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# MOSQUIRIX: JOURNEY FROM LAB TO PHASE 3 CLINICAL TRIALS

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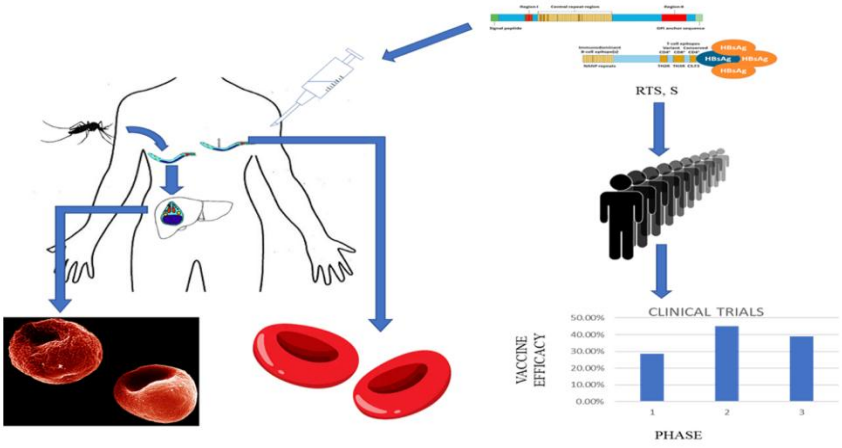
## ABSTRACT

**Background:** Malaria is the major public health threat in endemic regions. However, the recent advances in control efforts has reduced the burden of malaria to some extent but it is still responsible for vast number of deaths in other African countries. RTS, S/AS01 provides active immunization against the most vulnerable group i.e. infants and children aged 6 weeks to 17 months. Development of Mosquirix was a long process with difficulties in identifying malaria antigen related to the induction of immune responses and development of adjuvant system. **Main body:** Mosquirix was approved by European medicine agency in July 2015 and WHO launched pilot project in three malaria endemic countries in 2019. Phase 3 clinical trial suggested that it only provides partial protection against malaria infection in infant and children and considering the high burden of disease it is currently considered for use in malaria endemic countries.Currently GSK is planning Phase IV studies for identification of rare adverse events by a well-planned pharmacovigilance system. Safety profile of vaccine is acceptable but there is risk of convulsions after third dose of vaccine. **Conclusion:** From clinical trial the vaccine efficacy for phase 1, 2 and 3 was found 28.60%, 45% and 39% respectively. The 4-dose regimen of RTS, S has been found efficacious and provide greatest protection among infants and children. RTS,S/AS01E believe to shows promise results as a candidate malaria vaccine.

**KEYWORDS:** Malaria, Mosquirix, RTS, S/AS01, Endemic.

## GRAPHICAL ABSTRACT




## BACKGROUND

Malaria is one of the notable infectious disease and caused around 405000 deaths globally in 2018. The most vulnerable age groups affected by malaria are infants and children aged between 6 weeks to 17 months and accounts for approximately 20% of childhood deaths in Africa. Apart from children and infants the other risk groups it include pregnant women and non-immune people like tourists or military personnel travelling to or posted to malaria endemic countries.[1][2] As per the fourth world malaria report of world health organization (WHO) 228 million cases of malaria has occurred worldwide, 93% of which happened in Africa.[3] Malaria had once affected most of the temperate regions

and was eliminated by the 1950s, 1960s and early 1970s by anti-malarial chemotherapy and vector control programs. Though, control and elimination have been much more difficult in tropical regions because of the fact that excellent vectors, uninterrupted transmission and other factors that led to rates of transmission in tropical areas far exceeds the minimum rate required to maintain the parasite in human reservoir. Thus, an effective vaccine against the lethal parasite would be a valuable tool in the fight against malaria in tropical regions.[2]

Malaria is caused by four species of the protozoal parasite Plasmodium, endemic in most parts of India and other tropical countries. The four species that effect humans are plasmodium falciparum, plasmodium vivax, plasmodium malariae and plasmodium ovale. Last three produces the mild forms of malaria by destroying red blood cells in peripheral capillaries and thus, causing anaemia. However, the most dangerous is the P. falciparum. In this case, the infected red blood cells become sticky and form lumps in the capillaries of the deep organs of the body and cause microcirculatory arrest.[4]

