Open Access

Epidemiology & Public Health

Article Type: Review Article

Volume 3 Issue 1 - 2025

The Three Musketeers, The Sequel, and The Novel Malaria Vaccines: RTS, S/AS01$_E$ and R21/Matrix-M

***M Sanni Ali*[1]*; *Dennis Adu-Gyasi*[2,3]**

[1]*Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK.*

[2]*Kintampo Health Research Centre, Research and Development Division, Ghana Health Service, Kintampo North Municipality, Bono East Region, Ghana.*

[3]*Centre for Research in Applied Biology, School of Sciences, University of Energy and Natural Resources, Sunyani, Bono Region, Ghana, West Africa.*

Abstract

WHO recommended two novel vaccines, RTS,S/AS01$_E$ and R21/Matrix-M, against malaria caused by *Plasmodium falciparum*. For both vaccines, the recommended schedule is 4-doses starting from the age of 5 months, in children living in regions with moderate to high malaria transmission, with an optional 5-dose schedule for areas with highly seasonal malaria transmission. Both vaccines have favourable safety profile, are highly cost-effective, can be delivered through routine national Expanded Program on Immunizations (EPI) and can substantially reduce severe malaria burden. Although the choice between the two vaccines is dependent on several factors such as availability, cost, and country's context and malaria epidemiology, understanding of the similarity and differences between the two vaccines is essential. In this review, we described the historical development of both vaccines and their similarities and differences using an analogy from the famous historical adventure novels by Alexandre Dumas, The three musketeers and 20 years after.

***Corresponding author: M Sanni Ali**

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK.

Email: sanni.ali@lshtm.ac.uk

Received: Dec 09, 2024

Accepted: Jan 08, 2025

Published: Jan 15, 2025

Epidemiology & Public Health - www.jpublichealth.org

Citation: Sanni Ali M, Adu-Gyasi D. The Three Musketeers, The Sequel, and The Novel Malaria Vaccines: RTS, S/AS01$_E$ and R21/Matrix-M. Epidemiol Public Health. 2025; 3(1): 1063.

Introduction

Alexandre Dumas's creation of the adventurous "The three musketeers", published in French as *Les Trois Mousquetaires,* has the heroic, chivalrous swordsmen (Aramis, Porthos, Athos, and D'Artagnan) who fight for justice and defeated Milady who goes by numerous aliases [1]. It was reignited with its sequel "twenty years after" [2] when they reunited to fight the forces of evil – this epic novel shares a lot with the fight against malaria: military and war, swords, wine, and protection of the most vulnerable and loved ones - children!

Since human civilisation, malaria not only threatened the survival of children but also the success of several empires including the Romans [3], and advanced militaries despite the availability of antimalarial drugs, mosquito-repellents, bed nets and other counter measures. For example, during World War I, more than 100,000 British and French troops on the Macedonian front were sidelined due to malaria [4]. During World War II, malaria was the most important health threat encountered by U.S. troops in the pacific where approximately 500,000 men were infected [5]. Later, in the American-Vietnam war, more than 80,000 cases of malaria were detected among U.S. troops between 1965 and 1973 [4]. Hence, the US military at the Walter Reed Army Institute of Research (WRAIR) started the Malaria Vaccine Program with a goal of developing a safe, well-tolerated, licensed multi-antigen, multi-stage, and highly effective vaccine that prevents all symptomatic manifestations of malaria or very significantly limits the severity of disease in vaccinees who develop malaria and confers sustained protection [6].

Malaria vaccine research

Although malaria vaccine research has been an intense area of research since the 1960s [7], the significant sprint started in the 1980s when the Circumsporozoite Protein (CSP), a sporozoite surface antigen, was identified as the likely target of the protective immune responses [8]. In 1984, scientists at the US National Institutes for Health and the WRAIR cloned and sequenced the gene encoding the CSP of *Plasmodium falciparum* ushering the era of subunit vaccine development for malaria [9]. The same year, WRAIR entered an important collaboration

GSK to produce a malaria vaccine using GSK's recombinant Escherichia coli expression systems [10].

