

23 July 2015
EMA/CHMP/439337/2015
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Mosquirix™

International non-proprietary name: *Plasmodium falciparum* and hepatitis B vaccine (recombinant, adjuvanted)

Procedure No. EMEA/H/W/002300/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.



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List of abbreviations

ACD Active case detection

ACT Artemisinin-combination Therapies

ADI Active detection of infection

AE Adverse event

AQL Acceptable quality level

AS Adjuvant system

AS01 Liposome-based adjuvant system

AS01E Two immune enhancers MPL [3'-O-desacyl-4'-monophosphoryl lipid A] and QS-21

[Quillaja saponaria Molina, fraction 21]), in a liposome suspension (adjuvant system)

AS02 Oil-in-water emulsion-based adjuvant system

ATP According-to-protocol

BCG Bacille Calmin-Guerette

BPT Bordetella pertussis toxin

CHMI Controlled Human Malaria Infection

CHMP Committee for Medicinal Products for Human Use

CI Confidence Interval

CLB Concentrated liposome bulk intermediate

CMI Cellular mediated immunity

CoA Certificate of Analysis

Col Case of Interest

CPA Critical Process Attributes

CPP Critical Process Parameter

CQA Critical Quality Attributes

CS Circumsporozoite protein of P. falciparum

D Diphtheria

DLP Data lock point

DNA Deoxyribonucleic acid

DOC Day of challenge

DOPC Dioleoyl phosphatidylcholine

DOPC 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine

DP Drug product

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DSMB Data Safety Monitoring Board

DTP Diphtheria tetanus pertussis

DTPa Diphtheria tetanus acellular pertussis

DTPw Diphtheria tetanus whole cell pertussis

ELISA Enzyme-linked immunosorbent assay

ELISPOT Enzyme-linked immunosorbent spot

EMA European Medicines Agency

EPI Expanded Program on Immunisation

EU Elisa Unit

EU European Union

FC Final container

FDA Food and Drug Administration

FTIR Fourier transform infrared spectroscopy

FU Follow-up

GACVS Global Advisory Committee for Vaccine Safety

GCP Good Clinical Practice

GMC Geometric Mean Concentration

GMP Good Manufacturing Practice

GMT Geometric Mean Titre

GSK GlaxoSmithKline

HAZ Height for age z-score

HBs, HBsAg Hepatitis B virus surface antigen

HCP Host cell protein

HepB Hepatitis B

Hib Haemophilus influenzae type b

HIC Hydrophobic interaction chromatography

HIV Human immunodeficiency virus

HPSEC High Performance Size Exclusion Chromatography

ICH International Committee on Harmonization

ICS Intracellular cytokine staining

IDMC Independent Data Monitoring Committee (previously Data Safety Monitoring Board -

DSMB)

IEC Ion exchange chromatography

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IFN-γ Interferon-gamma

IgG Immunoglobulin G

IL-2 Interleukin-2

INF Infinite

IPC In-process control

ITT Intention to treat

IU International Unit

LB Liquid bulk

LL Lower limit

Me Measles

MedDRA Medical Dictionary for Regulatory Activities

MenC Meningococcal C conjugate vaccine

mIU milli-International Unit

mL millilitre

MPAC WHO Malaria Programme Advisory Committee

MPL 3-O-desacyl-4'-monophosphoryl lipid A

MSL Master Seed Lot

MVI Malaria Vaccine Initiative

OCABR Official Control Authority Batch Release Testing

OPV Oral Polio Vaccine

P. falciparum Plasmodium falciparum

PACMP Post approval change management protocol

PATH Program for Appropriate Technology in Health

PBMC Peripheral blood mononuclear cell

PCD Passive case detection

PCS Product Control Strategy

PCV Pneumococcal conjugated vaccine

PDef Primary case definition for malaria

PEG Polyethylene glycol

PETG Glycol-modified polyethylene terephthalate

Ph. Eur. European Pharmacopoeia

PHI Public Health Impact

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PIL Patient Information Leaflet

pIMD (potential) Immune-mediated disorder

PM Process monitoring

PMSF phenyl methyl sulfonylfluride

PPQ Process performance qualification

PRP Polyribosylribitol phosphate

PYAR Person years at risk

QD Quality decision

QS-21 'Quillaja saponaria 21': a triterpene glycoside purified from the bark of the Quillaja

saponaria Molina

RMP Risk management plan

RPN Risk priority number

RR Relative risk

RSI Reference safety information

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

RTS,S Particulate antigen, containing both RTS and HBs proteins

RTS,S/AS Candidate RTS,S adjuvanted vaccine formulations

S protein see HBsAg

SA Scientific advice

SAGE WHO Strategic Advisory Group of Experts on immunization

SD Standard Deviation

SDef Secondary case definition for malaria

SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SEC Size exclusion chromatography

SmPC Summary of product characteristics

SOP Standard operation procedure

SP Seroprotection

T Tetanus

TNF-a Tumor necrosis factor-alpha

TRA Technical risk assessment

TSE Transmissible spongiform encephalopathy

TVC Total vaccinated cohort

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UC Ultracentrifugation

UF Ultrafiltration

UL Upper limit

US United States

VE Vaccine efficacy

VLP Virus-like particle

WAZ Weight for age z-score

WHO World Health Organisation

WRAIR Walter Reed Army Institute of Research

WSL Working Seed Lot

YF Yellow fever

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1.1. Submission of the dossier

The applicant GlaxoSmithKline Biologicals S.A. submitted on 26 June 2014 an application to the European Medicines Agency (EMA) for a scientific opinion in the context of cooperation with the World Health Organisation (WHO) for Mosquirix, in accordance with Article 58 of Regulation (EC) No 726/2004.

The eligibility by the World Health Organisation was agreed upon on 26 March 2010 and CHMP on 23 March 2010.

Mosquirix is exclusively intended for markets outside the European Union.

The applicant applied for the following indication: active immunisation of children aged 6 weeks up to 17 months against malaria caused by *Plasmodium falciparum* and against hepatitis B.

Legal basis for this application

This application is submitted under Article 58 of Regulation (EC) No 726/2004 and includes a complete and independent dossier, by analogy to Article 8(3) of Directive 2001/83/EC.

Scientific advice

The applicant received Scientific Advice from the CHMP on 21-06-2007, 02-07-2007, 19-11-2009, 17-02-2011, 19-01-2012, 21-06-2012. The Scientific Advice pertained to quality and clinical aspects of the dossier.

2. Background information on the procedure

2.1. Manufacturers

Manufacturer of the biological active substance

GlaxoSmithKline Biologicals S.A. Rue de l'institut 89 1330 Rixensart Belgium

Manufacturer responsible for batch release

GlaxoSmithKline Biologicals S.A. Rue de l'institut 89 1330 Rixensart Belgium

2.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Jan Mueller-Berghaus Co-Rapporteur: Greg Markey

- The application was received by the EMA on 26 June 2014.
- The procedure started on 23 July 2014.
- · The Rapporteur's first Assessment Report was circulated to all CHMP members on 13 October

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2014. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 3 October 2014.

- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 6 November 2014.
- During the meeting on 20 November 2014, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 20 March 2015.
- The summary report of the GCP inspection carried out at the following sites: Gabon, Tanzania and Malawi between 10 November to 12th December 2014 was issued on 16 February 2015.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 28 April 2015.
- The PRAC Rapporteur Risk Management Plan (RMP) Advice and assessment overview was adopted by PRAC on 7 May 2015.
- During the CHMP meeting on 21 May 2015, the CHMP agreed on a list of outstanding issues to be addressed by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 19 June 2015.
- During a SAG meeting on 26 June 2015, experts were convened to address questions raised by the CHMP.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 30 June 2015.
- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 9 July 2015.
- During the CHMP meeting on 20 July 2015, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the CHMP meeting on 23 July 2015, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive scientific opinion to Mosquirix.

3. Scientific discussion

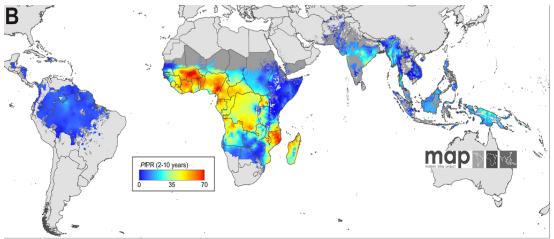
3.1. Introduction

Malaria caused by Plasmodium falciparum

Malaria is a life threatening disease caused in humans by five species of the genus Plasmodium: Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi.

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The spatial distribution of Plasmodium falciparum malaria endemicity map in 2010



The mapped variable is the age-standardised *P. falciparum* Parasite Rate (PfPR2-10) which describes the estimated proportion of 2-10 year olds in the general population that are infected with *P. falciparum* at any one time, averaged over the 12 months of 2010.