**Life cycle of plasmodium**

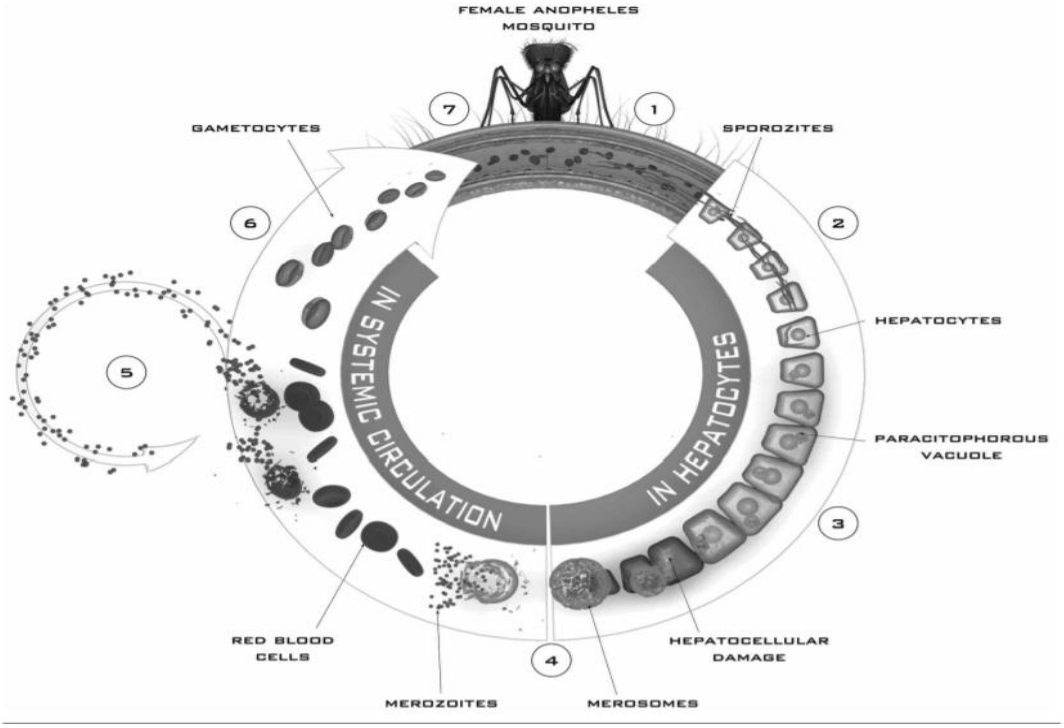



**Figure 1: Life cycle of malaria.**

The life cycle of protozoal parasite plasmodium in the human host is complex and involves asymptomatic liver stage (pre-erythrocytic stage) infection, which leads to symptomatic blood stage (erythrocytic) infection. Infection cycle, shown in figure 1, is briefed below:

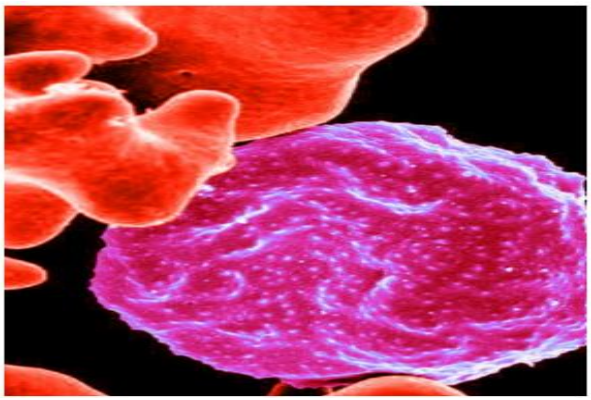


**Figure 2: A red blood cell infected with malaria.**

(1) Sporozoites enters the systemic circulation by the bite of an infected Anopheles mosquito;(2) Sporozoites then enters the hepatic circulation and invade the hepatocytes;(3) Inside hepatocytes they form merozoites and this stage is known as pre erythrocytic schizogony. In case of plasmodium vivax, merozoites convert into hypnozoites and this stage is known as exoerythrocytic cycle. Hypnozoites are dormant forms and best known for the relapse of malaria infection in human. (4) Merozoites are liberated from the hepatocyte in small cellular vesicles called merosomes, which disintegrate in the systemic circulation releasing the merozoites. (5) Further these Merozoites invade the erythrocytes, where they continue maturation and division. This results in rupturing of RBC and release more systemic merozoites, that invades more RBC; an infected RBC is shown in figure 2. (6) Some merozoites distinguish into male and female gametocytes. (7) Gametocytes is taken up by Anopheles mosquito during blood meal and induce new malaria cycle.

### 1.1. Mosquirix

Mosquirix (RTS,S/AS01) is the first malaria vaccine and provide active immunization in infants and children aged 6 weeks to 17 months against the protozoan parasite plasmodium falciparum, responsible for vast majority of deaths due to malaria.[5] Measures currently employed as an effort to reduce malaria incidence include rapid diagnostic testing, highly effective artemisinin combination therapy, use of insecticide treated bed nets and indoor residual spraying. In spite of these measures to reduce malaria incidence, development of an effective vaccine against P. falciparum could substantially reduce the malaria cases.[6] Mosquirix was invented in 1987 by the scientists working in the laboratories of British pharma giant GSK(GlaxoSmithKline). Mosquirix was approved in EU under the article 58. The European medicine agency (EMA) analysed the quality, safety and efficacy of RTS,S/AS01, although the vaccine was meant to be used only outside the EU.[5] It is the first malaria vaccine that made its way towards phase 3 clinical trial and is currently being studied in ongoing clinical trials in Africa.[1] The infants and children are vaccinated in three intramuscular doses at month 0, 1 and 2 and a booster dose administered at month 20 after the first dose to increase the protection. However, the duration of protection provide by RTS,S/AS01 is unclear and the vaccine efficacy wanes over time.[5] The WHO has recommended large scale pilot implementation of RTS,S/AS01 in children 5 to 9 months in African regions with moderate to high transmissions of malaria parasite.[7] The pilot project for the Mosquirix vaccination has been recently launched in three malaria endemic countries that is Malawi(on 23 April 2019), Ghana(on 30 April 2019), and Kenya( on 13 September 2019). [8]

