THE EVIDENCE

E-ISSN:3048-7870
Vol. 2 No. 4 (2024)

Author Introduction | Vaccine

Check for updates

A Review of WHO-Recommended R21/Matrix-MTM vaccine for the prevention of malaria

Lipigwe Lauya[1*], Abednego Samuel Dahal[1]

[1] Department of Medical Microbiology, College of Health Sciences University of Jos, Jos, Nigeria.

*Correspondence: lauyalipigwe@gmail.com

Abstract

Background: The regulatory-approved R21/Matrix-MTM malaria vaccine is the second of its kind and the product of more than 30 years of research by the Jenner Institute, University of Oxford, UK, and its partners.

Methods: We conducted unsystematic online search for relevant literature and the retrieved materials were then critically studied and synthesised to provide deeper insight into the development, components, clinical trial results and limitations of the R21-Matrix-MTM malaria vaccine.

Results: In clinical trials, the R21-Matrix-MTM elicited the highest levels of antibodies at a 10 µg dose per injection compared to other tested doses, reaching an efficacy of 77%. Researchers administered the vaccine intramuscularly as a three-dose primary series, with intervals of four weeks each, followed by a booster 12 months after the third dose was completed. The elicited antibodies target the antigen NANP, which is a repeated amino acid sequence asparagine-alanine-asparagine-proline (NANP) embedded within the circumsporozoite protein (CSP) of Plasmodium falciparum.

Conclusion: The R21/Matrix-MTM vaccine is a revolutionary intervention to prevent malaria in children between the ages of 3 and 5. We strongly encourage malaria vaccines for the adult group to overcome inequality and form a more formidable force for the eradication of malaria globally. There seem to be less excitement about the vaccine, possibly due to enduring familiarity with malaria and availability of existing interventions, this is likely to interfere with the vaccine uptake and/or adherence to the required doses. There's needs for public awareness in targeted areas.

Keywords: *Antibodies, malaria vaccine, NANP, plasmodium falciparum, R21, Matrix-M*

www.evidencejournals.com

Cite this Article

Lauya L, Dahal AS. A Review of WHO-Recommended R21/Matrix-MTM vaccine for the prevention of malaria. THE EVIDENCE. 2024:2(4):1-10.
DOI:10.61505/evidence.2024.2.4.90
Available From https://the.evidencejournals.com/index.php/j/article/view/90

Received:	2024-07-11
Revised:	2024-07-28
Accepted:	2024-08-11
Published:	2024-12-20

Evidence in Context

- The R21/Matrix-MTM malaria vaccine, developed over 30 years by the Jenner Institute, is the second approved malaria vaccine.
- Clinical trials showed 77% efficacy with the highest antibody levels at a 10 µg dose.
- It's given as a three-dose series, with a booster after 12 months.
- The vaccine targets the NANP antigen in Plasmodium falciparum.
- Vaccine uptake may be hindered by familiarity with malaria and existing interventions, requiring increased public awareness.

To view Article

Introduction

Globally, malaria has killed an estimated 15,861,000 people between 2000-2022 [1]. In 2022, 249 million cases of malaria were reported, with 608,000 global deaths [1]. The most vulnerable age groups to malaria are children, especially under 5 years [2]. By way of intervention, the R21/Matrix-M^{TM} vaccine emerged as the second malaria vaccine to receive regulatory approval to alleviate the burden of the disease in these age groups [1,2]. The vaccine was examined in several trials and showed satisfactory outcomes, which led to its approval by the WHO in 2021 [2]. Similarly, a counterpart malaria vaccine, RTS/AS01, was the first to be approved by the same organization for the same purpose in 2015 [3]. These milestones in malaria vaccine developments were achieved in not less than 60 years [4,5]. The University of Oxford alone dedicated minimum of 30 years of consistency and relentless research to the novel R21/Matrix-M^{TM} malaria vaccine [2]. This breakthrough and rapid rollout of the new

Malaria vaccine aligns with one of the four key objectives of WHO's Global Malaria Programme strategy for 2024-2030: "Developing and Delivering New Tools and Innovation [1]. This objective prioritizes the evaluation and swift rollout of promising malaria interventions. This focus on new control and prevention strategies propels WHO and its partners towards achieving the ambitious goal of eradicating malaria by 2030. This includes a 90% reduction in malaria deaths and eliminating malaria in not less than 30 countries. And of course, we wouldn't want the disease to return to countries already free from it, places like Sri Lanka, Cabo Verde, Saudi Arabia and more. That's the aim of WHO [1]. This article presents the history of R21/Matrix-M^{TM} vaccinedevelopment,its efficacy, findings from clinical trials, and its limitations.

Historical Development of Malaria Vaccines

For decades, scientists have relentlessly waged a war against malaria, a top killer disease from a single parasite. This commitment has yielded both setbacks and triumphs. The journey began in the 1960s and 70s with pre-erythrocytic vaccines on the spotlight [6,7]. In the 1970s, Clyde and colleagues launched an investigative human trial of radiation-attenuated sporozoites and observed progressive evidence, even though the trial results did not meet expectations [8].