Of these five, *P. falciparum* is recognised as the major cause of severe morbidity and mortality [WHO World Malaria Report 2013]. P. falciparum is present predominantly in sub-Saharan Africa where it causes 98% of malaria in humans.

The Plasmodium parasite is transmitted to man via the bite of infected female mosquitoes of the genus Anopheles. *P. falciparum* sporozoites are injected into the circulation and rapidly target the liver. They invade hepatocytes where schizonts containing 10,000-30,000 merozoites develop. After release into the blood stream the disease-associated asexual erythrocytic phase of the infection is initiated. The merozoites infect and multiply within erythrocytes at an estimated 10-fold increase in number each 48 hours.

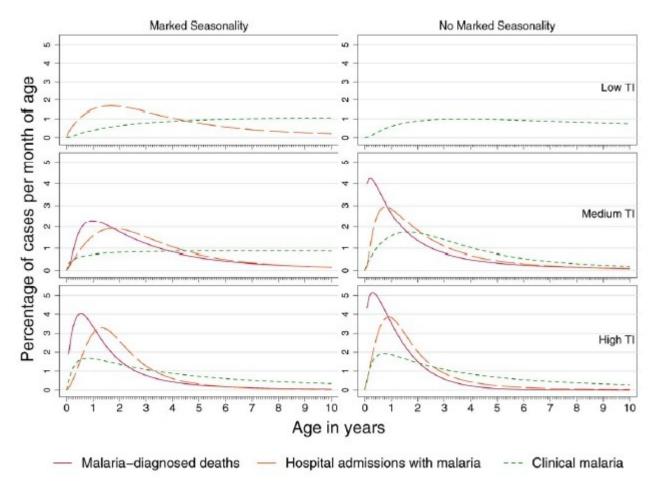
Clinical manifestations appear around the time that erythrocytes become infected. These include fever, chills, headache, joint and muscle pain, sweating and vomiting. As the infection develops in erythrocytes, acute complications may occur including severe anaemia, respiratory distress, cerebral malaria, jaundice, renal failure, shock and acidosis. If not treated within 24 hours, *P. falciparum* malaria can progress to a severe and potentially fatal illness.

In sub-Saharan Africa, entomological inoculation rates can be as high as 1000 per year. The malaria burden (clinical malaria, hospitalisation with parasitaemia and mortality) shifts towards younger ages with increasing transmission intensity, although marked seasonality moderates this effect. With repeated exposure protection is acquired, first against severe malaria, then against illness with malaria and, much more slowly, against microscopy-detectable parasitaemia. Hence most malaria infections in adults are asymptomatic and the greatest burden of morbidity and mortality is observed during early childhood.

The global prevalence of malaria began to decline in the early part of this century, predominantly due to strengthened control measures but the global burden of malaria remains significant. Of the estimated 207 million cases of malaria reported in 2012, 167 million (~80%) were reported in Africa. Almost all deaths are caused by *P. falciparum*. The World Malaria Report 2013 estimated that of 627,000 deaths reported globally, 562,000 (~90%) occurred in the African region where 462,000 (82%) deaths occurred in children under the age of 5 years.

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Age patterns of P. falciparum malaria in Sub-Saharan Africa



Age distribution of uncomplicated clinical malaria, hospital admissions with malaria and malaria-diagnosed deaths per month of age in children under ten years of age, by transmission intensity (TI) and seasonality of malaria transmission [2010]

The 2015 Millennium Development Goals updated by Roll Back Malaria (RBM) are to:

Reduce global malaria deaths to near zero

Reduce global malaria cases by 75% from levels in 2000 and

Eliminate malaria in ten new countries

Challenges for reaching these goals include the limited access to and capabilities of local health care services, limiting access to quality diagnosis and treatment services. There has also been some emergence of resistance to artemisinins, the basis of Artemisinin Combination Therapies (ACT) recommended by the WHO as first line treatment for *P falciparum* malaria. An alarming spread of resistance to the insecticides commonly used to control the mosquito malaria vector has also been reported.

In addition to insecticide-treated bednets (ITNs) specific therapeutic strategies currently in use to prevent malaria infection in young children include:

IPT - the administration of a full course of an effective antimalarial treatment at specified time points to a defined population at risk of malaria, regardless of whether they are parasitaemic, with the objective of reducing the malaria burden in the specific target population. IPT delivered alongside licensed vaccines in infants within the context of the routine EPI at 6, 10 and 14 weeks, is called IPTi.

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In a meta-analysis, the administration of IPTi through the standard EPI showed 30% efficacy against clinical malaria and 23% against all-cause hospitalization. In 2009 WHO recommended that all infants at risk of *P. falciparum* infection in sub-Saharan Africa areas with moderate-to-high transmission and low levels of parasite resistance to sulfadoxine-pyrimethamine (SP) should receive preventive malaria treatment through immunization services at intervals that correspond to routine vaccination schedules.

SMC - previously called Intermittent Preventive Treatment in children (IPTc), this was recommended by the WHO in 2012 for areas of highly seasonal malaria transmission in Africa. This involves intermittent administration of full treatment courses of an effective antimalarial during the malaria season to prevent illness in children aged 3 to 59 months. It aims to maintain therapeutic antimalarial drug concentrations in the blood throughout the season with the highest malaria risk. A meta-analysis of SMC studies in which a therapeutic course of SP plus amodiaquine (SP-AQ) was given once per month to children under 5 years of age during the peak malaria transmission season showed an 82% reduction in the incidence of clinical malaria episodes and a protective effect of 57% against all-cause mortality during the transmission season.

P. falciparum requires a specific temperature range in the mosquito to develop whilst *P. vivax*, the second most dominant cause worldwide tolerates lower temperatures as well as higher altitudes. Another factor is the dominance of the Duffy negativity trait in the African population that causes resistance of the red blood cells versus *P. vivax*. In contrast to other plasmodia, *P. falciparum* causes severe disease in 5% and has already developed resistances to antibiotics. Also, an age<3 years is an indicator for poor prognosis in severe malaria.

Diagnosis is made using blood film, rapid tests and PCR for blood stages.¹

Severe malaria

Severe malaria shows the following clinical symptoms, singly or in combinations:

- impaired consciousness (including unarousable coma);
- prostration, i.e. generalized weakness so that the patient is unable to sit, stand or walk without assistance:
- multiple convulsions: more than two episodes within 24h;
- deep breathing and respiratory distress (acidotic breathing);
- acute pulmonary oedema and acute respiratory distress syndrome;
- circulatory collapse or shock, systolic blood pressure < 80mm Hg in adults and < 50mm Hg in children;
- acute kidney injury;
- clinical jaundice plus evidence of other vital organ dysfunction; and
- abnormal bleeding
- Laboratory and other findings:
 - o hypoglycaemia (< 2.2mmol/l or < 40mg/dl);</p>
 - metabolic acidosis (plasma bicarbonate < 15mmol/l);

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¹ {World malaria report 2013 2013 #2}

- severe normocytic anaemia (haemoglobin < 5g/dl, packed cell volume < 15% in children; <7g/dl, packed cell volume < 20% in adults);
- o haemoglobinuria;
- o hyperlactataemia (lactate > 5mmol/l);
- renal impairment (serum creatinine > 265µmol/l); and
- o pulmonary oedema (radiological).

Parasitaemia is not directly correlated to the clinical severity as lower parasite densities (< 2,5% parasitaemia) already can be deadly in low-transmission areas whilst much higher densities are still tolerated in high-transmission areas. Nevertheless, parasitaemia >20% is always associated with a high risk of death.²

Transmission areas and risk groups

High transmission area (=hyperendemic/holoendemic)

- Prevalence of P. falciparum > 50% most of the year in children 2-9 years of age.
- Maximum risk for infants and children

Moderate transmission area (=mesoendemic)

- Prevalence of P.falciparum 11-50% most of the year in children 2-9 years of age
- Maximum risk for children, adolescents, pregnant women (2nd+3rd trimester) and HIV+/AIDS persons

Low transmission area (=hypoendemic)

- Prevalence of P.falciparum ≤10% most of the year in children 2-9 years of age
- low risk in all age groups³

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² {Management of severe and complicated 2012 #1}

³ {Management of severe and complicated 2012 #1}

Country profiles for countries partaking in the pivotal studies

Table 1. Transmission rates in the different study countries and % of cases caused by P.falciparum (source: WHO Malaria Report 2013)

	Transmission per Population (%)			
Country	High	Medium	Malaria-free	
Burkina Faso	100	0	0	100
Gabon	100	0	0	75
Ghana	100	0	0	100
Kenya	36	40	24	100
Malawi	100	0	0	100
Mozambique	100	0	0	100
Nigeria	100	0	0	100
Tanzania	73	27	0	100

Table 2. Confirmed and estimated Malaria cases and deaths in the countries partaking in the pivotal studies (source: WHO data repository)