**Table 1: Recommendations for the use of mosquirix.[4]**

| |
|---|
| **What is its indication?**<br>Mosquirix provides active immunization against plasmodium falciparum malaria and hepatitis B in infants and children. |
| **Availability and storage**<br>Mosquirix will be available as a 25-µg powder and solvent for suspension for injection in vials, after reconstitution each vial contains two 0.5ml doses.<br>It should be stored in a refrigerator temperature ranging from 2-8 ℃.<br>It should be used immediately after reconstitution, if not used immediately it should be store for no longer than 6 hours at 2-8℃. |
| **Administration regimen**<br>Infants and children are vaccinated in three 0.5ml intramuscular doses at monthly intervals, followed by a fourth 0.5 ml booster dose 18 months after the third dose.<br>Preferred sites for intramuscular injection are the anterolateral thigh in infants aged <5 months and the deltoid muscle in children aged ≥5 months. |
| **Composition**<br>Each 0.5 ml dose contain 25 µg of RTS, [portion of P. falciparum circumsporozoite protein fused with hepatitis B surface antigen (RTS), combined with hepatitis B surface antigen (S)] adjuvanted with AS01E. |

### 1.2. Development of RTS,S

The development of RTS,S malaria vaccine has been ongoing since 1960s. It was ready for clinical trials only after 3 decades of efforts from the scientists, working at GlaxoSmithKline laboratories. For further vaccination development and conduction of early clinical development GlaxoSmithKline collaborated with the Walter Reed Army institute for research. In January 2001, GSK and PATH's Malaria Vaccine Initiative (PATH/MVI), with partly funding from Bill and Melinda Gates foundation & PATH's Malaria Vaccine Initiative, formed a public-private partnership to develop RTS,S for infants and young children living in malaria-endemic regions in sub Saharan Africa.[7][8]

Both groups, the British pharma giant GSK and Walter Reed Army Institute for Research in early 1984 were attempting to develop a vaccine based on the evidence that the radiation-attenuated sporozoites protects against the malaria infection. Immune response generated by radiation-attenuated sporozoites targets the CSP (Circumsporozoite protein) antigen and this led to the identification of CSP antigen. This CSP antigen was cloned and sequenced by the U.S. National Institutes of Health (NIH) and WRAIR. GlaxoSmithKline Escherichia coli elaboration system was used by researcher team to produce a subunit antigen based on the central repeat region of the CSP. Many attempts were made to advance the independent CSP subunit vaccine, but none showed the significant clinical efficacy.

Valenzuela et al. gave the concept of polyvalent vaccine. They developed a hybrid HBsAg particles having insertion site at pre-surface region(pre-S) for the insertion of second antigen. This new hybrid HBsAg improved the epitope presentation of foreign antigens.[9] To test this hypothesis Rutgers et al. used the hepatitis B surface antigen (HBsAg) as a carrier matrix for the CSP central repeat region to increase the immunogenicity of vaccine against plasmodium falciparum parasite and achieve high-level protection against malaria infection.[10]

The first CS protein construct created at GSK/WRAIR was result of the fusion of PFCSP NANP to the N-terminus of HbsAg and produced in yeast cells (Saccharomyces cerevisiae). However, the first construct of CS protein lacked the T cell epitopes at the C-terminal flanking region of CS protein, which would be required for the induction of specified T cell responses. This led to the generation of second CS protein that contain T and B cells epitopes, at C terminal region, based on the plasmodium NF54 strain. This resulted in RTS,S (Figure 3) product comprising of 25% fusion protein RTS (B cell Repeats +T cell epitopes + HBsAg (S) antigen) and 75% wild-type HBsAg (S) antigen. In RTS,S 'R' represents the central repeat region, a single polypeptide chain analogues to highly-conserved tandem repeat tetrapeptide NANP amino acid sequence and 'T' represents T-cell epitopes. The combined RT peptides is genetically fused at the N-terminal of the Hepatitis B surface antigen (HbsAg), 'S' represents the surface portion co-expressed in yeast cells, yielding virus-like particles that shows both CSP and S at their surfaces. A second "S" portion is an unfused HBsAg that spontaneously fuses to the RTS component.[2][6]

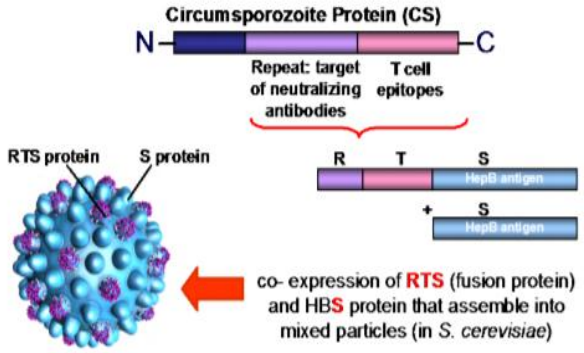



**Figure 1: RTS, S**

### 1.3. CS Protein

In the early 1960's, Nussenzweig et al. at New York university discovered that the immunization of animals with the bites of irradiated attenuated infected mosquitos could potentially protect against the sporozoite infection, consequently this discovery was applied to human volunteers. Though, soon it was realised that the approach of irradiated attenuated sporozoite was unfeasible for human vaccination because of lack of ability to generate sporozoites other than within the mosquito, and problems related to immunization via mosquito bite. The discovery of Nussenzweig led to the identification of the CS protein expressed on sporozoites and liver stage schizonts. The molecular weight of CS protein is 58 KD and consist of a central repeat region surrounded by a non-repetitive region. There are approximately 41 repeats of NANP (N, asparagine; A, alanine; p, proline) and some extent of NVDP (V, valine; D, aspartic acid) amino acid sequence (Figure 4 shows the CS protein structure).[2]