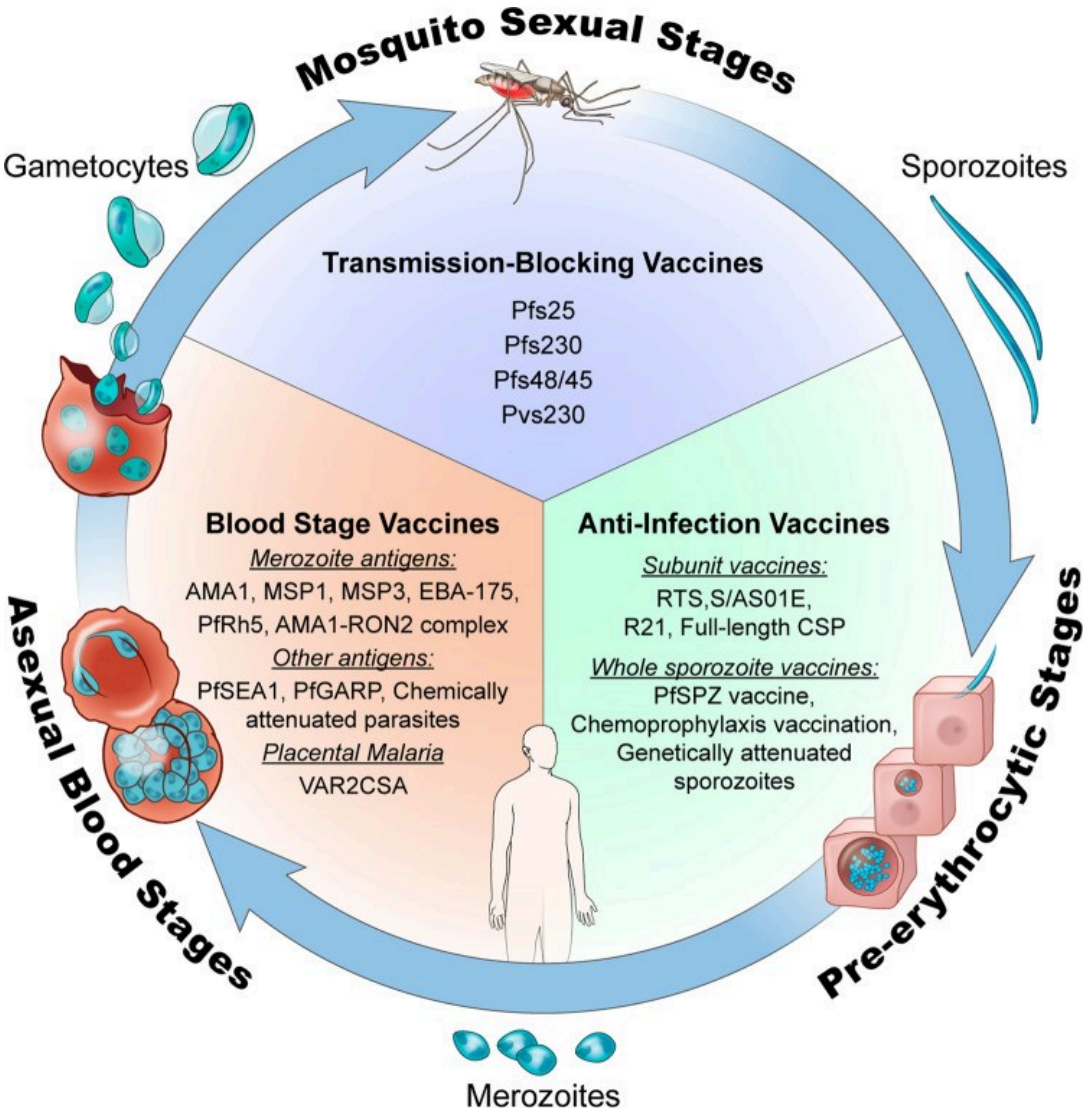

Figure 1: Life cycle of the malaria parasite and various vaccine types targeting different stages of the life cycle [23].

During the 1980s, the first malariaantigenknown as CSPwas identified in*Plasmodium falciparum,* leading to the development of the subunit vaccine, RTS,S in 1987 [9,10,11]. At that time, a range of vaccines that target the erythrocytic stage of the parasite (merozoites-derived antigens) also emerged [12,13], but trials efficacy was unsatisfactory [9,14,15]. In 2009 through 2019, the

Subunit vaccine candidate, RTS,S, which targets the CSP antigen,demonstrated promising efficacy in clinical trials and was approved for human use by the WHOin 2015 [3,11].However, the RTS,S was imperfectand needed tobe improvedupon. This need birthed the R21/Matrix-M™ vaccine by the University of Oxford, UK and its partners,and was approved for use in children in 2021 [4,5,16]. The key milestones in the malaria development processare highlightedin Figure 1.