In 1987, the GSK malaria vaccine program was transferred from its laboratories in Philadelphia, PA, to its vaccine division in Belgium to benefit from the research and development expertise in the field of vaccinology that existed in the Belgian division [10]. The same year, scientists working in GSK laboratories in Belgium conceived of and pioneered the use of the hepatitis B surface antigen (HBsAg) as a carrier matrix for the central repeat region of the *P. falciparum* CSP [11]. Initially, the R16-HBsAg construct was developed that was abandoned after Phase I testing [10], followed by a more promising CSP–HBsAg fusion protein of the carboxyl-terminal half of the *P. falciparum* (strain NF54, laboratory clone 3D7) Circumsporozoite Protein (CSP) [12].

When this fusion protein (CSP-HBsAg) is co-expressed in yeast cells (*Saccharomyces cerevisiae)* with the non-fused HBsAg antigen, it self-assembles to form virus like particles (VLP), the RTS,S [12] was created: the CSP protein, the HBsAg, and the yeast expression system – the three Musketeers!

"It is the down of the 17th century. After the assassination of his father, the young king Louis XIII ascends through the French throne. His once peaceful nation is surrounded by enemies on all sides. Within the boarders of France itself, Cardinal Richelieu picking his advisor plots to cease power for himself. The inexperience Louis and his new bride, Queen Anne, find themselves without a single friend. Europe is a powder cake wating to explode in a war that will engulf the entire continent and only a few men can prevent the incoming apocalypse" - the three Musketeers [1,10,13].

The dourest Athos, who obscured his identity and refused to tell anyone about his past was once part of Milady de Winter, the formidable foe. Although he has a deep hatred to Milady, their secrets are inextricably linked to one another. Athos is also the best swordsman of all the musketeers, with the best military mind – comparable to the CSP, part of the *Plasmodium falciparum parasite*. There are often stretches of times where Athos drinks nonstop, yet he is the most likely of the friends to act as a leader [1,14].

The extrovert Porthos loves to dress well, the most concerned with his looks, and is extremely dedicated and loyal toward his friends and fellow Musketeers. He stands out for his physical strength, size and love for wine! women and song [1,14]. HBsAg, the first vaccine to be produced using recombinant DNA technology is the most complex recombinant protein in forming nanoparticles that resemble patient-derived virus particles in both structure and immunogenicity [15]. Porthos is the easiest musketeer to read, as HBsAg is the easiest antigen to express in bulk using yeast cells or Escherichia coli expression vectors.

The attractive Aramins is a talented writer and has the best expression skills among the group helping them in secretive letters and helping them out of several binds. He re-joined the Musketeers when he decided that he is better off as a soldier rather than a monk; he is constantly torn between his life as a musketeer and his desire to join the church [1,14]. Needless to mention that monasteries were historically excellent fermenters and were associated with the best of wines and beers, especially in Belgium. The yeast *Saccharomyces cerevisiae* is not only the species most essential to winemaking but also the expression system for CSP and HBsAg to forms the RTS,S recombinant protein.

The expressed RTS,S protein contains 189 amino acids from a single allele of the *P. falciparum* CSP including NANP conserved repeats from the central region and the C-terminus of the non-repeat region which contains T-cell epitopes. The NANP repeats contain the immunodominant B-cell epitopes while the C-terminal region contains numerous polymorphisms and three known T-cell epitopes consisting of highly variable CD4+ and CD8+ T-cell epitopes and a conserved 'universal' CD4+ T-cell epitope [15].

The creation of RTS,S protein was an important advance, however, critical to the success of this RTS,S vaccine was the development of GSK's innovative adjuvant systems (AS). Adjuvants have an essential role in the development of vaccines, they are critical for determining the magnitude and direction of the immune response by mechanisms including increased antigen presentation, uptake, distribution and selective targeting [16]. The GSK's proprietary adjuvant systems have passed through a series of comparative tests in several human challenge trials, the oil-in-water emulsion plus MPL and QS21 (AS01) adjuvant system proved the greatest CS-specific antibody and CD4+ T-cell responses [10].

The adjuvant more or less parallels the story of the young and handsome man from Gascony, D'Artagnan – the rider!, who is known for his intelligence and bravery which makes him a perfect candidate for M. de Tréville's musketeers. However, before he can become a musketeer, he must prove himself worthy: through a series of duels, political intrigue along with Athos, Porthos, and Aramis. D'Artagnan often acts as the leader of the group despite his young age; he's largely a heroic character, but not without his faults [1,10].