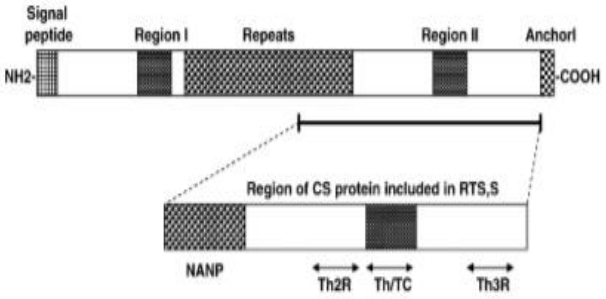



**Figure 2: Structure of CS protein.**

The exact function of central repeat region is still not stated clearly, but most likely it provides a significant adaptive advantage to the parasite. Most of the mutations are found in the central repeat region of the CS protein. These mutations result in the contraction and expansion in the number of amino acid sequence repeats and lead to the conversion of NANP to NVDP sequences or vice versa. The N-terminus region of CS protein is maintained among different strains of plasmodium falciparum and contain a pattern of five amino acids (93KLKQP97). This sequence of amino acid plays a major role in sporozoite invasion to mosquito salivary glands as well as binding to the hepatocytes before invasion. The C-terminus region is responsible for the invasion of mosquito salivary glands, sporozoite mobility, and invasion of hepatocytes. The central repeat region consists of B cell epitopes and the flanking C-terminal region consists both B and T cell epitopes.

Clinical trials were initiated to test the immunogenicity of the plasmodium falciparum CS protein vaccine (PFCSP). The data of clinical trial showed the poor immunogenicity of PFCSP which were strengthen by the addition of potent adjuvant.[2]

### 1.4. Adjuvant system

The soluble recombinant plasmodium falciparum circumsporozoite protein (PFCSP) is a poor immunogenic for humans, thus for a better vaccine platform potent adjuvant was required to achieve better protection against malaria. Immune system is generally tolerant to antigen to protect from allergies and autoimmune reactions, unless antigens trigger danger signals that activates the immune system to response antagonistically against antigen. Adjuvants are capable of inducing immune responses and have been widely used for immunization purposes, however the mechanism by which the adjuvant trigger immune response is still not understood properly. Adjuvants are prepared as aluminium salts, or emulsions such as oil-in-water-emulsion (o/w), water-in-oil (w/o) or water-in-oil-in-water (w/o/w), the function of these preparation is to stabilize the antigen as well as to slow its release. However, the rate of release does not necessarily corelate with immunogenicity. Adjuvants additionally contain stimulants such as toll-like-receptors (TLRs) ligands,

cytokines, bacterial toxins, or saponins.[1] There were total of 11 adjuvant system were developed and tested on animals for immunogenicity by GSK, out of these 11 only AS04 (SBAS4), AS03 (SBAS3), AS02 (SBAS2), AS02A were further tested on human and demonstrated increase in protection provided by RTS,S. Adjuvant AS04 contains alum and MPL (monophosphoril lipid A) and the AS03 is squalene-in-water emulsion, MPL is an endotoxin capable of inducing immune responses in macrophages and dendritic cells and promotes the release of inflammatory cytokines by binding to the lipid binding protein (LBP) in serum, CD14 and toll-like-receptor-4 (TLR4). Inflammatory responses generated by macrophages and dendritic cells further activate the CD 4 T cells that assists B cells to produce cytotoxic antibodies and activates cytotoxic CD8 T cells (CTLs). AS04 is currently employed as an adjuvant for human papillomavirus/cervical cancer vaccine. AS02 contains MPL and QS21 a saponin derived from the bark of the Quillaja Saponaria. The function of QS21 is to trap components of the vaccine by hydrophobic interactions and thus slow the release of antigen to the immune system. Thimerosal a mercury-based preservative has been removed due to concern over potential neurotoxicity. New adjuvant name AS02A now have lactose as a cryopreservant later on the GSK replaced the o/w emulsion of AS02A by liposomes and it was named as AS01B. Immunization of mice with RTS,S/AS01B did not increase the concentration of CS antibodies as compared to RTS,S/AS02A, but it did increase the frequency of CD4 T and CD8 T cells specific for the CS protein.[2]

### 1.5. Mechanism of action

RTS,S is a pre-erythrocytic vaccine that targets the plasmodium falciparum Circumsporozoite protein and thus limits its ability to infect, mature and multiply in the liver. The active substance of the RTS,S/AS01 is plasmodium falciparum circumsporozoite protein (PFCSP) fused with hepatitis B surface antigen (RTS) and free 'S' protein spontaneously assemble in 'RTS,S' adjuvanted with AS01 to increase the immune system response. Mosquirix triggers danger signals and led to the formation of anti-circumsporozoite antibody and circumsporozoite-specific CD4-positive T cells. Mosquirix also prevents from hepatitis B virus through induce humoral response but it should not be used only for this purpose.[5][12]

## Main body

### 2.1. Clinical trials

As of July 2015, there were ~22 preclinical malaria vaccine trials and 42 clinical trials being conducted across the world, as per "malaria vaccines under development" report of WHO. The vaccine had also undergone numerous preclinical and early phase clinical trials in non-endemic countries as well as human trials in endemic regions. Research on clinical trials identified 5 randomized controlled trial and 4 follow-up extension studies assessing the effect of RTS,S with variations of either AS01 or AS02 on development of clinical disease in the field. Populations were limited to African infants and children.[12][13] The studies are described below and results summarized in Table 2.[1]