Historical Background of the R21/Matrix-M™ Vaccine

R21 is a subunit vaccine constructed by Katherine A. Collins to overcome the limitations of the initial CSP-basedRTS,Svaccine [16]. Itwasconstructedby the fusion of the N-terminus of hepatitis B virus surface antigen (HBsAg) with*Plasmodium falciparum* circumsporozoite protein (PfCSP), thus the name virus-like protein (VLP) [16-18]. After R21 was constructed in 2012, researchers subsequently combined it with Matrix-M™ adjuvant to create the protein-adjuvanted vaccine R21/Matrix-M™ [17]. The use of the Matrix-M™ adjuvant in combination with R21 is due to its impressive historical safety and immunogenicity when used in combination with seasonal influenza vaccines in clinical trials [19,20]. The adjuvant was also well-trusted when it was usedin the development ofvaccine against SARS-CoV-2 and Ebola virus [21,22]. The original target of the R21/Matrix-M™ vaccine isthe CSP from the*P. falciparum* strain NF54 [16]. The CSP, expressed on the parasite's sporozoites, is responsible for enhancing gliding motility and hepatocellular invasion of the malaria parasite [23-25].

Katherine A. Collins and the Jenner Institute, University of Oxford, UK, in collaboration with Novavax, Inc., developed the R21/Matrix-M™ vaccine and manufactured it on a commercial scale by the Serum Institute of India (SII) Private Ltd [2,16]. The R21/Matrix-M™ program was led by Professor Adrian Hill, the director of the University of Oxford's Jenner Institute at the Nuffield Department of Medicine [2]. As of May 2024, 80 million doses of the R21/Matrix-M™ vaccine are underway in 22 African, delivered by the GAVI vaccine alliance [26]. GAVI planned to immunize 9.7 million children by the end of 2024. The vaccine was designed to induce hyperactivation of the anti-CSP antibody specific for the antigen NANP, which is a repeated amino acid sequence, asparagine-alanine-asparagine-proline, embedded within the *P. falciparum* circumsporozoite protein (pfCSP) [16,27], and to be administered in low doses to cut down the cost of production [28-30]. Additionally, the vaccine is targeted at children between the ages of 3 and 5, which gives credence to its potential to protect the most vulnerable individuals from malaria [1,29].

Purpose of the R21/Matrix-M™ Vaccine

Plasmodia sporozoites move so quickly (within ≤30–60 minutes) from the bite site to the liver that the body does not generate enough immunity to overcome this process [31,32]. The purpose of the R21/matrix-M™vaccineismainly to outsmart this crafty maneuver by targeting sporozoites at the pre-erythrocytic stage, which ultimately stops disease and transmission [33].

Compared to the RTS,S vaccine, which has limited CSP content and only recognizes specific *P. falciparum* epitopes, R21/matrix-M™ contains a higher CSP content, offering broader epitope recognition of various Plasmodium strains [22]. The Matrix-M™ adjuvant has a high immunostimulatory ability to induce the production of T helper cells [22], leading to a high yield of anti-CSP immunoglobulin G (IgG) antibodies that can directly neutralize sporozoites in the bloodstream, preventing their progression to invade liver cells [16,22]. The adjuvant can also stimulate antigen-presenting cells (APCs) at the site where the injection is given [22]. This enhances efficient presentation of the antigen in local lymph nodes, thus intercepting the parasite at the early stage of infection [22].

Ideally, when mosquitoes bite, they introduce hundreds to thousands of sporozoites into the victim's skin [34,35]. The mosquito species and plasmodia strain involved determine the exact number of sporozoites to be released [34,35]. Nevertheless, since these sporozoites migrate from the bite site too quickly, the ability of the R21/Matrix-M™ vaccine to completely clear sporozoites at the pre-erythrocytic stage may not always be achieved. This allows the parasite's life cycle to progress, eventually leading to the clinical manifestation of symptoms. Alternatively, a multistage vaccine has been proposed to close this gap [16]. One such candidate, chimpanzee adenovirus 63 (ChAd63) and modified vaccinia Ankara (MVA), encoding CSP and ME-TRAP (Multiple-Epitope Thrombospondin-Related Adhesion Protein), demonstrates remarkable antigenicity against controlled human malaria, reducing parasite levels in the liver by an impressive 79–85% [36,37].

This is especially typical for the massive production of CD8+ cytotoxic T lymphocytes (CTLs) and interferon-gamma (IFNγ), a powerful immune signaling molecule [36,37].

Components of R21/Matrix-M™

R21/Matrix-M™ is an improved version of the RTS,S vaccine. The main differences between these vaccinesare intheir CSP content, HBsAg content, adjuvant, and expression host. Table 1 summarizes the key differences between them.