	# confirme	ed cases		# estimated c	ases	# confirr	ned deat	hs	# estimat	ed deaths
Country	2012	2011	2010	2012	2010	2012	2011	2010	2012	2010
Burkina Faso	3858046	428113	804539	5600000 [2500000- 8900000]	5416849 [2829562- 8160019]	7963	7001	9024	17000 [12000- 21000]	31423 [23489- 39141]
Gabon	19753	Not avail.	13936	410000 [210000- 620000]	348509 [202948- 499693]	182	74	134	1100 [620- 1500]	589 [311- 770]
Ghana	3755166	1041260	1071637	6900000 [3900000- 10000000]	6527901 [4195914- 9002752]	3859	3259	2855	17000 [13000- 22000]	12575 [9137- 15979]
Kenya	1453471	1002805	898531	3500000 [2200000- 5200000]	3454057 [2232710- 4656424]	785	713	26017	12000 [4700- 22000]	2074 [943- 7157]
Malawi	1564984	304499	Not avail.	4400000 [2200000- 6800000]	4004127 [2249857- 5856906]	5516	6674	8206	10000 [7200- 13000]	7571 [5926- 10459]
Mozambique	1813984	1756874	1522577	7000000 [3700000- 11000000]	7471146 [4445213- 10626710]	2818	3086	3354	18000 [14000- 22000]	29197 [22052- 36626]

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15183
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] 21490]
0

3.2. Quality aspects

3.2.1. Introduction

The final commercial RTS,S/AS01E vaccine consists of a powder (RTS,S lyophilised antigen) and a suspension (AS01E Adjuvant System) in two separate preservative-free multidose (two-dose) vials as described in the SmPC. Other ingredients are: Powder- sucrose, polysorbate 80, disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate; Suspension- Monophosphoryl lipid A (MPL), *Quillaja saponaria* Molina Fraction 21 (QS-21), dioleoyl phosphatidylcholine (DOPC), cholesterol, sodium chloride, disodium phosphate anhydrous, potassium dihydrogen phosphate and water for injection.

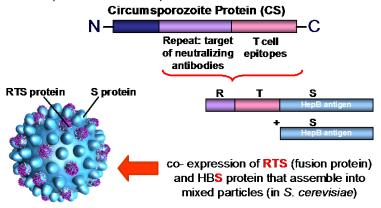
The final drug product for administration is obtained by reconstituting the powder containing the antigen with the suspension containing the Adjuvant System, providing an opalescent, colourless to pale brownish liquid, to be injected intra-muscularly. After reconstitution, one dose (0.5 ml) contains 25 μ g RTS,S antigen and 25 μ g of each of the two immunoenhancer components (MPL and QS-21) of the ASO1E Adjuvant System. This final formulation was used in the pivotal studies.

3.2.2. Active Substance

General information

The active substance represents viral-like particles comprised of the 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) and S proteins (hepatitis B surface antigen) co-expressed in *Saccharomyces cerevisiae*. The RTS,S antigen consists of two proteins, RTS and S, that intracellularly and spontaneously assemble into mixed polymeric particulate structures. A structural representation is shown below in Figure 1.

Figure 1: RTS,S recombinant protein virus-like particle



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Manufacture, characterisation and process controls

Manufacture of the drug substance (DS) is performed at GlaxoSmithKline Biologicals S.A., Rue de l'Institut, 89, 1330 Rixensart, BELGIUM. QC testing is undertaken at GlaxoSmithKline Biologicals S.A., Parc de la Noire Epine, Avenue Fleming, 20, 1300 Wavre, BELGIUM.

Commercial production of RTS,S purified bulk antigen (drug substance) is a continuous process, which starts with the fed-batch fermentation of the recombinant yeast strain *Saccharomyces cerevisiae* RIX4397 from a two-tiered cell bank system, followed by harvesting of the yeast cells, disruption, extraction and purification.

The purification process consists of several steps including different types of chromatography ultracentrifugation, and filtration. The DS is stored at -70°C in sterile containers.

A single fermentation produces one single fermentation broth from which one single extraction is performed. This leads to one single DS batch of purified RTS,S antigen. There is no blending at any stage of the process and no re-processing at any stage of the DS manufacturing process. The production process of RTS,S DS is a continuous process and therefore no intermediates are produced. See section on 'Control of critical steps and intermediates' for description of in-process controls used. Maximum storage times for individual process steps do not exceed 24 hours before proceeding to the next manufacturing step.

The commercial manufacturing process for the RTS,S DS is sufficiently described. Specific details have been provided regarding equipment and consumables. Column resins were named and any changes to these critical consumables which may impact the quality of the product will be managed by variation. During the procedure, the control strategy to change other consumables (e.g. filters), key reagents and equipment has been provided. In conclusion, the manufacturing process is acceptable.

Control of materials

The development and generation of the recombinant *S. cerevisiae strain* including the history and origin of the genes has been provided. The HBsAg has already been used in licensed GSK Hepatitis B vaccines. Information on the relationship of this construct to the licensed products has been provided.

The RTS protein is a fusion protein derived from parts of the CSP protein (a sporozoite surface antigen of the malaria parasite *Plasmodium falciparum* strain NF54) fused to the amino terminal end of the Hepatitis B virus S protein (HBsAg). The CSP encoding sequences were cloned in frame to the encoding sequence of the HBsAg to obtain the RTS gene sequence. Then this RTS gene sequence was cloned in an expression cassette which was inserted into an integrative vector.

The *Saccharomyces cerevisiae* strain used for production of particles containing both the S and RTS polypeptides, carries separate expression cassettes for each protein integrated into the genome. A two-tiered cell banking system is used. Preparation of the master and working seed lots were described and were satisfactorily controlled. An overview of the tests performed on the seeds, along with the methods of analysis was provided. All test results (including identity by culture and southern blot analysis, purity) provided for the current master and working Seed met the specifications set. Genetic stability of the recombinant strain was sufficiently demonstrated from master seed beyond the end of fermentation of the cells. No rearrangements and constant copy numbers of the integrated gene cassettes were reported. The comparability protocol defining the control and qualification of new working seed lots is described in detail and covers all critical aspects (QC release testing, production of commercial scale bulk lots and genetic characterisation) to ensure continuous production.

All raw materials, media components and buffers for cell banking, fermentation, extraction and purification have been listed, along with the analytical references for each. The applicant has stated

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that none are of animal or human origin. Vendors or Certificates of Analysis (CoAs) are not provided. However, since material from new vendors must comply with the analytical references (Ph.Eur. grade or GSK internal monograph) and an appropriate system is in place for the qualification of key reagents from new vendors, this is acceptable. Filters and resins are specified as process parameters.

Control of critical steps and intermediates

In-process controls are applied during the RTS,S manufacturing process. They are intended to provide a means of monitoring product purity, yield and integrity and are classified in two categories:

- 1. Quality decision (QD) tests, which are used to demonstrate that the process is controlled and to take the decision to proceed to the next manufacturing step. These tests are validated and have defined specifications.
- 2. Process monitoring (PM) tests, which are used for process consistency evaluation and for data accumulation (to be used in case of investigation)

A major objection was raised during the procedure on the consistency of the DS manufacturing process for critical aspects of the manufacturing process (protein and antigen yield, protein load on columns), absence of satisfactory validation of critical process parameters and the lack of reliability of protein and antigen content tests employed as process monitoring tests indicating insufficient control of the manufacturing process. The consequences of excursions from Critical Process Parameters (CPP), non-CPP, QD and process-monitoring in-process controls (PM-IPCs) had not originally been adequately explained.

The applicant has now provided further details of its Product Control Strategy (PCS) and explained that it is driven by a risk analysis of the manufacturing process which is based on identification of Critical Quality Attributes (CQAs) and Critical Process Attributes (CPAs). A technical risk assessment (TRA) is then conducted focusing on the impact each unit operation in the manufacturing process can have on the identified CQAs and CPAs. CQAs/CPAs were identified by using a risk assessment tool which considers prior product and process knowledge, characterisation data, *in vivo* non-clinical and clinical evaluations. CQAs pertain to purity, antigen integrity, antigen content and physical description. CPAs pertain to yield, filterability and process time for an individual manufacturing step. Proposed CPPs include filter and column lifetimes, storage conditions and a centrifuge flow rate. The TRA to classify CPPs was described and the outcome was graphically presented in a matrix format for each manufacturing step. Appropriate testing has now been defined to verify that CPPs are within established operating ranges as well as to ensure that CQAs/CPAs remain in their respective ranges to achieve the desired product quality.

IPC tests are applied during fermentation, extraction and purification steps. Performance of two IPC tests was found to be unreliable for demonstration of process consistency of the commercial process. During the procedure more detailed information was given that these test issues have been resolved. Furthermore, the applicant provided further information to justify the conduct and control of the fermentation process.

For certain IPCs, control levels will be established after manufacture of an initial 6 batches using the optimised process with the refitted facility (see post approval change management protocol information in the section- manufacturing and process development). These control levels will be reassessed and updated after 30 batches produced during routine manufacture.