The intelligent Milady Laurence de Winter is Cardinal Richelieu's chief spy and assassin. She is a manipulative, ruthless, and relentless spy with a brilliant blue eyes and black lashes and brows, and a voice that can seduce and bewitch - the classic example of a femme fatale [10]. At a young age, she was branded with a *fleur-de-lis,* a brand given to criminals that represents their removal from polite society, because she stole sacred objects from a church, hid her criminal status to marry Athos. Afterall, "Athos is the only one who seemed genuinely happy after her death, also the wisest member of the group and the most likely of the friends to act as a leader along with d'Artagnan. Athos is often the musketeer who is matched up with more than one enemy at once in combat and he always manages to come out unscathed" [1,10]. Throughout the novel, Milady successfully seduced many characters and got them to do her bidding except d'Artagnan, the only person who gets the better of her and who tricks her into thinking that he is the Comte de Wardes, [10].

The furiously self-absorbed, powerful and cunning Cardinal Richelieu who tortured and severely taxed the citizens will go outside the bounds of traditional morality to get his way. He works hard to maintain the reputation and power of King Louis XIII, the king of France, since this is the stock on which his own status is based – just like the anopheles mosquito sucks human blood, to use the protein for developing its eggs, reproduction! To help locate their prey, anopheles mosquito engages their antennae and palps to detect carbon dioxide and odour. Hence, people who have a high metabolic rate and emit more carbon dioxide, including those who are pregnant, working out, or drinking alcohol tend to be more attractive to mosquitoes [17]. Cardinal Richelieu hates the vulnerable and lonely Queen Anne, he wants to diminish her influence, and she is the is the victim

of an assassination attempt.

The sequel, "Twenty Years After" – nearly two decades have passed since the musketeers triumphed over Milady and her Master Cardinal Richelieu. Time went on, yet "treasons and stratagems still cry out for justice: civil war endangers the throne of France, while in England Cromwell threatens to send Charles I to the scaffold. Dumas brings his immortal quartet out of retirement to cross swords with time, the malevolence of men, and the forces of history. But their greatest test is a titanic struggle with the vengeful son of Milady, Mordaunt, who wears the face of Evil and the sinister Cardinal Mazarin" [2]. d'Artagnan is a musketeer who has changed a lot with the death of his beloved Constance, poisoned by the evil Milady. He has to find the three other musketeers in return for a promised promotion. By the end of the novel, the musketeers were able to convince Mazarin, by kidnapping him, to agree to the demands made by the people of Paris, thus bringing peace to the city and all well rewarded!

2012 - that's also nearly twenty years after the design of RTS,S, scientists at Jenner Institute, University of Oxford created the novel R21/Matrix-M [18]: 1) the CSP protein of *P. falciparum*, 2) the carrier hepatitis B surface antigen (HBsAg) but without the unfused HBsAg found in RTS,S and 3) an improved yeast expression system, *Pichia pastoris*, rather than *Saccharomyces cerevisiae* of RTS,S, and an altered adjuvant system, the Matrix-M [19].

The R21, like RTS,S vaccine, is a virus-like particle-based vaccine based on CSP and hepatitis B surface antigen. It is formed solely from HBsAg fused to the C-terminus and central repeats of the CSP (CSP-HBsAg fusion), which self-assemble into virus-like particles in yeast cell. R21 comprises only fusion protein moieties, in contrast to RTS,S which comprises 20% fused HBsAg with the remaining 80% being HBsAg monomers expressed alone, thereby diminishing CSP coverage of the virus-like particle surface [18]. Hence, a large proportion of the antibody response induced by RTS,S is towards the HBsAg. R21 is an improved HBsAg-CSP-based construct, but without any unfused HBsAg protein which is present at a four-fold molar excess in RTS,S particles. This excess of HBsAg was required for RTS,S to form a particle; however, R21 forms particles without requiring excess HBsAg. This was achieved by expressing R21 in an improved yeast expression system, *Pichia pastoris*, rather than in Saccharomyces cerevisiae of RTS,S. As a result, the majority of R21 surface is coated in CSP antigen and predicted to yield a greater humoral response through enhanced surface recognition by B-cell receptors [18,19]. The removal of the unfused S particles and the increased density of CSP antigen on the Virus-Like Particle (VLP) is believed to improve the immune response against the CSP in R21 than in RTS,S. As a result, a 50 µg dose of R21 contains about 25µg of CSP antigen compared with 10 µg of CSP in a standard adult 50µg dose of RTS,S. In addition, R21 was developed to induce a lower immune response against the HBsAg fraction. Table 1 compares RTS,S/AS01$_E$ and R21/Matrix-M and Figure 1 compares the two vaccines.