Table 1: Key differences between R21 and RTS,S vaccines

Point of Difference	RTS, S	R21
Development year	1987	2012
Developer	Glaxosmithkline and the walter reed army institute of research	Jenner institute, university of Oxford, UK + Novavax, Inc.
Construct	CSP and HBsAg fused + excess unfused HBsAg (S)	Hybrid (CSP-HBsAg), no excess HBsAg
CSP content	Lower density	Higher density
HBsAg content	Higher	Insignificant
CSP antigen per dose	Lower	Higher
Expression system	Saccharomyces cerevisiae	Pichia pastoris [8]
Adjuvant	AS01 (QS-21 + MPL)	Matrix-MTM (saponin-based)
Efficacy	Lower (56%) at 12 months after the 3rd dose	Higher (77%) at 12 months after the 3rd dose
Antibody Response	Induces antibodies toward both CSP and HBsAg	Predominantly induces antibodies to CSP

In RTS,S, the CSP fragment is attached to the carrier HBsAg and co-expressed in the yeast*Saccharomyces cerevisiae*along with an additional free unmodified recombinant HBsAg (S) that has a fourfold molar excess (1:4) compared to that of the attached CSP protein. This results in a greater immune response against HBsAg than against the target plasmodium CSP [3,18,38,39]. In contrast, R21 contains a CSP fragment directly fused to HBsAg and co-expressed in the yeast*Pichiapastoris* to form a hybrid particle (CSP-HBsAg) with no additional excess HBsAg. This fusion increases the concentration of CSP and is believed to enhance the immunogenic power of the CSP antigen [11,16,18,40]. In other words, R21 contains a higher concentration of the CSP antigen per dose than does RTS,S. R21 maintains an inconsequential (if at all) immune response against the HBsAg fraction. See Figure 2. However, during the preclinical investigation, adjustments were made to resolve technical issues and make the vaccine easier to purify. This involves the addition of four amino acids, EPEA (Glu-Pro-Glu-Ala) and a c-tag (R21c), which was later removed during large-scale production of the vaccine [18,41,42]. The RTS,S is combined with adjuvant Mosquirix™ also known as AS01. This adjuvant has two immunity triggers, namely, liposome-based monophosphoryl lipid A (MPL) derived from *Salmonella minnesota* and QS-21, a natural saponin molecule purified from the bark of the South American tree*Quillaja saponaria* [40,43]. While effective in stimulating the T-helper cells, MPL has the disadvantage of safety issues because of its bacterial origin [43]. Similarly, the R21 vaccine uses theMatrix-M™ adjuvant, a 40 nm complex containing the saponin from Quillaja extract, phospholipids and cholesterol [40,41,43], but, it does not contain the bacterial-based MPL, offering a safer profile compared to the AS01 adjuvant [41].

Mechanism of Action of R21/Matrix-M™

The mechanism of action of the R21/Matrix-M™ vaccine follows the natural principle of immunologic induction and activity through multiple pathways [22,44].

Part A: Intramuscular Injection

When the R21/Matrix-M™ vaccine is injected into muscle tissue (event 1), it initiates rapid release of local immune cells and signalling cytokines, some of which includes interferon gamma (IFN-γ), interleukin (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α) [22,44,45]. These cytokines facilitate the accumulation of immune cells, including the antigen-presenting cells (APCs) like dendritic cells (DCs), at the injection site and in the draining lymph nodes (dLNs) [22,44,46]. This initial event also triggers the release of specific chemokines. For instance, chemokines like CXCL1 and CCL2 attract monocytes and granulocytes, while CXCL9 and CXCL10 recruit T cells [22,44,45].

The Matrix-M adjuvant enhances this immune response by promoting antigen uptake, transport, and efficient presentation to T-cells by the APCs in the immune-equipped dLNs (event 2-3). When the CD4+ T cells are activated, they differentiate into three special cells (event 4): Th1 cells, which orchestrate cellular immunity; Th2 cells, which stimulate antibody production; and T follicular helper (Tfh) cells, which assist in the maturation of B cells within germinal centers (event 5). Inside these germinal centers, activated B cells interact with Tfh cells and other immune cells, leading to the selection of the most effective B cells (event 6) [22]. This process results in the production of long-lasting and memory-inducing antibodies and plasma cells that neutralize the malaria parasite (event 7). The R21 was designed to induce antibody response predominantly towards the parasites CSP [16]. Additionally, memory T-cells of CD8+ types are generated, ensuring a rapid immune response during future encounters with the parasite (event 8) [22,44].