**Table 2. Vaccine Efficacy Results from Clinical Trials.**

| Reference | Population | Follow-up Duration | Study Period | Outcome | Vaccine Efficacy (95%CI) (Per-protocol) | Vaccine Efficacy (95% CI) (ITT) |
|---|---|---|---|---|---|---|
| **Phase 1 & 2 trials** | | | | | | |
| Alonso (2004) | 2022 children from Mozambique | 8.5 months after 1st dose<br>6.5 months after 3rd dose | April 2003-May 2004 | First episode of fever and parasitemia>2500 parasites/ µL (Cohort 1)<br>First episode of parasitemia>0 parasites/ µL (Cohort 2)<br>Several episode of fever and parasitemia>2500 parasites/ µL (Cohort 1) | 29.9% (11.0 to 44.8); n= 1490<br>45.0% (31.4 to 55.9); n= 367<br>27.4% (6.2 to 43.8); n=1490 | 30.2% (14.4 to 43.0); n=1605<br>NR<br>NR |

| | | | | | | |
|---|---|---|---|---|---|---|
| Alonso (2005) | 2022 children from Mozambique | 18 months after 3rd dose | | First or only episode of fever and parasitemia>2500 parasites/ µL (Cohort 1)<br>Several episode of fever and parasitemia>2500 parasites/ µL (Cohort 1) | 35.3% (21.6 to 46.6); n=1442<br>29.8% (13.8 to 42.8); n=1442 | 32.8% (20.1 to 43.4); n=1605<br>32.4% (17.6 to 44.5); n=1605 |
| Sacarlal (2009) | 2022 children from Mozambique | 45 months after 1st dose | | First or only episode of fever and parasitemia>2500 parasites/ µL<br>Several episode of fever and parasitemia>2500 parasites/ µL | 30.5% (18.9 to 40.4); n=1490<br>25.6% (11.9 to 37.1); n=1490 | NR<br>NR |
| Bejon (2008) | 894 children from Kenya/ Tanzania | Mean 7.9 months | March 2007-august 2007 | First or only episode of fever and parasitemia>2500 parasites/ µL<br>Multiple episode of fever and parasitemia>2500 parasites/ µL | 53% (28 to 69); n=809<br>56% (31 to 72); n=809 | 49% (26 to 65)(unadjusted); n=894<br>54% (31 to 69) (unadjusted) ; n = 894 |
| Olotu (2011) | 894 children from Kenya/ Tanzania | 12 months after 3rd dose 15 months after 3rd dose (Kenyan cohort only) | | First or only episode of fever and parasitemia>2500 parasites/ µL<br>Multiple episode of fever and parasitemia>2500 parasites/ µL<br>First or only episode of fever and parasitemia>2500 parasites/ µL<br>Multiple episode of fever and parasitemia>2500 parasites/ µL | 39% (20 to 54); n=835<br>42% (22 to 57); n=835<br>46% (24 to 61); n=415<br>51% (29 to 66); n=415 | 39% (20 to 53) (unadjusted) ; n=894<br>44% (24 to 58); n=894<br>37% (14 to 55); n=447<br>44% (20 to 61); n=44 |
| Abdulla (2008) | 340 infants from Tanzania | 6 months after 3rd dose | July 2006-February 2008 | First or only episode of fever and parasitemia>500 parasites/ µL<br>Any infection (any level of parasitemia) | 43.2% (–47.1 to 78.0); n=297<br>65.2% (20.7 to 84.7); n= 297 | 41.8% (–32.9 to 74.6); n=340<br>NR |
| Aponte (2007) | 214 infants from Mozambique | 6 months after 3rd dose | June 2005-march 2007 | First or only episode of fever and parasitemia>500 | 65.8% (25.3 to 84.4); n=185<br>65.9% (42.6 to | NR |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | parasites/ µL<br>Any infection (any level of parasitemia) | 79.8); n=185 | NR |
| Aide (2010) | 214 infants from Mozambique | 1 year after 3rd dose | | First or only episode of fever and parasitemia>500 parasites/ µL<br>Multiple episode of fever and parasitemia>500 parasites/ µL | 33.0% (–4.3 to 56.9); n=177<br><br>25.9% (–15.7 to 52.6); n=177 | 25.9% (–9.9 to 50.0); n=214<br>24.3% (–12.9 to 49.2); n=214 |
| **Phase 3 trials** | | | | | | |
| Agnandji (2011) | 6000 children aged 5-17 months from 7 African countries (total study population: 15,460) | 12 months after 3rd dose | Randomization from march 2009 to January 2011 | First or only episode of fever and parasitemia>5000 parasites/ µL<br>First or only episode of fever and parasitemia>0 parasites/ µL<br>All episode of fever and parasitemia>5000 parasites/ µL | 55.8% (97.5% CI 51.3 to 59.8); n= 4296<br><br>54.1% (49.9 to 57.9); n=4296<br><br>55.1% (50.5 to 59.2); n=4296 | 50.4% (45.8 to 54.6); n=6000<br><br>NR<br><br>53.9% (49.6 to 57.8); n=6000 |

**2.2. Phase 1 and 2 trials**

The result of phase II proof-of-concept in children was conducted in Mozambique in 2004, paving the way for subsequent Phase II and III trials in 2007 and 2009, respectively. The results of this study showed that RTS, S was likely to provide partial protection against malaria in children age 6 weeks to 17 months. A randomized double-blinded phase 2b controlled trial was performed on 2022 children in Mozambique to evaluate the safety and efficacy of RTS,S/AS02 against plasmodium falciparum parasitic infection and illness over the period of 6.5 months after entire vaccination. Two groups of children aged 1-4 years living in distinct zones were included in the study. Each group was randomized to receive either 3 doses of control or 3 doses of RTS,S/AS02 vaccine.