IPCs, release testing and characterisation tests, process measurements, process performance qualification (PPQ) and continued process verification have been discussed in further detail as further elements to establish consistency and comparability (see also process validation section). Upon

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request a more detailed description of the technical risk assessment (TRA) leading to the quantitative derivation of CQA/CPAs has been provided. Numeric examples for some quality attributes were given. Moreover information on the quantitative derivation of the risk priority number (RPN) and the process of defining the criticality of process parameters has been explained. In summary, the approach on classification of CQA/CPA and CPP in place was then found to be comprehensive and acceptable.

Process validation

The applicant's approach to process validation consists of identification and validation of the critical parameters of the manufacturing process and demonstration of process consistency for at least three consecutive batches which must show compliance with pre-established quality standards. Manufacturing of these batches must also show consistency of the unit-step performances and of the residual clearance profiles. Process consistency has been evaluated on eleven lots manufactured in commercial production facilities at commercial scale. As described above, the major objection also included deficiencies in the process validation of critical process parameters.

Process consistency data were originally provided for fermentation, extraction and purification steps. Predefined criteria were only defined for a very limited number of parameters assessed. In the applicant's response to the Day120 questions, further information was provided on the establishment of the optimal growth conditions that resulted in the defined feeding curve. In addition, further characterisation data employing new or improved test methods confirmed that no intra-particle aggregation or post-translational modifications are present in the purified RTS,S antigen.

Validation and qualification data presented for transport between facilities showed that appropriate procedures are in place.

Validation of critical process parameters was demonstrated only for stability of the working seed and DS, when stored. No validation was presented for the other critical process parameters defined. The applicant plans to validate these critical process parameters concurrently with commercial production after implementation of a series of changes to optimise the process, which have been described in the change management protocol provided (see manufacturing and process development section).

Impurity clearance was demonstrated for the host cell impurities. Impurity clearance was demonstrated for the host cell impurities, DNA and other product-related impurities. Process-related impurities were also investigated. Clearance studies on two process related impurities initially failed. Preliminary data suggest that the optimised purification process is capable of removing these impurities to acceptable levels. Moreover the applicant is committed to re-evaluating the clearance of one of those impurities concurrently with the production of batches using the optimised commercial process (see recommendations). With respect to the second process-related impurity, the applicant provided further data demonstrating that the residual amount in DS is present at an acceptable level. The quality and safety of the product authorised now is thereby assured with respect to these impurities. Product related impurities are discussed in the DS 'Specification' section.

Process performance qualification (PPQ) using the optimised commercial process was on-going at the time the scientific opinion was granted. It aims to confirm that the optimised commercial manufacturing process performs consistently by evaluation of 6 batches. These 6 consistency batches will be compared to product manufactured with the commercial process prior to optimisation. Data are expected as part of the type IB variation to approve the improved process using the refitted facility. Continued process verification will be applied during routine commercial production. Control levels applied to certain IPCs will be set after the initial 6 consistency batches and will then be updated after 30 batches. It is recognised that data from earlier batches, not all of which passed validation acceptance criteria, have been submitted to support authorisation. This approach has been considered

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acceptable because a) the reasons for the failures have been adequately explained and b) the applicant has undertaken a comprehensive characterisation exercise of DS using the commercial process and the developmental process, demonstrating comparability (see below). It should however be noted that the intended changes in the manufacturing process are not approvable without the cited additional validation data to support process verification.

Manufacturing process development

During clinical development of the RTS,S/AS01E candidate vaccine, the production process of the RTS,S drug substance evolved and changes in the manufacturing scale, facility and the manufacturing process were introduced. Lots produced from these different processes were employed in different clinical studies and consistency of the commercial manufacturing process was demonstrated with commercial scale lots. A comprehensive exercise was performed to demonstrate that the physicochemical, antigenic and immunogenic properties of the antigen produced at different stages of the clinical development are comparable.

The majority of this product characterisation was performed at the DS level on clinical consistency material. Scientific advice was largely followed and generally, comparability was demonstrated by these analyses although slight differences were observed for some tests which caused concern in case it reflects changes in the particle composition or structure. The applicant provided further assurance that the minor differences seen are due to the test methods employed at different stages during development and that they are not the result of varying gene expression levels or post-translational modifications. This was found acceptable.

Differences found in the physico-chemical and antigenic properties were not significant as shown by data from non-clinical and clinical studies. Non-inferiority was demonstrated in the clinical lot-to-lot consistency study in terms of the anti-CS antibody response of the groups receiving RTS,S/AS01E formulated from commercial scale DS lots in comparison to vaccine formulated with DS lots produced with the former manufacturing process.

Purity assessment by SDS-PAGE revealed a specific host cell protein (HCP) impurity in commercial scale lots, which was not reported for clinical development scale lots. Further investigations identified this impurity as yeast cytosolic catalase which has some homology to human catalase. This impurity was detected at levels of up to 2%. Studies in mice indicated low immunogenicity against yeast catalase, whereas evaluation of an anti-catalase response in humans showed that one child, of the 300 children investigated, had antibodies against human catalase following vaccination, but no clinical symptoms (see clinical evaluation). This finding is addressed in the non-clinical and clinical assessments. Risk minimisation measures have been requested by the PRAC. Consistency of precommercial and commercial material has subsequently been examined. Using an optimised purification process it was shown that the HCP impurities are consistently cleared. The HCP profiles are comparable for batches produced by different scales, however the intensity of some HCPs, including the catalase impurity, are less prominent in batches manufactured by the optimised process. The applicant has however committed to repeating the HCP clearance evaluation on commercial lots produced using the optimised manufacturing process. Quantification of residual catalase on a defined number of commercial lots will also be performed as part of the characterisation testing of the RTS,S DS to confirm the consistency of catalase removal during the process.

The comparability protocol provided for the assessment of the intended changes in the commercial manufacturing process is generally acceptable. Additional tests and evaluation of the clearance of HCP will be included as supporting characterisation tests. Depending on the data set further non-clinical or clinical data might be warranted.

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The current manufacturing commercial process is not the final process for DS (purified bulk) (See Regional Information section). The applicant has proposed two post approval change management protocols (PACMPs) for DS and the applicant will seek approval of each change before marketing final commercial material in Sub-Saharan African countries. The first PACMP is due to a re-fit of the manufacturing facility where the DS is made. New equipment was installed and some changes to the process implemented with the purpose of optimisation. The second PACMP regards changes to microbial control during DS manufacture.

Characterisation

Characterisation of the RTS,S antigens and self-assembled particles was conducted by employing a broad panel of physico-chemical and immunological analyses. The analyses include the evaluation of the primary and secondary antigen structure, particle structure and size, the electrophoretic profiles and the antigenic properties. Data were presented for seven commercial scale RTS,S DS lots. These characterisation studies confirm consistent physico-chemical and antigenic properties of the RTS,S DS of the lots evaluated. In conclusion, the analytical results of RTS,S DS are consistent with the proposed structure and the DS has been satisfactorily characterised.

Specification

The drug substance release specifications include: appearance (visual); pH (Ph.Eur.); identity and antigenic activity; purity; protein content; S to RTS ratio; endotoxin (Ph.Eur.); sterility (Ph.Eur.); size distribution profile; lipid content; polysaccharide content. In general, the analytical test panel proposed for the routine release testing of RTS,S DS lots is acceptable and complies with WHO Guidelines to assure the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum*" (TRS 980).

With respect to the current specification for the antigenic activity, Hepatitis B by ELISA, further justification for the specification limits was given upon request. The proposed specification limits take into account performance of batches used in clinical trials. The specification limits for the determination of the lipid content were redefined and significantly tightened.

Regarding DS specification, a tolerance interval approach was initially proposed in the setting of limits. Based on the number of batches which contribute data towards calculating mean +/- tolerance interval, potentially very wide ranges were initially proposed. This approach was not accepted and the applicant has recalculated the specification limits using the mean +/- 3 standard deviation approach, or in some instances, further justified the use of tolerance intervals on relevant batches. In addition, the newly defined specification limits were compared to the range of data from lots used clinically (In addition, some batch release specifications at both substance and product levels, which are not calculated from tolerance intervals were tightened, either based on manufacturing experience or on manufacturing considerations. This approach is acceptable currently and the applicant will reassess the specification limits RTS,S drug substance when data from more than 30 batches become available (see recommendations).

Two types of impurities can potentially be found in RTS,S DS:

- Impurities originating from the yeast cell system (examples: residual DNA, host cell proteins (HCP))
- Impurities resulting from substances added during fermentation or purification.