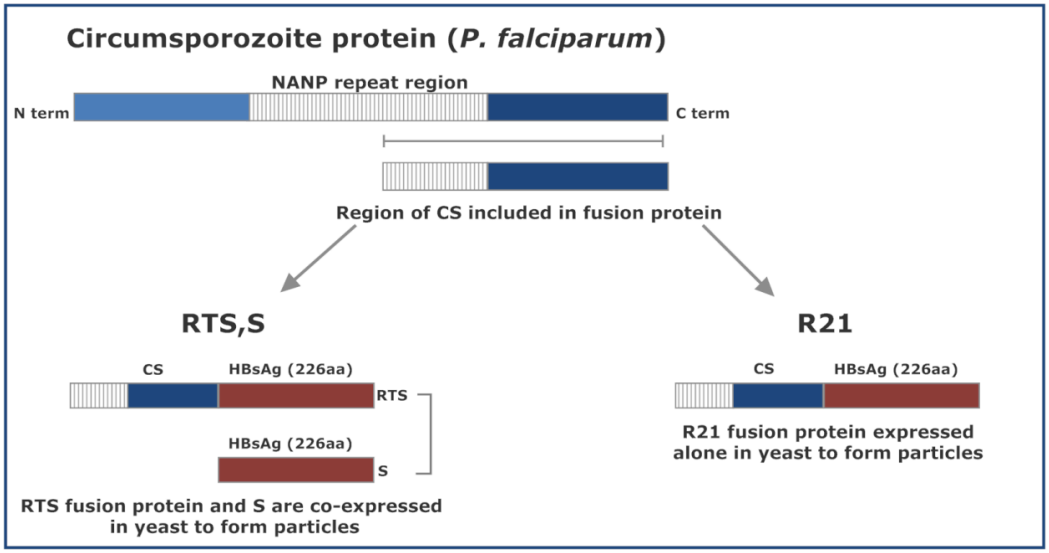

Figure 2: Schematic diagram showing the RTS,S and R21 fusion proteins. Both RTS,S and R21 include the fusion protein of HBsAg to the C-terminus and central repeats of the CSP of *P. falciparum*. These repeats comprise many copies of the four amino acid sequence NANP [49,50].

Part B: After Phagocytosis

The APCs engulf the antigen (event 1) and deposit it in the phagosome. Saponin components of the matrix-m adjuvant is believed to disrupt the phagosome membrane and the antigen is translocated into the lysosomes (event 2) [22]. The antigen is processed in the lysosome by lysosomal conditions (acidity and lysosomal enzymes) into small peptide fragments and is loaded onto major histocompatibility complexes [22]. The release of saponins from Matrix-M in the lysosomes increases the permeability of the lysosomal membrane (event 3). This allows intact and partially degraded antigen fragments to escape into the cytosol (event 4). Matrix-M also induces upregulation and expression of co-stimulatory molecules (CD86, CD69) on APCs (event 5) [22], improving their ability to present antigen fragments to T-cells via two pathways: fragments of antigen in the lysosome bind to MHC class II molecules to be recognized by CD4+ T lymphocytes (event 6), whereas, intact antigens that escape lysosomal degradation are further processed in the cytosol and presented by MHC class I molecules to CD8+ T lymphocytes (event 7) [22,47]. In addition, Matrix-M activates the NLRP3 inflammasome, a crucial structure responsible for releasing pro-inflammatory cytokines like IL-1β and IL-18 (event 8) [22]. This activation also stimulates the production of other cytokines, including TNF-α and IL-6, by APCs [22]. The combined release of these cytokines amplifies the immune response, creating a robust defence against the malaria parasite.

Key Observations from Clinical Trials ofR21/Matrix-M^{TM}

The efficacy of the R21/Matrix-M^{TM} malaria vaccine was assessed in several clinical trial phases, which provided key data on the vaccine's efficacy, safety, and immunogenicity under real-world conditions and represented the data upon which the WHO relied to recommend the use of the vaccine in children who are at greater risk of malaria [17,27,29,41,42,48-50].

Thevaccine effectively elicited immune responses specific for NANP repeats on the pfCSP for up to 12 months in children. However, the immunity lowered at 24 months [17,27,29]. Younger age groups had the highest 12-month vaccine efficacy of 79% and 80% [17,29]. This is correlated with better vaccine effectiveness at a dose ratio of 10 µg/50 µg (R21/Matrix-M^{TM}) administered 3 times at intervals of 4 weeks, followed by a booster shot 12 months after the third dose [17,27,29]. However, the immune induction power depends on the quality of the CSP contained in each vaccine [17]. The safety profile of the vaccine was satisfactory, with only mild reactions such as fever and pain at the injection site. Post-vaccination fevers vary from mild to none across regions and within groups included in the studies. Severe adverse effects (AEs) were mild and resolved within 48 hours [41]. These symptoms are less common with the R21/Matrix-M^{TM} vaccine than with the RTS,S/AS01 vaccine [3,32,51]. This combination of efficacy and safety is unique and positions the R21/Matrix-M^{TM} vaccine as a smartintervention for malaria prevention in children living in malaria-endemic regions.

Limitations of the R21/Matrix-M^{TM} vaccine

First, the ability of the R21/Matrix-M^{TM} malaria vaccine to completely clear Plasmodium sporozoites in the early stages of infection is limited. This is because of its long-term window for producing higher titers of antibodies. Even though the window period is not explicitly stated in clinical trials, higher titers of malaria-specific anti-NANP antibodies were observed 28 days after the third vaccination, indicating the vaccine's delayed impact, during which the plasmodium sporozoites must have advanced [17,27,29,41]. Second, the efficacy of the R21/Matrix-M^{TM} vaccine was sustained for up to two years after the booster was administered [17], suggesting continued monitoring of the vaccine to establish the extent of its protection beyond this timeframe [4]. Third, R21/Matrix-M^{TM} vaccination should be further evaluated for potential changes in age-related effectiveness. For example, younger children (5–17 months old) had greater NANP-specific antibody titers than older children (18–36 months old), showing that age may influence the immune response and effectiveness [29]. Furthermore, participants' voluntary withdrawals across clinical trials can explain the potential reluctance of taking the vaccine or incomplete adherence to the required doses, as already observed by Datoo and colleagues among participants in Burkina Faso [27]. Finally, the R21/Matrix-M^{TM} is limitedby its restricted applicability in children aged 3 to 5 years. No malaria vaccine for use in the adult population hasbeen licensed.