For subjects <24 months, the control consisted of 2 doses of pneumococcal conjugate vaccine and 1 dose of Haemophilus influenza type b (Hib) vaccine; those aged 24 months and older obtain 3 doses of paediatric hepatitis B vaccine. The vaccine efficacy of RTS,S/AS01 was found to be 28.6% for the first episode of infection and 29.9% for the clinical disease in group 1. When changed the age, use of bed nets, health facility distance and geographic region greatly increased Vaccine efficacy; 31.8% for the first episode of infection and 35.3% for clinical disease in group 1.

Vaccine efficacy rate for group 2 was found to be 45% for the first episode of parasitemia >0 parasites/µL (a measure of infection). Test results for waning efficacy were not significant. A single-blind follow-up study was performed to evaluate the safety and efficacy of RTS,S/AS02A at 18 months after the third dose of vaccine. The number of children with clinical malaria was lower and yielding a vaccine efficacy of 48.6%. All-cause hospitalization rates were alike between groups and no communication was found between age and vaccine efficacy. An open-label follow-up study was performed to evaluate safety and efficacy at 45 months after first vaccine dose. Results were in favour of RTS,S/AS02 with a vaccine efficacy of 31.9% for the first episode of infection and 30.5% for clinical illness. Vaccine efficacy for clinical malaria remained significant and the number of hospitalizations was insignificantly lower in the RTS,S/AS02A group.[14]

To evaluate the efficacy and safety a double-blind randomized controlled trial was performed on 894 Tanzanian and Kenyan children aged 5-17 months over the average follow up period of 7.9 months. Participants were randomized to received 3 doses of either RTS,S/AS01E or human diploid vaccine and monitored by weekly home visits and local staff in health facility. Vaccine efficacy was found to be 53% for first episode and 56% for multiple episode of clinical malaria. Vaccine efficacy was also significantly in favour of RTS,S/AS01E for all episodes of clinical malaria. Anti-CS antibody titres were calculated throughout the study, but no relation was found between the titre level and

protection from clinical malaria 1 month following the third dose of vaccine.[15-20]

A Phase 2b, single-centre, double-blind randomized trial evaluate RTS,S/AS02D in 340 infants in Tanzania and Bagamoyo over a 9-month observation period in combination with the WHO Expanded Program on Immunization (EPI) vaccines. Infants were randomized to receive 3 doses of RTS,S/AS02D, or hepatitis B vaccine via injection within the left anterolateral thigh. According to the EPI immunization programme, all volunteer were also received a vaccine containing diphtheria and tetanus toxoids, whole-cell pertussis, and conjugated Hib. The oral polio vaccine was provided at birth and administered with subsequent doses of DTPw/Hib. Participants were monitored by home visits. Vaccine efficacy for the development of any infection was significantly in favour of RTS,S/AS02D .Results for analysis of EPI vaccine titers met pre-specified standards and responses were deemed non-inferior when administered with RTS,S or control vaccine. It is concluded from the clinical trial data that the RTS,S/AS02D doesn't interfere with antibodies responses generated by other immunization and could possibly be given as a part of a routine vaccination schedule. Outcomes were assessed through both active and passive discovery of infection and clinical disease.[15-20]

### 2.3. Phase 3 trials

The phase III clinical trials were conducted at eleven sites in seven African countries. The sites involved in the phase III clinical trials were located in Burkina Faso, Mozambique, Gabon, Ghana, Tanzania, Kenya, and Malawi. The clinical trials were conducted on 15459 participants consist of 6537 infants aged 6 to 12 weeks and children aged 5 to 17 months at first dose of vaccination. Children in each age category were randomly distributed into 3 groups: one group received 3 doses of RTS,S at months 0, 1 and 2 and a booster dose after 18 months of the third dose; second group received an equivalent schedule without the booster; and an control group received coparator vaccine at months 0, 1, and 2 and 20. Comparator vaccine includes meningococcal serogroup C conjugate vaccine for infants and rabies vaccine for children.

The phase III clinical trial was conducted in a double-blind manner so that neither the researchers nor the patients know about the study treatment assigned. The infants and children were followed for an average of 48 months after the first dose of vaccination. The main aim of the study was to evaluate the RTS, S efficacy and the secondry aim was to evaluate the booster dose of vaccine at month 20. All Paricipants were provided with insecticide treated bed nets and almost 80% of trial participants were using insecticide treated bed nets. The phase III clinical trials results over the follow up study for the one year after the first dose of vaccine showed that the RTS, S reduced the number of clinical malaria cases by approximately half in children and by one third in infants. Vaccine efficacy waned over time but could be enhanced by a booster dose. Final result of phase III clinical trials was published in The Lancet(in april 2015), showed that vaccine efficacy against clinical malaria in children with booster dose was 39% and without booster dose was 26%, over an median 48 months.