Table 1: Summary of characteristic of RTS, S/AS01$_E$ and R21/Matrix-M.

	RTS, S/AS01$_E$ (Mosquirix™)	R21/Matrix-M™
Stage	Pre-erythrocytic malaria vaccine	Pre-erythrocytic malaria vaccine
Developer	GSK (1997) The Walter Reed Army Institute of Research (WRAIR)	Jenner Institute, Oxford University (2012)
	GSK and PATH	University of Oxford and the Serum Institute of India
Components		
	Central repeat region and the C terminal region of the CSP protein (CSP) + HBsAg + AS01$_E$	Central repeat region and the C terminal region of the CSP protein + HBsAg + Matrix-M™
The CSP	Central repeat region and the C terminal region of the CSP protein (CSP): Amino acids 207 to 395 of the CSP from NF54 strain of P. falciparum	Central repeat region and the C terminal region of the CSP protein
HBsAg	Fused HBsAg and unfused HBsAg	Only Fused HBsAg
Adjuvant	Liposome based adjuvant. AS01$_E$: Saponin (QS-21) + liposome (MPL)	Novavax's adjuvant technology: Matrix-M™ Matrix-M (MM): Saponin (QS-21)
Adjuvant Developer	GSK Biologicals	Novavax
The yeast expression System	*Saccharomyces cerevisiae*	*Pichia pastoris*
Vaccine Manufacturer	GSK Biologicals	Serum Institute of India Pvt. Ltd
Dose	(0.5ml) 25 microgram RTS, S adjuvanted with AS01$_E$	5microgram R21 + 50 microgram Matrix-M™
Storage	−70°C in sterile containers, After reconstitution with the liquid Adjuvant, it should be stored at 2-8^{0}C.	2-8^{0}C
The vaccine and adjuvant	Comes in two parts: Powder or lyophilized form containing RTS, S presented in a 3 mL glass vial closed with rubber stoppers and aluminium caps (2 dose) AND the liquid suspension of the AS01$_E$ adjuvant system in a 3 mL glass vial (2 doses)	Single and multiple dose vials (co-formulated antigen-adjuvant) in a single vial
Reconstituted product	Preservative-free liquid suspension for injection that appears opalescent, colorless to pale brown. 1 vial of each produces 2 doses of vaccine for intramuscular injection (1ml)	Clear colorless to mildly turbid solution for injection.
Route of administration	IM, deltoid muscle	IM, deltoid muscle – if small anterolateral thigh muscle
Approved	6 October 2021	2 October 2023

Delivered	As a lyophilized injection, IM	
WHO recommendation	Using a 4-dose schedule in 3 to 5 moderate-to-high transmission settings sub-Saharan Africa.	Using a 4-dose schedule in 3 to 5 moderate-to-high transmission settings sub-Saharan Africa.??
	The first dose given at 5 months of age (monthly interval) Third dose should be completed by the 9 months. Fourth dose should be administered at 15–18 months.	The first dose given at 5 months of age (monthly interval) Third dose should be completed by the 9 months. Fourth dose should be administered at 15–18 months.
Price range per dose	$9 to $10	$2 to $4 per dose
Manufactured advantage?	By GSK	At mass scale and modest cost by the Serum Institute of India.
Piloted	In children 5–17 months of age in Ghana, Kenya and Malawi.	-
Financial Support	GSK, PATH Malaria Vaccine Initiative (MVI) with the support of the Bill and Melinda Gates Foundation.	The European and Developing Countries Clinical Trials Partnership ('EDCTP'), the Welcome Trust, and the European Investment Bank ('EIB').

