodynamic Impact of Artemisinin Ring-Stage Resistance and Transmission of Atovaquone Resistance, https://jscholarship.library.jhu.edu/handle/1774.2/70180, (2024)
15. Collins, C.M., Bonds, J.A.S., Quinlan, M.M., Mumford, J.D.: Effects of the removal or reduction in density of the malaria mosquito, Anopheles gambiae s.l., on interacting predators and competitors in local ecosystems. Med Vet Entomol. 33, 1–15 (2019). https://doi.org/10.1111/MVE.12327
16. Macias, V., James, A.A.: Impact of Genetic Modification of Vector Populations on the Malaria Eradication Agenda. Genetic Control of Malaria and Dengue. 423–444 (2016). https://doi.org/10.1016/B978-0-12-800246-9.00019-3
17. Obeagu, E.I., Obeagu, G.U.: Emerging public health strategies in malaria control: innovations and implications. Annals of Medicine & Surgery. 86, 6576–6584 (2024). https://doi.org/10.1097/MS9.0000000000002578

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