Conclusion

The R21/Matrix-M^{TM} vaccine is a revolutionary intervention against malaria. It has the ability to elicit an immune response toward the NANP central repeat of *P. falciparum* and intercept the Plasmodium sporozoites in the pre-erythrocytic stage of the malaria life cycle. This is a wonder the world has been expecting. The vaccine is only allowed to be used in children between the ages of 3 and 5. No definite evidence of the vaccine's efficacy in adult. We strongly encourage continued search for malaria vaccines for the adult population to overcome inequality and form a more formidable force for the eradication of malaria globally. Furthermore, since the WHO announced the approval of the R21/Matrix-M^{TM} vaccine in 2021, the world seems to be less excited about this novel intervention. Could the enduring familiarity with malaria and availability of other existing interventions be the reason for this? As wider rollout is set to begin, should this be a concern? Does this factors have the potential to interfere with the R21/Matrix-M^{TM} uptake and/or adherence to the required doses? But the good news is that, after decades of fervent research marked by repeated setbacks, and now an efficacy of 77%, the R21/Matrix-M^{TM} is simply a wonder. There's need for public awareness about this vaccine as soon as possible in order to minimize likely underutilization in targeted regions.

Abbreviations

APCs: Antigen-presenting cells

ChAd63: Chimpanzee adenovirus 63

CSP: Circumsporozoite protein

PfCSP: *Plasmodium falciparum* circumsporozoite protein

Tfh: T follicular helper

VLP: Virus-like protein

Supporting information: None

Ethical Considerations: Not applicable

Acknowledgments: None

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author contribution statement: All authors (Initials of authors) contributed equally and attest they meet the ICMJE criteria for authorship and gave final approval for submission.

Data availability statement: Data included in article/supp. material/referenced in article.

Additional information: No additional information is available for this paper.

Declaration of competing interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Clinical Trial: Not applicable

Consent for publication: Note applicable

References

[1] World malaria report 2023. Geneza: World Health Organization; 2023. Licence: CC BY-NC-SA 3. 0 IGO. *[Crossref][PubMed][Google Scholar]*

[2] University of oxford. R21/Matrix-MTM malaria vaccine developed by University of Oxford receives regulatory clearance for use in Ghana, 2023. [Internet]. [cited 2024 Feb 18]. *Available from: [Article][Crossref][PubMed][Google Scholar]*

[3] RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: Final results of a phase 3, individually randomised, controlled trial. The Lancet. 2015;386(9988):31-45. *[Crossref][PubMed][Google Scholar]*

[4] Parums DV. Editorial: Current Status of Two Adjuvanted Malaria Vaccines and the World Health Organization (WHO) Strategy to Eradicate Malaria by 2030. Med Sci Monit. 2023;29. *[Crossref] [PubMed][Google Scholar]*

[5] Genton B. R21/Matrix-MTM malaria vaccine: a new tool to achieve WHO's goal to eliminate malaria in 30 countries by 2030? J Travel Med. 2023;30(8). *[Crossref][PubMed][Google Scholar]*

[6] Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. Nature. 1967; 216(5111), 160–162. *[Crossref] [PubMed][Google Scholar]*

[7] Ballou WR, Cahill CP. Two decades of commitment to malaria vaccine development: GlaxoSmithKline Biologicals. Am J Trop Med Hyg. 2007;77(6 Suppl), 289–295. *[Crossref][PubMed] [Google Scholar]*

[8] Clyde DF, Most H, McCarthy VC, Vanderberg JP. Immunization of man against sporozite-induced falciparum malaria. Am J Med Sci. 1973;266(3), 169–177. *[Crossref][PubMed][Google Scholar]*

[9] Ballou WR, Hoffman SL, Sherwood JA, et al. Safety and efficacy of a recombinant DNA Plasmodium falciparum sporozoite vaccine. The Lancet. 1987;1(8545), 1277–1281. *[Crossref] [PubMed][Google Scholar]*

[10] Hoffman SL, Goh LM, Luke TC, et al (2002). Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. J Infect Dis. 2002;185(8), 1155–1164. *[Crossref][PubMed][Google Scholar]*

[11] El-Moamly AA, El-Sweify MA. Malaria vaccines: the 60-year journey of hope and final success—lessons learned and future prospects. Trop Med Health. 2023;51(1):29. *[Crossref][PubMed][Google Scholar]*

[12] Goodman AL, Draper SJ. Blood-stage malaria vaccines - recent progress and future challenges. Ann Trop Med Parasitol. 2010;104(3), 189–211. *[Crossref][PubMed][Google Scholar]*

[13] Sirima SB, Cousens S, Druilhe P. Protection against malaria by MSP3 candidate vaccine. N Engl J Med. 2011;365(11), 1062–1064. *[Crossref][PubMed][Google Scholar]*

[14] Thera MA, Doumbo OK, Coulibaly D, et al. A field trial to assess a blood-stage malaria vaccine. N Engl J Med. 2011;365(11), 1004–1013. *[Crossref][PubMed][Google Scholar]*