Both types of impurities were tested in RTS,S DS and results provided for seven DS lots. As regards host cell impurities, residual amounts or consistently low levels were reported. Although the residual host cell protein levels complies with the specification given in the WHO TRS 980, a specific yeast cell

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protein (catalase) of ~65kDa was identified to be present in lots. This impurity was further addressed and it has been demonstrated that the optimised purification process will most likely consistently reduce this HCP impurity to an acceptably low level. The tests for the determination of HCP and the DNA content will be retained, as part of characterization tests, until more experience is gained through routine manufacture.

Given the initial failure of the validation of some process related impurities, the applicant will monitor residual levels in the new process performance qualification. The data will be assessed as part of the PACMP. Since supporting data are strongly indicative that these additives will be removed by the optimised process, the proposed strategy is accepted.

Impurities resulting from the substances added during manufacture were either measured on RTS,S DS lots or calculations were performed on the process input of the impurity to calculate the worst case concentrations per vaccine dose (see comments above regarding PV studies of clearance of process related impurities). There was only one process related impurity originating from substances added that was not considered initially. The applicant provided preliminary clearance data and a toxicological assessment indicating that the residual level, calculated in the DS, are far below the level of toxicological concern. The applicant will re-evaluate clearance of this impurity during the commercial manufacturing campaign and submit a variation accordingly.

Analytical methods

The tests performed in accordance with Ph.Eur. are considered validated by the applicant and therefore, apart from the endotoxin test, no additional validation information has been provided. This is acceptable.

The potency test for RTS,S is an ELISA with a capture antibody directed against the CS (RT) part of the Virus Like Particle (VLP), and detection antibody raised against the S protein. Development follows the principle in the relevant WHO document. Potency testing for Hepatitis B is an inhibition ELISA which is based on the test used for licensed GSK Hepatitis B vaccines.

However, for these critical tests, only very brief descriptions and unclear validation summaries had originally been supplied. Consequently, better descriptions (e.g. SOPs) and full validation protocols and reports were sought and supplied for both drug substance and drug product testing and found acceptable.

During the procedure, the applicant provided further details of critical reagents and consumables and explained how changes to the analytical procedures will be managed to ensure adequate control is maintained. Data were also provided to show the stability indicating potential of the stability assays by demonstrating the capability of the assays to detect alterations of the RTS,S product.

Batch analysis

General information including the dates of manufacture and batch size of RTS,S DS lots that were used to formulate RTS,S drug product commercial lots has been presented in the dossier. All lots fulfil the specification set for the release testing. Data from nine RTS,S DS lots manufactured at full-scale in the proposed facility are also included. The data showed compliance to the specification in force at that time, showing consistency of the manufacturing process. These lots were used in Phase III clinical studies.

Reference materials

The reference material used for identity and antigenic activity of the purified RTS,S bulks is a final container lot which was employed in phase III clinical trials and is appropriately characterised. In

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addition, procedures are in place to control performance of the reference and to qualify new reference material.

Stability

Data from real-time, real-condition stability studies include three commercial scale batches used to formulate commercial scale consistency lots as well as Phase III clinical consistency lots. These data indicate no impact of the evaluated 48 month period of storage at -70°C on the physicochemical characteristics, antigenic properties, electrophoretic profiles, sterility, purity or RTS to S ratio of the DS. The study is ongoing up to 60 months.

Preliminary data are available for the proposed 60 months shelf-life for two of the three consistency DS lots as well as statistical analyses of the data obtained throughout the 60 months storage period.

Data from an accelerated stability study of the three PB lots, conducted at $+37^{\circ}C\pm2^{\circ}C$ for 7 days demonstrate a marked increase in in vitro potency (CS-S and Hepatitis B), the S/RTS ratio and changes in the electrophoretic profiles.

As the 60 months data for one out of the three lots included in the long-term real-condition stability study are still outstanding due to ongoing investigations of unexpected results for one test, a shelf-life of 48 months is proposed and justified for the time being. The applicant intends to reconsider the shelf-life depending on the results of the investigation. In accordance with EU GMP guidelines, any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

3.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The RTS,S/AS01E vaccine contains as active substance the RTS,S antigen which is formulated with the applicant's AS01E proprietary Adjuvant System. The latter consists of two immune enhancers MPL [3'-O-desacyl-4'-monophosphoryl lipid A] and QS-21 [*Quillaja saponaria* Molina, fraction 21]), in a liposome suspension.

Immunoenhancer QS-21 is considered a novel excipient because it is not yet approved in the European Union as an excipient in any licensed drug product for human use by the intramuscular route. DOPC and cholesterol have been previously used in other EU licensed injectables. MPL has also been used in the centrally approved vaccines, Fendrix and Cervarix. Novel excipients require full details of manufacture, characterisation, and controls, to be provided according to the drug substance format and cross references to supporting safety data (nonclinical and/or clinical). Also the Guideline on Adjuvants in vaccines for human use (EMA/CHMP/VEG/134716/2004) states that complete quality information on the components of an Adjuvant System should be provided. Satisfactory information on all adjuvant components (MPL, QS-21, DOPC and cholesterol) has been provided.

The pharmaceutical form of the reconstituted RTS,S/AS01_E is a liquid suspension for injection which is an opalescent, colourless to pale brownish liquid. The RTS,S/AS01_E vaccine is preservative-free and consists of two fractions:

• The powder or lyophilised fraction containing the RTS,S antigen, which is presented in a 3-millilitre (mL) clear glass vial (Type 1, Ph. Eur.) closed with rubber stoppers and aluminium caps. Each vial contains two doses of RTS,S antigen.

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⁴ 6.32 of Vol. 4 Part I of the Rules Governing Medicinal products in the European Union

• The liquid suspension consisting of AS01_E Adjuvant System. AS01_E is presented in a 3-mL glass vial (Type 1, Ph. Eur.) closed with rubber stoppers and aluminium caps. Each vial contains two doses of AS01_E Adjuvant System.

The liquid ASO1_E Adjuvant System is used to reconstitute the RTS,S lyophilised antigen, extemporaneously prior to administration. Reconstitution of one vial of lyophilised RTS,S with one vial of ASO1_E Adjuvant System delivers two human doses of the RTS,S/ASO1_E vaccine for intramuscular administration. Satisfactory information has been provided to justify that no preservative is included in this 2-dose product and according to the SmPC, the reconstituted product should be used immediately.

The QS-21 and 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) components are controlled according to the applicant's internal monograph. All other excipients are well-known pharmaceutical ingredients and their quality is compliant with Ph.Eur. standards. These include: Powder- sucrose, polysorbate 80, disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate; Suspension-, cholesterol, sodium chloride, disodium phosphate anhydrous, potassium dihydrogen phosphate, water for injection..

The DP is also subject to PACMPs (see 'comparability exercise for finished medicinal drug product' section). Each of the following sections in the DP report are divided firstly into a section for the AS01_E (Adjuvant (Adjuvant System suspension) and the RTS,S (lyophilised antigen). Information on the reconstituted DP is given in the DP stability section only.

AS01_E Drug Product

Non-clinical and clinical studies demonstrated the need for specific adjuvantation to increase immunogenicity of the RTS,S antigen. This was observed for both humoral and cell mediated immune (CMI) responses. Among all tested adjuvant Systems, the ones including MPL and QS-21 as immunoenhancers provided the highest efficacy in a proof-of-concept clinical trial. The AS01E Adjuvant System was selected for use with RTS,S antigen based on successful clinical evaluation, showing a well-tolerated safety profile and good enhancement of a specific immune response.

Besides the immunoenhancers, MPL, and QS-21, the Adjuvant System formulation contains additional excipients: cholesterol and DOPC, in a phosphate based buffer system.

Key biological properties of AS01E Adjuvant System are related to the immunostimulatory properties of QS-21 and MPL. Briefly, in antigen presenting cells, MPL induces the release of pro-inflammatory cytokines and increases the number and the expression level of co-stimulatory molecules. QS-21 adjuvant activity is associated with the stimulation of an innate immune response characterised by the direct activation of antigen-presenting cells and the stimulation of the inflammasome pathway upon endocytosis. The liposomes serve as carriers for the two immunoenhancers.

The AS01E Adjuvant System formulation process consists of mixing the concentrated liposome bulk intermediate (CLB) with the formulation buffer (PO4/NaCl), followed by the addition of QS-21 liquid bulk (LB).

The main process changes applied between AS01E Phase III consistency lots and AS01E commercial consistency lots at the formulation step are a scaling up and changing the filtration step together with a transfer from clinical to commercial formulation facilities. Comparability between commercial and clinical formulations of AS01E was satisfactorily demonstrated.

Additionally, significant changes planned for commercial manufacturing of $ASO1_E$ were implemented in Industrialisation (see below for definition), between the Phase 3 efficacy and the Phase 3 consistency lots. These changes involved the two intermediate steps of CLB and QS-21 LB.

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No process changes were implemented in the manufacturing process of the intermediate QS-21 LB between Phase 3 efficacy and Phase 3 consistency lots other than QS-21 supplier changes which have been justified. Changes were applied at the time of process transfer to commercial facilities (scale up and change in storage conditions).