The primary efficacy outcome was the development of the clinical disease for each age category. Other outcomes includeded changing parasitmetia level, clinical malaria, and anti-CS antibody titers. Schoenfeld residuals showed that vaccine efficacy waned over time, with greater efficacy at the start of the follow-up period. Vaccine efficacy for all episodes during this population was 55.1%. The results of ITT analysis were also significant. Vaccine efficacy for severe malaria was 47.3% for children 5-17 months (n=4296) and 34.8% for children 6 weeks -17 months (n=12,961). No difference in mortality was demonstrated after one month from the third vaccine dose, but this may have been the result of extremely low rates of malaria-related mortality (6.6%). It is possible that close monitoring and follow from patients contributed to a reduction in overall mortality rates and more power is required to detect differences between the groups. These early results prove the validity of efficacy rates observed in Phase 2 trials. Future analyses will conclude whether vaccine response is sustainable over a 32month follow-up period.[19]

### 2.4. Preparation for phase iv malaria vaccine trial

Post-approval Phase IV studies will be required to identify rare adverse events that were not identified in Phase III studies. Phase IV studies are large scale studies and consist of observing over 10,000 volunteers by a well-planned pharmacovigilance system. Various efforts have been made in creating such systems in Africa.[22] For proper functioning of such system various institutes came forward such as WHO collaborating with centres of excellence in pharmacovigilance and efforts of IN-DEPTH Network, a non-governmental organization made from research institutions having health and surveillance systems.[23] Main problem in phase IV clinical trial is the absence of baseline disease profile of rare disease due to the inadequate diagnostic tools in many health facilities in sub-Saharan Africa. To carry out phase IV studies in African countries would require evaluation of some rare diseases related to vaccines such as intussusceptions and Kawasaki Syndrome.[24][25] GSK is currently planning for Phase IV studies of the vaccine in sub-Saharan African countries. The study aims to enrol several thousands of youngsters to permit the identification of potential rare adverse events.[26]

### 2.5. Safety of RTS,S

EMA in its assessment report concluded that the safety profile of vaccine is acceptable and similar to others, but there is high risk of convulsion in the older age group within seven days after the third dose of vaccine.

The most common side effects of vaccine were swelling, local pain, and mild fever, similar to some standard vaccine given to children. It was found in the phase III studies that the incidence of fever after the week of vaccination was higher in those children who received the booster dose than those receiving the control vaccine. In some cases febrile convulsion were seen with generalized convulsion; however, all seizures were sort out soon and no long-term effects were observed.[27]

The occurrence of meningitis as a possible risk is going to be followed closely in extended follow-up in 3 of the 11 study sites that conducted the phase III studies and in the planned pharmacovigilance study included in the Risk Management Plan approved by the EMA. However, it was found that the children protected by RTS,S develop their innate immunity against malaria more slowly than unvaccinated children. There is greater risk for disease in vaccinated children in malaria endemic regions when the protection of vaccine wanes over time.[11]

Overall the RTS,S has been found to be safe. In a phase II safety assessment report of RTS,S, there was a better frequency of upper tract infections, also as rash and diaper rashes among RTS,S vaccinated infants that were mild to a fair in intensity and unrelated to the vaccinations. This wasn't found in subsequent phase III clinical trial studies. The incidence of post-vaccination febrile convulsion was similar in both vaccine and control groups.[28] Meningitis was more common among children who received the RTS,S vaccine but wasn't associated with vaccinations.[29]

**2.6. Efficacy of RTS,S**

It was found in the Clinical study results that RTS,S has the capabilities to protect infants and children living in Sub-Saharan Africa. In phase III trials, RTS,S reduced half the cases of malaria in children and one third in infants over the first year after the vaccination. Vaccine efficacy was high after vaccination but can be increased by a booster dose.

Vaccine efficacy for clinical malaria was 51.3% after short duration of the vaccination and waned over time. After the follow up of 18 months from the 3$^{rd}$ dose, vaccine efficacy was 45.7%. vaccine efficacy against clinical malaria in children with booster dose and without booster dose was 39% and 26%, over a median of 48 months.[30]

At the end of study period it was found that statistically significant vaccine efficacy against clinical malaria was found in those that received the fourth dose, but not in those that didn't.[31]

**2.7. Immunogenicity**

A major question that needs to be addressed is the longevity of protection. It is currently unknown how long the vaccine will provide immunity against the development of the clinical disease. Limited data from Phase 1 and 2 follow-up studies have suggested that clinical efficacy is maintained up to 12 months after the third dose of the RTS,S AS01E formulation,17 and 45 months with AS02A. Theoretically, the AS01E adjuvant should provide a similar duration of protection, but there are no published reports beyond 12 months.[32] The immunogenicity profile of RTS,S has been extensively investigated, including attempts to correlate vaccine immunogenicity with protection . In summary, anti-CSP antibody titers against the NANP repeat region, and increased titers and responses have been correlated with a reduced risk of clinical malaria, but no threshold defining protection has been established. CD4+ T cell responses are also increased after RTS,S/AS01 immunization, & may be associated with protection.[33][34]

Immunogenicity has also been explored in vulnerable groups, including children living with HIV. A study of RTS,S/AS01 in Kenyan children with WHO stage 1 or 2 HIV disease reported anti-CSP antibody geometric mean titre (GMT) was greater in vaccines than controls 1 month after dose 3 (329.2 EU/mL versus 0.3 EU/mL), but was lower than children in the Phase 3 study (621 EU/mL). Although children in the RTS,S group experienced fewer episodes of clinical malaria, severe malaria, hospital admission due to malaria, and anaemia than controls, these differences were not significant, and the study was not powered for these outcomes.[35]