[15] Payne RO, Milne K, Elias SC, et al. Demonstration of the Blood-Stage Plasmodium falciparum Controlled Human Malaria Infection Model to Assess Efficacy of the P falciparum Apical Membrane Antigen 1 Vaccine, FMP2 1/AS01. J Infect Dis. 2016;213(11), 1743–1751. *[Crossref][PubMed][Google Scholar]*

[16] Collins KA, Snaith R, Cottingham MG, Gilbert SC, Hill AVS. Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. Sci Rep. 2017;7. *[Crossref][PubMed][Google Scholar]*

[17] Datoo MS, Natama HM, Somé A, et al. Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: a phase 1/2b randomised controlled trial. Lancet Infect Dis. 2022;22(12):1728-1736. *[Crossref][PubMed][Google Scholar]*

[18] Mukhopadhyay E, Brod F, Angell-Manning P, et al. Production of a high purity, C-tagged hepatitis B surface antigen fusion protein VLP vaccine for malaria expressed in Pichia pastoris under cGMP conditions. Biotechnol Bioeng. 2022;119(10):2784-2793. *[Crossref][PubMed][Google Scholar]*

[19] Cox RJ, Pedersen G, Madhun AS, et al. Evaluation of a virosomal H5N1 vaccine formulated with Matrix MTM adjuvant in a phase I clinical trial. Vaccine. 2011;29(45):8049-8059. *[Crossref][PubMed][Google Scholar]*

[20] Portnoff AD, Patel N, Massare MJ, et al. Influenza Hemagglutinin Nanoparticle Vaccine Elicits Broadly Neutralizing Antibodies against Structurally Distinct Domains of H3N2 HA. Vaccines. 2020;8(1):99. *[Crossref][PubMed][Google Scholar]*

[21] Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, et al. Phase 1–2 Trial of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine. N Engl J Med. 2020;383(24):2320-2332. *[Crossref][PubMed][Google Scholar]*

[22] Stertman L, Palm AE, Zarnegar B, et al. The Matrix-MTM adjuvant: A critical component of vaccines for the 21st century. Hum Vaccin Immunother. 2023;19(1). *[Crossref][PubMed][Google Scholar]*

[23] Duffy PE, Sahu T, Akue A, Milman N, Anderson C. Pre-erythrocytic malaria vaccines: identifying the targets. Expert Rev Vaccines. 2012;11(10):1261-1280. *[Crossref][PubMed][Google Scholar]*

[24] Coppi A, Natarajan R, Pradel G, et al. The malaria circumsporozoite protein has two functional domains, each with distinct roles as sporozoites journey from mosquito to mammalian host. J Exp Med. 2011;208(2):341-356. *[Crossref][PubMed][Google Scholar]*

[25] Rathore D, Nagarkatti R, Jani D, et al. An Immunologically Cryptic Epitope of Plasmodium falciparum Circumsporozoite Protein Facilitates Liver Cell Recognition and Induces Protective Antibodies That Block Liver Cell Invasion. J Biol Chem. 2005;280(21):20524-20529. *[Crossref][PubMed][Google Scholar]*

[26] Gavi vaccine alliance. Gavi's impact in Africa since 2000 [Internet]. [cited 2024 May 11]. Available from: *[Article][Crossref][PubMed][Google Scholar]*

[27] Datoo MS, Natama MH, Somé A, et al. Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial. The Lancet. 2021;397(10287):1809-1818. *[Crossref][PubMed][Google Scholar]*

[28] Tsoumani ME, Voyiatzaki C, Efstathiou A. Malaria Vaccines: From the Past towards the mRNA Vaccine Era. Vaccines (Basel). 2023;11(9):1452. *[Crossref][PubMed][Google Scholar]*

[29] Datoo MS, Dicko A, Tinto H, Ouédraogo JB, Hamaluba M, Olotu A, et al. Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, phase 3 trial. The Lancet. 2024;403(10426):533-544. *[Crossref][PubMed][Google Scholar]*

[30] Schmit N, Topazian HM, Natama HM, et al. The public health impact and cost-effectiveness of the R21/Matrix-M malaria vaccine: a mathematical modelling study. Lancet Infect Dis. 2024;24(5):465-475. *[Crossref][PubMed][Google Scholar]*

[31] Vanderberg JP, Sidjanski S. Delayed Migration of Plasmodium Sporozoites from the Mosquito Bite Site to the Blood. Am J Trop Med Hyg. 1997;57(4):426-429. *[Crossref][PubMed][Google Scholar]*

[32] Sinnis P, Coppi A. A long and winding road: The Plasmodium sporozoite's journey in the mammalian host. Parasitol Int. 2007;56(3):171-178. *[Crossref][PubMed][Google Scholar]*

[33] Baer K, Klotz C, Kappe SHI, Schnieder T, Frevert U. Release of Hepatic Plasmodium yoelii Merozoites into the Pulmonary Microvasculature. PLoS Pathog. 2007;3(11):e171. *[Crossref][PubMed][Google Scholar]*

[34] Medica DL, Sinnis P. Quantitative Dynamics of Plasmodium yoelii Sporozoite Transmission by Infected Anopheline Mosquitoes. Infect Immun. 2005;73(7):4363-4369. *[Crossref][PubMed][Google Scholar]*