The comparability exercises were also performed in two steps: first, Phase 3 consistency lots were compared to Phase 3 efficacy lots; second, the commercial consistency lots were compared to all relevant industrialisation lots ("industrialisation" refers to the facilities used to produce $ASO1_E$ FC GMP lots during product development) including Phase 3 lots. This approach, for comparability assessment, was applied to the two intermediates (CLB and QS-21 LB) and to $ASO1_E$ final bulk (FB) and final containers (FC). The comparability between the commercial consistency lots and previous industrialisation lots has been established at the level of the intermediates CLB and QS-21 Liquid bulk (LB) and on $ASO1_E$ FB and FC.

Because the exact nature of the adjuvant activity of MPL and QS-21 in the $ASO1_E$ formulation is complex, comparable quality attributes alone cannot ensure comparable biological activity. Therefore two experiments were conducted in the mouse model to support key manufacturing changes between Phase III consistency and commercial lots. The study design of the two experiments was based on the *in vivo* potency assay used for the release and stability follow up of the RTS,S candidate vaccine lots.

The proposed containers for storage of QS-21 LB, CLB and AS01E FC have been evaluated against current guidelines and found to be suitable.

RTS,S Drug Product

The development of the lyophilised RTS,S antigen manufacturing process took place in parallel with clinical development.

The initial RTS,S freeze-dried formulation used in initial trials contained lactose manufactured from bovine milk. To avoid the use of an excipient of animal origin, sucrose replaced lactose as cryoprotectant. This formulation was used in clinical trials up to the phase III efficacy studies. An increase of the sucrose content allowed reduction of the duration of the lyophilisation cycle. RTS,S phase III consistency FC lots were formulated from commercial scale RTS,S DS lots, whereas RTS,S phase III efficacy FC lots were formulated from small scale DS lots. Additionally, the Phase III consistency lot is a single-dose preparation whereas the Commercial Consistency lot is identical except it is a two-dose preparation. The main process changes applied between RTS,S Phase III consistency lots and RTS,S commercial consistency lots are a scaling up in formulation, filling and lyophilisation operations and a transfer from clinical to commercial facilities. A comparability exercise was performed including quality and non-clinical assessments. Two experiments were conducted in the mouse model to support key manufacturing changes between Phase III consistency and commercial lots. The study design of the two experiments was based on the in vivo potency assay used for the release and stability follow up of the RTS,S candidate vaccine lots. Comparability between commercial and clinical lots of RTS,S was satisfactorily demonstrated. Major manufacturing changes will continue to be supported by in vivo potency testing performed in mice with RTS,S/AS01_E reconstituted vaccine.

Manufacture of the product and process controls

AS01_E Drug Product

 $ASO1_E$ drug product is formulated at GSK, Parc de la Noire Epine, Avenue Fleming 20, Wavre, Belgium. It is filled, labelled and packaged at GSK, 637 Rue des Aulnois, Saint-Amand-Les-Eaux, France and QC tested at the GSK Parc de la Noire Epine site or Rue des Aulnois site. QA release is from GSK, 89 Rue de l'Institut, Rixensart, Belgium.

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The $ASO1_E$ Adjuvant System formulation process consists of mixing the concentrated liposome bulk intermediate (CLB) with the formulation buffer (PO4/NaCl), followed by the addition of QS-21 liquid bulk. After pH check and sterile filtration, the final bulk (FB) is filled into the final containers (FC). The proposed containers for storage of QS-21 LB, CLB and $ASO1_E$ FC have been evaluated against current guidelines and found to be suitable.

Overall the description of the manufacturing process is satisfactory. The details of the manufacturing process of ASO1_E and its intermediates are sufficiently described. In response to questions raised, the applicant provided a detailed description of the control strategy. Depending on the nature of the test and the number of batches tested, control and action limits are set by defined calculations. In general, the analytical test panel proposed for the routine release testing of ASO1_E lots is acceptable and complies with WHO Guidelines to assure the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum*" (TRS 980).

The critical process parameters that are identified are considered suitable to ensure the manufacture of consistent and acceptable product. The manufacturing processes has been satisfactorily evaluated and validated.

Additional quality information requested during the procedure, on the excipients of the adjuvant system (i.e. MPL, QS-21, DOPC and cholesterol) has been provided. Impurity levels for all components of the adjuvant system have been sufficiently described and their presence/levels were justified by clinical data. Monophosphoryl lipid A (MLA) is a purified, non-toxic endotoxin derivative obtained from *S. minnesota* manufactured by Corixa Corporation (GSK Vaccines), Montana, USA. Details of the production and purification, in-process controls and critical process parameters are provided. Detailed information on the development of the manufacturing process is provided. The changes to the manufacturing process to accommodate the increased scale of manufacture using new equipment in two buildings have been assessed. The comparability data and the process validation data demonstrate that MPL Powder manufactured in both is comparable. MPL Powder manufactured in both buildings may thus be used interchangeably to manufacture MPL-containing vaccines. Satisfactory storage periods and information on containers has been provided.

MPL is a well characterised substance, potential impurities are identified. The specification and analytical tests applied for the release and stability monitoring of the substance are appropriate. However, the applicant was requested to revise the specification to align it with the current Ph. Eur. monograph for 3-O-Desacyl-4'-Monophosphoryl Lipid A <2537>. Since sound justification of the deviations was provided by the applicant, no change of specifications is deemed necessary. The MPL lyophilised powder can be stored at +2 to $+8^{\circ}$ C for 60 months.

QS-21 contains a mixture of structurally-related saponins obtained by chromatographic purification of an aqueous extract of the bark of the soap bark tree *Quillaja saponaria* Molina. A satisfactory description of the manufacturing process together with suitable in-process controls has been provided. The different manufacturing processes have been compared. Comparability data for material produced from the current supplier and the original supplier are satisfactory. Available stability data demonstrate the stability of QS-21 when stored at -20°C for the proposed storage period of 36 months. Testing is ongoing and will continue to 48 and 60 months.

DOPC has a well-established manufacturing process. It is purified by chromatography and recrystallisation. D,L- α -tocopherol is added as an antioxidant to protect the unsaturated fatty acid of DOPC from oxidation during storage. The specification and analytical methods are considered appropriate. Lipoid PC remains stable for 3 years when stored at -20 \pm 5°C.

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Satisfactory details of the manufacturing process are provided for cholesterol. This is a well-established material and the specification and control tests are satisfactory. The applicant adequately justified the use of cholesterol that is compliant to Ph. Eur monograph Cholesterol <993>, instead of the monograph Cholesterol for Parenteral Use <2397> and will develop an endotoxin test for cholesterol raw material testing. Data generated during the long-term stability study support a re-assay period of 36 months from date of manufacture for product stored at -20 °C.

The release specification for the intermediate CLB has been satisfactorily justified based on batch release data and stability studies. The overall stability data support the proposed shelf life of 36 months for concentrated liposome bulk lots when stored at 2 to 8 °C. Any out of specification results from the on-going stability study of the CLB consistency lots will be reported to the EMA.

The release specification for the intermediate QS-21 LB has been satisfactorily justified based on batch release data and stability studies. The proposed shelf life of 12 months for QS-21 LB lots when stored in at 2 to 8 °C is supported by the stability data provided.

RTS,S Drug Product

RTS,S, S drug product is formulated at GSK, Parc de la Noire Epine, Avenue Fleming 20, Wavre, Belgium. It is filled, labelled and packaged at GSK, 637 Rue del Aulnois, Saint-Amand-Les-Eaux, France and QC tested at the GSK Parc de la Noire Epine site. QA release is from GSK, 89 Rue de l'Institut, Rixensart, Belgium.

The manufacturing process for RTS,S drug product comprises, currently, thawing of the DS, formulation with a solution of sucrose, sodium dihydrogen phosphate/ disodium phosphate (NaH₂PO₄/Na₂HPO₄) buffer, polysorbate 80 and water for injection, followed by a filtration step from the formulation tank into a shipping tank. Excipients used comply with Ph. Eur.

All steps are performed at room temperature. After thawing, the bulk may then be stored at 2-8 °C. Stirring, mixing and filtration steps are conducted at room temperature. The shipping tank is stored at +2°C to +8°C until transport to the filling site.

The final bulk is aseptically filled under Class 100/Grade A laminar flow into washed, siliconised, depyrogenated and sterilised glass (type 1) vials using an automated filling/stoppering machine under isolators. Filled vials are partially stoppered and aseptically transferred to the lyophiliser. Lyophilised vials are capped and stored until labelling and packaging. Contrary to GMP guidelines, the final sterilising filtration seems to be temporally and spatially distant from vial filling and closure. Data support the current process, however there are plans to improve this issue and it is intended to implement the filtration immediately prior to filling for the RTS,S component. Initial study results provided during the procedure confirmed the feasibility to move the filtration step. The time frame for implementation however depends on a series of further activities including complete qualification of filters and equipment and media simulations. Full implementation in routine manufacture is expected to be achieved by the applicant by the end of 2016.