The Phase 3 clinical trial demonstrated greater vaccine-induced immunogenicity in older children versus infants. Among children aged 5-17 months, anti-CSP antibody increased to 318.2 EU/mL 1 month after the booster dose, compared with 34.2 EU/mL in children who did not receive the boost. One year later, levels dropped to 52.4 EU/mL and 19.3 EU/mL, respectively. In young infants 6-12 weeks of age, anti-CSP antibody increased to 169.9 EU/mL 1 month after the booster dose, compared with 6.2 EU/mL in infants not boosted. One year later, levels dropped to 15.9 EU/mL and 3.7 EU/mL, respectively. Infants in the top tertile of anti-CSP antibody response after the primary series experienced a 36.9% reduction in the risk of clinical malaria compared with infants in the lowest anti-CSP antibody tertile. This association was not seen in older children. The rapid anti-CSP antibody decline in all participants was characterized by an initial rapid half-life of ~40 days followed by a more gradual loss of vaccine-induced antibody ~600 days.[36]

**2.8. Cost of RTS,S**

Despite being partially effective, RTS,S was predicted to have a substantial public health impact on disease burden, based on the results of modelling studies. Cost data helps the policy makers and countries to evaluate the value of this new intervention in relation to malaria control strategies. For any country to make decision at its level is concerned with financing, ease of mobilizing and

maintaining the level of resources to reinforce the new vaccine programme and to support these decisions it is necessary to estimate the cost of RTS,S introduction in EPI(Expanded Programme on Immunization) program. Costing include various factors like generic activities, assumptions, methods adapted by specific country for delivery of immunization service, structure of the EPI program, geography, demographics, cost of labour, distribution model, spare capacity to accommodate vaccine.

RTS,S is lyophilized monovalent recombinant protein vaccine reconstituted with adjuvant and both are need to store in cold chain storage at 2-8℃. The vaccine and diluent are packed together with a total packed volume of 9.68 $cm^3$ per two-vial packages. After reconstitution two doses per vial is produced. From the data of clinical trial, it is suggested that three doses of RTS,S/AS01 at 6-9 months of age without or with a fourth booster dose after 18 months from 3rd dose is required for protection against malaria. It is assumed that the first and third dose is administered along with vitamin A and measles vaccine and the first and third dose are considered under routine immunization visits for costing purposes but second dose at 6-9 months and fourth dose are considered under out of schedule visits. K. Galactionova et al in 2015 estimated both the economic and financial cost of introducing the RTS,S into EPI. The financial costs are estimated under an assumption of 100% spare capacity to accommodate the vaccine. Input prices and unit cost data were gathered from various sources including wages of EPI staff, per-diems, vaccine related equipment, rental, hotel rooms, fuel and cost of cold chains and vehicles. For each activity formulas were defined to calculate price and unit cost. The methodology is applied to estimate the cost of RTS,S introduction in Burkina Faso, Ghana, Kenya, Senegal, Tanzania, and Uganda. At an assumed vaccine cost of $US5 per dose, the total economic cost of introducing RTS,S/AS01 would be $US23.11–28.28 per fully vaccinated child.[5][37]

## CONCLUSION

RTS,S/AS01 4 dose schedule has been found efficacious and provides the greatest benefits against malaria infection. It has been reported that the vaccine efficacy in children is greater who received a fourth dose than those who didn't receive it. In phase 3 trial, RTS,S/AS01 vaccine was shown to have acceptable efficacy and tolerability profile in both infants and children. Development of a malaria vaccine is a medical breakthrough and approval of RTS,S by EMA makes history as the first human parasite vaccine that has potential to prevent malaria infection worldwide. However, there are many problems that need to be resolved in the areas of cold chain systems, community acceptance, duration of immunity, cost effectiveness, use in special population, distribution in endemic regions and monitoring of adverse events post-licensure to ensure a successful rollout of the new vaccine.

## DECLARATION

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and material**

Not applicable

**Competing interests**

Not applicable

## FUNDING

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**Authors' contributions**

All authors have made equal contribution in collecting the data.
KD did the literature work and prepared the manuscript.
SC helps in chosing the topic.
Dr BP helps in Stream lining the article.
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## ABBREVIATIONS

1. RTS: Fusion protein of a portion of the circumsporozoite protein from P. falciparum and the amino terminal end of the Hepatitis B virus S protein
2. RTS,S: Particulate antigen, containing both RTS and HBs proteins
3. AS01: Liposome-based adjuvant system
4. WHO: World Health Organisation
5. GSK: GlaxoSmithKline
6. EU: Eurpean Union
7. EMA: European Medicines Agency
8. CSP: Circumsporozoite protein
9. NIH: National Institutes of Health
10. WRAIR: Walter Reed Army Institute for Research
11. HBsAg: Hepatitis B Surface Antigen
12. PFCSP: Plasmodium falciparum Circumsporozoite Protein
13. NANP: N, asparagine; A, alanine; P, proline
14. NVDP: V, valine; D, aspartic acid
15. TLR: Toll-Like-Receptors
16. MPL: Monophosphoril lipid A
17. QS21: Quillaja saponaria Molina, fraction 21
18. CI: Confidence interval
19. ITT: Intention to treat
20. Hib: Haemophilus influenza type b
21. EPI: Expanded Program on Immunization
22. DTPw: Diphtheria tetanus whole cell pertussis
23. EU: Elisa Unit

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