[35] Kebaier C, Voza T, Vanderberg J. Kinetics of Mosquito-Injected Plasmodium Sporozoites in Mice: Fewer Sporozoites Are Injected into Sporozoite-Immunized Mice. PLoS Pathog. 2009;5(4):e1000399. *[Crossref][PubMed][Google Scholar]*

[36] Hodgson SH, Ewer KJ, Bliss CM, et al. Evaluation of the Efficacy of ChAd63-MVA Vectored Vaccines Expressing Circumsporozoite Protein and ME-TRAP Against Controlled Human Malaria Infection in Malaria-Naive Individuals. J Infect Dis. 2015;211(7):1076-1086. *[Crossref][PubMed][Google Scholar]*

[37] O'Hara GA, Duncan CJA, Ewer KJ, et al. Clinical Assessment of a Recombinant Simian Adenovirus ChAd63: A Potent New Vaccine Vector. J Infect Dis. 2012;205(5):772-781. *[Crossref][PubMed][Google Scholar]*

[38] Gordon DM, McGovern TW, Krzych U, et al. Safety, Immunogenicity, and Efficacy of a Recombinantly Produced Plasmodium falciparum Circumsporozoite Protein-Hepatitis B Surface Antigen Subunit Vaccine. J Infect Dis. 1995;171(6):1576-1585. *[Crossref][PubMed][Google Scholar]*

[39] Heppner DG Jr, Kester KE, Ockenhouse CF, Tornieporth N, Ofori O, Lyon JA, et al. Towards an RTS,S-based, multi-stage, multi-antigen vaccine against falciparum malaria: progress at the Walter Reed Army Institute of Research. Vaccine. 2005;23(17-18):2243-2250. *[Crossref][PubMed][Google Scholar]*

[40] Laurens MB. RTS,S/AS01 vaccine (MosquirixTM): an overview. Hum Vaccin Immunother. 2020;16(3):480-489. *[Crossref][PubMed][Google Scholar]*

[41] Venkatraman N, Tiono AB, Bowyer G, et al. Phase I assessments of first-in-human administration of a novel malaria anti-sporozoite vaccine candidate, R21 in matrix-M adjuvant, in UK and Burkinabe volunteers. MedRxiv. 2019:19009282. *[Crossref][PubMed][Google Scholar]*

[42] Sang S, Datoo MS, Otieno E, et al. Safety and immunogenicity of varied doses of R21/Matrix-MTM vaccine at three years follow-up: A phase 1b age de-escalation, dose-escalation trial in adults, children, and infants in Kilifi-Kenya. Wellcome Open Res. 2023;8:450. *[Crossref][PubMed][Google Scholar]*

[43] Reed SG, Bertholet S, Coler RN, Friede M. New horizons in adjuvants for vaccine development. Trends Immunol. 2009;30(1):23-32. *[Crossref][PubMed][Google Scholar]*

[44] Reinke S, Pantazi E, Chappell GR, et al. Emulsion and liposome-based adjuvanted R21 vaccine formulations mediate protection against malaria through distinct immune mechanisms. Cell Rep Med. 2023 Nov 21;4(11):101245. *[Crossref][PubMed][Google Scholar]*

[45] Didierlaurent AM, Collignon C, Bourguignon P, et al. Enhancement of adaptive immunity by the human vaccine adjuvant AS01 depends on activated dendritic cells. J Immunol. 2014;193(4):1920–30. *[Crossref][PubMed][Google Scholar]*

[46] Magnusson SE, Altenburg AF, Bengtsson KL, Bosman F, de Vries RD, Rimmelzwaan GF, Stertman L. Matrix-M adjuvant enhances immunogenicity of both protein- and modified vaccinia virus Ankara-based influenza vaccines in mice. Immunol Res. 2018;66(2):224–33. *[Crossref] [PubMed][Google Scholar]*

[47] den Brok MH, Bull C, Wassink M, et al. Saponin-based adjuvants induce cross-presentation in dendritic cells by intracellular lipid body formation. Nat Commun. 2016;7:13324. *[Crossref] [PubMed][Google Scholar]*

[48] Venkatraman N, Bowyer G, Edwards NJ, et al. High level efficacy in humans of a next-generation plasmodium falciparum anti-sporozoite vaccine: R21 in Matrix-M™ adjuvant. In: Abstract of the 66th Annual Meeting of the American Society of Tropical Medicine and Hygiene; Baltimore, Maryland USA; 2017 Nov 5-9. Abstract 1911. *[Crossref][PubMed][Google Scholar]*

[49] Hill A. Clinical Trial Protocol: VAC053 Protocol v5. 0. University of Oxford, 2019. *[Crossref] [PubMed][Google Scholar]*

[50] Hill, A. Clinical Trial Protocol: VAC060 Protocol v3. 0. University of Oxford, 2019. *[Crossref] [PubMed][Google Scholar]*

[51] RTS,S Clinical Trials Partnership, Agnandji ST, Lell B, et al. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. N Engl J Med. 2011;365(20):1863-1875. *[Crossref] [PubMed][Google Scholar]*