The applicant validated a holding time of the formulated bulk for 14 days at 2 - 8 °C in a stainless steel tank. Validation of aseptic formulation operations during the RTS,S drug product manufacturing process has been performed by media challenge. Similarly, media fill runs have been conducted to validate aseptic filling and lyophilisation. The change management protocol (CMP) introduces changes to the current process that will allow an upscale of formulated bulk. These changes are deemed acceptable.

During the manufacture of drug product (formulation, filling), appropriate process parameters or IPCs are given. More detailed specification of the freeze-drying procedure was requested to ensure

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consistent control of this complex operation and this was raised as part of the major objection on the control strategy since this is a critical step in the drug product manufacturing process. The applicant outlined that relevant in-process control parameters are tracked during operation and are compared to established operating ranges. Actions if any parameter operates out of this range were then outlined and found acceptable. Final product testing and acceptable quality level (AQL) specifications also provide assurance that the freeze-drying operation is under control on a batch by batch basis. The list of critical process parameters has been revised to comply with the applicants internal requirements and an updated list was provided during the procedure. For each CPP the target and/or operating ranges were defined and information on the knowledge and detectability according to the risk priority number (RPN) was given. No impurities are generated by the formulation and filling processes of the RTS,S final container. Drug substance-related impurities may be detected in the RTS,S drug product; (See RTS,S 'drug substance' section).

In summary, the manufacturing process has been appropriately validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

Product specification

AS01_E Drug Product

Overall, the AS01_E Adjuvant System preparation is adequately controlled. The release specification for the AS01_E final bulk includes a sterility test. Nonetheless, in order to take due account of the performance of the commercial production process, the applicant will review the DP specification after data from 30 batches become available (see recommendation).

The release specification for the $ASO1_E$ final container includes tests for appearance; pH, identity, content of relevant constituents; particle size profile; osmolality and sterility.

Analytical data for the reference standards MPL, QS-21, DOPC and cholesterol are provided.

The content of potential impurities is satisfactorily controlled during the manufacturing process, at release of final product and in stability studies. Therefore, the current control strategy is considered justified.

RTS,S Drug Product

The release specification for the RTS,S final bulk includes a sterility test.

The release specification for the RTS,S final container includes tests for appearance, pH, volume, identity, purity/ content of relevant constituents, particle size, osmolality and sterility. Appropriate limits have been set. In general, the analytical test panel proposed for the routine release testing of RTS,S DP lots is acceptable and complies with WHO Guidelines to assure the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum*" (TRS 980).

Stated impurities have been studied in nonclinical and clinical studies as relevant.

Regarding RTS, S DP specification, a tolerance interval approach was originally proposed by the applicant in the setting of limits (see DS section). In addition, some batch release specifications at both substance and product levels, which were not calculated from tolerance intervals were very wide originally and were requested to be tightened, based on manufacturing experience (e.g. endotoxins) or on manufacturing considerations (e.g. sucrose). The applicant has now provided acceptance criteria which are based on ±3SD and in future will also further tighten the limits, if necessary. A review of DP

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specifications is planned after 30 batches have been manufactured using the final commercial routine process. This approach is deemed acceptable.

Tests proposed to be repeated by an independent test laboratory (Official control authority batch release testing)

The CHMP has made recommendations for Official Control Authority Batch Release Testing (OCABR) of this product.

According to the Administrative Procedure for European Official Medicines Control Laboratory Certification of Compliance of Batches under Article 58 [PA/PH/OMCL (04) 140 DEF], batch compliance control of individual batches should be performed before release on to the market for a given biological medicinal product licensed in a third country. In this context a list of key tests to be repeated by an independent laboratory for the purpose of batch compliance control should be proposed within the CHMP scientific opinion.

The following tests are proposed for the independent control laboratory testing:

On the drug substance (purified bulk antigen):

RTS,S purity and RTS to S ratio

On the RTS,S final container (lyophilised component of the drug product):

- Appearance
- Identity and *in vitro* potency assay (serves as an identity test)
- Endotoxin

Analytical methods

AS01_F Drug Product

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

RTS,S Drug Product

Tests have been adequately described and validated, though as for drug substance, protocols and reports were requested for critical assays. These requested data were provided. The tests performed in accordance with official pharmacopoeia monographs (refer to P.5.2 sections) are considered validated (except for endotoxin) and are therefore not described in these sections. In compliance with the WHO recommendations, discussion of concordance between the RTS,S potency ELISA and *in vivo* potency testing had been requested. Further evaluation of artificially stressed samples revealed an enhanced ability of the *in vitro* assays to detect sub-potent batches compared to *in vivo* potency tests. Considering the principles of Directive 2010/63/EU on the protection of animals, the enhanced capability of the *in vitro* potency tests to detect sub-potent batches and the generally lower variability of *in vitro* assays compared to animal tests, the use of *in vitro* potency assays for the CSP and HepB determinants was endorsed.

Potency CS-S of the RTS,S Final Container is essentially the same as the identity and activity assay CS-S by ELISA for drug substance. However, for DP, the measured CS-S content is expressed as a ratio to the label-amount of RTS,S. For the DS, it is expressed as a ratio to the measured protein content of the DS. The method for potency Hepatitis B of the RTS,S final container is the same as that used for DS.

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Batch analysis

AS01_F Drug Product

Batch analyses data from three $\mathsf{ASO1}_\mathsf{E}$ FB lots, used to fill three FC commercial consistency lots have been provided. Batch analyses data including three $\mathsf{ASO1}_\mathsf{E}$ FC commercial consistency lots, two dose consistency and lots involved in Phase III consistency studies, have been provided. Results comply with specification and confirm consistency of the manufacturing process.

RTS,S Drug Product

Batch analysis results of RTS,S drug products lots have been presented, including FB and FC: Phase III clinical consistency lots (4 lots), two-dose final container consistency lots (3 lots) and commercial consistency lots (44 lots). Results for all lots complied with their specifications and confirm consistency of the manufacturing process.

Reference materials

For $ASO1_E$ and RTS,S drug product, adequate information on reference standards used for all components has been provided.

Stability of the product

The RTS,S final container (FC) product comprises the lyophilised RTS,S antigen two-dose cake in 3-ml glass vials. The RTS,S lyophilised antigen is mixed with the liquid $ASO1_E$ Adjuvant System presented also in two-dose 3-mL glass vials, immediately prior to administration, to form the RTS,S/ASO1_E reconstituted vaccine. Stability data for both the RTS,S lyophilised drug product and the RTS,S/ASO1_E reconstituted vaccine were collected.

AS01_E Drug Product

The holding time for the formulated bulk between formulation and filling was validated through QC testing and stability studies performed on industrialisation and commercial ASO1E FC lots, filled from FB lots stored in stainless steel tanks

The currently available long-term, real-time stability data obtained on Phase III single dose efficacy and consistency lots, and two-dose industrialisation consistency lots show that, at up to 36 months storage at 2 to 8°C, all results comply with the specifications. Interim 6 months stability data from commercial consistency lots confirm the stability profile. Comparability between phase III efficacy lots, phase III consistency lots and two-dose industrialisation consistency lots has been demonstrated. Temperature cycling studies show that a temperature, up to 37°C, for up to 14 days during the storage period, has no deleterious impact on the stability of the ASO1_E Adjuvant System. These data support the proposed shelf-life of ASO1_E FC two-dose for up to 36 months at 2 to 8°C. A temporary exposure at 25°C and at 37°C for 14 days has been supported by data. However, in order to minimise the risk of uncontrolled product storage, the SmPC defines the final storage conditions for the finished product which is 2-8 °C for 3 years.

Thermal cycling studies were performed also with an interval of 14 days at -20°C between regular storage at 2-8°C. These studies showed that some parameters were altered compared to regular storage. Therefore, excursions below the recommended storage temperature of 2-8°C are not justified and the SmPC and PIL recommend that the product is not frozen.

RTS,S Drug Product

A comprehensive stability testing program has been presented for which data are presented up to the 36-month time point for single dose phase III efficacy and consistency lots, industrialisation

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consistency lots (2 dose). Twelve months data are available for 2-dose commercial consistency lots. The stability data on RTS,S Final container support a shelf life of 36 months at 2 °C to 8 °C.

However, all product batches which have been placed onto stability studies have less than 0.3% residual water content, whereas the specification is not more than 3%. Since the stability of freezedried product is linked to residual water, there were concerns that the stability of the product close to the proposed specification limit was not assured. The applicant has now provided QC test data which show that a higher water content, close to the acceptance criteria does not impact the results of other QC release tests. Therefore the acceptance criterion of 3% for water content is considered acceptable.