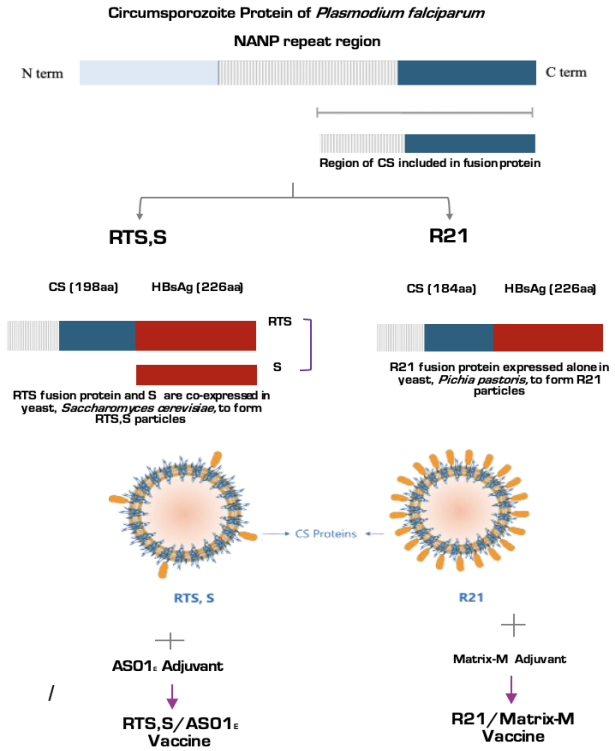

Figure 1: Comparison of RTS, S/AS01E and R21/Matrix.
aa: amino acids, CS: circumsporozoite.
Adapted from Collins et al. [18].

In 2015, following an RTS,S/AS01$_E$ [20] application for the Committee for Medicinal Products for Human Use (CHMP) through the European Medicines Agency and in anticipation of a positive opinion from the CHMP, WHO established a Joint Technical Expert Group to monitor progress with the RTS,S/AS01$_E$ trials with the intention that this group will provide advice to a joint committee of WHO's Malaria Policy Advisory (MPAG) Committee and the Strategic Advisory Group of Experts (SAGE) committees, which formulate WHO's recommendations on the use of RTS,S/AS01$_E$ [21]. The same year, RTS,S/AS01$_E$ received a favourable opinion from the European Medicines Agency (EMA) after review of its safety and efficacy to reduce clinical *Plasmodium falciparum* malaria episodes in young African children - a milestone in vaccine development as the first human parasite vaccine passed the highest level of regulatory scrutiny, referred to as WHO-listed authority maturity level 4 (WLA ML4)) [21,22].

The following year, WHO recommended further evaluation of RTS,S/AS01$_E$ in children aged 5–17 months in a large-scale pilot implementation program before rolling it out at country level. The pilot implementation programs was launched in 2019 in three countries (Ghana, Kenya and Malawi) and more than 830,000 children were vaccinated with over 2.4 million doses of RTS,S/AS01$_E$ in a little over 2 years [23]. In West Africa, scientists demonstrated the superiority of combining RTS,S with Seasonal Malaria Chemoprevention (SMC) in two highly seasonal site (Mali and Burkina Faso) among 6861 children aged 5-17 months compared to either of the interventions given alone [24].

October 2021, WHO's Strategic Advisory Group of Experts on Immunization (SAGE) and the Malaria Policy Advisory Group (MPAG) jointly reviewed all available evidence, on October 6, 2021, and after 35 years since the creation of RTS,S, the WHO and approved the first malaria and parasitic vaccine (RTS,S/AS01; RTS,S also known as Mosquirix™) for widespread use [25].

"The Long-Awaited Vaccine for Children is a Breakthrough for Science, Child Health, Malaria Control, and A Gift to The World".

---Dr. Tedros Adhanom Ghebreyesus, WHO Director-General

In 2023, the second malaria vaccine, R21/Matrix-M [26] was licensed by several African countries, and on December 21, 2023, it received a WHO policy recommendation and prequalification [27], offering large-scale supply to help reduce the great burden of malaria in sub-Saharan Africa.

Now, we have two malaria vaccines that have been rolled-out in several African countries in the fight against malaria. Setting aside their political differences, the musketeers are valiant and just in their efforts to save the doomed Charles I, so do the novel malaria vaccines to save the beloved children in Africa: "All for one and one for all, united we stand divided we fall."

— Alexandre Dumas, The Three Musketeers [1,2].

Author contributions: Both MSA and DAG conceptualised and reviewed the paper.

Competing interests: Both authors declare no competing interests.

References

1. Dumas A, Maquet A. The Three Musketeers. 1984.
2. Dumas A, Maquet A. Twenty Years After. 1985.
3. Marciniak S, et al. Plasmodium falciparum malaria in 1st–2nd century CE southern Italy. Curr Bio. 2016; 26: R1220.

4. Institute of Medicine (US) Committee for the Study on Malaria Prevention and Control. Malaria: Obstacles and Opportunities. Oaks SC Jr, Mitchell VS, Pearson GW, Carpenter CCJ, editors. Washington (DC): National Academies Press (US). 1991.

5. Bray RS. Armies of Pestilence: The Impact of Disease on History. 1st ed. The Lutterworth Press. 1996.

6. Heppner DG, 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: 2243-2250.

7. Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of X-irradiated sporozoites of Plasmodium berghei. Nature. 1967; 216: 160–162.

8. Otocnjak P, Yoshida N, Nussenzweig RS, Nussenzweig V. Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. J Exp Med. 1980; 151: 1504–1513.

9. Dame JB, et al. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite Plasmodium falciparum. Science. 1984; 225: 593–599.

10. Ballou WR, Cahill CP. Two Decades of Commitment to Malaria Vaccine Development: Glaxosmithkline Biologicals. Am J Trop Med Hyg. 2007; 77: 289–295.

11. Rutgers T, et al. Hepatitis B surface antigen as carrier matrix for the repetitive epitope of the circum-sporozoite protein of Plasmodium falciparum. Biotech. 1988; 6: 1065–1070.

12. Vreden SGS, Verhave JP, Oettinger T, Sauerwein RW, Meuwissen JHET. Phase I clinical trial of a recombinant malaria vaccine consisting of the circumsporozoite repeat region of Plasmodium falciparum coupled to hepatitis B surface antigen. Am J Trop Med Hyg. 1991; 45: 533–538.

13. Anderson PWS. The Three Musketeers: Adapted from Alexandre Dumas' Novel. 2011.

14. Sawyer I. The Three Musketeers. LitCharts. LitCharts LLC. 2023.

15. Almeida MEM, Vasconcelos MGS, Tarragô AM, Mariúba LAM. Circumsporozoite Surface Protein-based malaria vaccines: a review. Rev Inst Med Trop. 2021; 63: e11.

16. Sesardic D, Dobbelaer R. European union regulatory developments for new vaccine adjuvants and delivery systems. Vaccine. 2003; 22: 2452-2456.

17. What is a Mosquito? U.S. Centers for Disease Control and Prevention. 2020.

18. 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: 46621.

19. Venkatraman N, Bowyer G, Edwards N. High level efficacy in humans of a next-generation Plasmodium falciparum anti-sporozoite vaccine: R21 in Matrix-M (TM) adjuvant. Am J Trop Med Hyg. 2017; 97: 594.

20. 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. Lancet. 2015; 386: 31-45.

21. Laurens MB. RTS, S/AS01 vaccine (Mosquirix™): an overview. Hum Vaccin Immunother. 2020; 16: 480-489.

22. Syed YY. RTS, S/AS01 malaria vaccine (Mosquirix®): a profile of its use. Drugs Ther Perspect. 2022; 38: 373-381.

23. WHO. Global Advisory Committee on Vaccine safety. Weekly epidemiological record. 2022.

24. Chandramohan D, et al. Seasonal malaria vaccination with or without seasonal malaria chemoprevention. N Engl J Med. 2021; 385: 1005-1017.

25. WHO. WHO recommends groundbreaking malaria vaccine for children at risk. 2021.

26. Datoo MS, et al. Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, controlled, phase 3 trial. Lancet. 2024; 403: 533-544.

27. WHO. WHO recommends R21/Matrix-M vaccine for malaria prevention in updated advice on immunization. 2023.

28. WHO. Malaria vaccine: WHO position paper. 2024; 19: 225–248.

29. WHO. R21/Matrix-M malaria vaccine: Evidence to recommendations framework. 2023.