Furthermore, accelerated stability data show that the RTS,S FC is stable at 25°C for up to 193 days and up to 30 days at 37°C. Also, data from the temperature cycling stability study showed that RTS,S Drug Product remains within its specification. However, in order to minimise the risk of uncontrolled product storage, the SmPC defines the final storage conditions for the finished product which is 2-8 °C for 3 years.

RTS, S/AS01_F

Comparability between phase III efficacy lots, phase III consistency lots and two-dose consistency lots has been demonstrated. Results from the in-use stability study support the use of RTS,S/AS01 $_{\rm E}$ vaccine within a maximum of 6 hours after reconstitution at a temperature up to 37°C. However, in order to minimise the risk of uncontrolled product storage and subsequent deterioration, the SmPC states that if the product is not used immediately, in use storage times should not be longer than 6 hours at 2°C to 8°C.

Comparability exercise for finished medicinal drug product Post approval change management protocol

The current manufacturing process is not the final process for final bulk (after formulation) or final container, after filling (for AS01E) or after filling and freeze-drying (for RTS,S). The applicant has proposed two post approval change management protocols (PACMPs), and the applicant will seek CHMP scientific opinion of these data before marketing final commercial material. One PACMP is to add an additional site for formulation operations, increase scale of formulation activities at the new site and add additional freeze-driers to the fill/finish site (St Amand). Although complex and extensive, the comparability exercise would mirror the comparability exercise performed so far. No additional nonclinical or clinical studies are proposed. Several issues were raised on the strategy and test program. The applicant updated the change management protocol to extend the test program to include conduct of the two in-vivo potency assays in mice (CSP and S determinants), that were done for release purposes during vaccine development and also as part of the last comparability exercise between commercial consistency lots and clinical lots. A second PACMP has been presented for ASO1_F Adjuvant System to seek approval for changes in the storage conditions of one intermediate, as well as AS01_E formulation scale-up and transfer of formulation to a different site. Clarification was given to several questions on the PACMPs for the RTS,S and ASO1E drug products. The PACMPs were updated accordingly.

Adventitious agents

The RTS,S drug substance in the RTS,S/AS01_E vaccine is a product derived using biotechnology, from yeast seeds. Culture media that could support bacterial or fungi growth are used. Therefore, starting materials are tested for microbiological purity according to the relevant requirements and possible microbial contamination during production is monitored. The applicant has stated that except for casamino acids used in the manufacture of MPL immunoenhancer, no components of animal or human

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origin are used in the commercial manufacturing process of DS or DP. Casamino acids derive from bovine milk, which is sourced from healthy animals and which is fit for human consumption. In the light of current scientific knowledge, bovine milk is unlikely to present any TSE risk.

3.2.4. Discussion on chemical, pharmaceutical and biological aspects

As discussed above, a major objection was raised during the article 58 procedure on the control strategy regarding consistency of the DS manufacturing process, which had not been adequately demonstrated for critical aspects of the manufacturing process, the absence of validation of critical process parameters and concerns over the control of the performance of the manufacturing process. The consequences of excursions from CPP, non-CPP, and IPCs had not been adequately explained. The applicant subsequently provided a detailed description of the control strategy applied for the routine manufacture of RTS,S. The strategy follows, in principle, standard regulatory guidance and found to be acceptable. The applicant currently intends to implement the control strategy concurrently with the manufacture of lots using the optimised process at the newly refitted facility. Because of this, only concepts can be given, and actual data will be provided in the type IB variations associated with the Change Management Protocols, when they become available.

However, the initial approach of calculating alert and action limits for certain IPCs was found to be not appropriate to provide a meaningful tool for process monitoring. The applicant has revised this approach and is now committed to setting tighter provisional alert and action levels and to re-evaluate them once more commercial batches will have been produced.

The applicant confirmed that the first 6 batches produced with the optimised manufacturing process are intended for commercial use. The justification given can be accepted since the testing program applied exceeds the usual batch release program and it includes further extensive characterisation studies and demonstration of comparability with previous production campaigns.

All the questions initially raised were appropriately addressed and corroborated by additional information or new data. This includes questions on the validation of the removal of process and product related impurities, minor differences observed in the characterisation studies and information on the validation of assays. However, it is noted that the applicant is committed to re-evaluating the clearance of two process-related impurities concurrently with the production of batches using the optimised commercial process to provide confirmation of the preliminary data which suggested that the optimised purification process is capable of removing them to an acceptable level. The applicant will submit a variation accordingly (see recommendations).

Additionally, further to the finding of an anti-catalase response in one child out of 300 evaluated, following vaccination (also addressed by PRAC requirements), the applicant has committed to repeating the HCP clearance evaluation on 10 commercial lots produced using the optimised manufacturing process. Quantification of residual catalase on 30commercial lots will also be performed as part of the characterisation testing of the RTS,S DS to confirm the consistency of catalase removal during the process (see recommendations).

Outstanding issues such as stability data of the drug substance stored in the actual container/ closure system were provided to confirm that it does not impact the stability of the DS. Results of Ph. Eur. compliance testing on extractables and leachables for the container closure systems used for the storage of RTS,S DS lots used currently and in previous manufacturing campaigns should be submitted (see recommendations).

Additional data from the long-term real condition stability studies were provided, but final results on one test are still outstanding due to ongoing investigations of unexpected results observed on the 60-

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months sample of one of the three RTS,S DS stability lots. The applicant proposed to limit the shelf-life to 48-months for the time being, which is accepted and justified. The applicant intends to reconsider the shelf-life of DS once the results of the investigation on the unexpected results for one test are known.

At the Drug Product (ASO1 $_{\rm E}$, RTS,S Drug Product) level, consistency of pre-commercial and commercial material has been examined. With respect to the Drug Product, the applicant satisfactorily addressed the outstanding questions especially on the change management protocol for the ASO1 $_{\rm E}$ Adjuvant System. The PACMP for the RTS,S and ASO1 $_{\rm E}$ Drug Product was updated and is now also deemed acceptable.

Other outstanding issues concerned the re-definition of CPPs for the freeze-drying process of the RTS,S drug product and the implementation time lines of the filtration step prior to filling. The applicant satisfactorily addressed these questions and submitted an updated list of CPPs.

As regards the potency assay of the final container product for batch release evaluation of artificially stressed samples revealed an enhanced ability of the *in vitro* assays to detect sub-potent batches compared to *in vivo* tests. Considering the principles of Directive 2010/63/EU on the protection of animals, the enhanced capability of the *in vitro* potency tests to detect sub-potent batches and the generally lower variability of *in vitro* assays compared to animal tests the use of *in vitro* potency assays for the CSP and HepB determinants is supported.

Finally, although DS and DP specifications have been acceptably justified for the present time, in view of the number of process changes impacted, the applicant should review and where needed update and submit justifications accordingly for DS and DP further to manufacture of 30 batches using the commercial processes.

3.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

3.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

Area	Number	Description	Classificati on*
Quality	Quality 001	IPC controlcontrol levels should be reassessed/updated for RTS,S drug substance after 30 commercial batches have been manufactured	REC
Quality	Quality 002	RTS,S DS: The specification limits for RTS,S drug substance should be reviewed and submitted when data from more than 30 batches using thefinal, optimised commercial routine process become available.	REC
Quality	Quality 003	RTS,S DP: The specification limits for RTS,S drug product (vaccine) should be reviewed and submitted when data from more than 30	REC

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^{*} Recommendation

3.3. Non-clinical aspects

3.3.1. Introduction

RTS,S consists of the RTS hybrid polypeptide containing B and T cell epitopes from *Plasmodium falciparum* CSP fused to the hepatitis B surface antigen (S) proteins (hepatitis B surface antigen) co-expressed in *Saccharomyces cerevisiae*. These assembles as virus-like particles which are not infectious. The product is presented with an adjuvant, termed ASO1_E. Primary pharmacodynamics studies were undertaken in three different settings: 1) initial studies to decipher immunogenicity profile of RTS,S/ASO1 vaccine formulation, 2) subsequent studies to support manufacturing scaling-up and lot consistency, and 3) a series of in vitro and in vivo (mice) studies to characterize Mode of Action (MOA) of the ASO1 adjuvant system.

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Table 3. Adjuvant Systems and their components

Components (quantity per final human dose of 0.5 mL)
MPL (50 μg); QS-21 (50 μg); DOPC¹ (1000 μg); Cholesterol (250 μg)
MPL (25 μg); QS-21 (25 μg); DOPC¹ (500 μg); Cholesterol (125 μg)
MPL (50 μg); QS-21 (50 μg) in oil in water emulsion
MPL (25 μg); QS-21 (25 μg) in oil in water emulsion

^{1.} DOPC: Dioleoyl phosphatidylcholine

In addition, stand-alone safety pharmacology studies were conducted with RTS, S/AS01 $_{\rm B}$, AS01 $_{\rm B}$ or MPL alone.

3.3.2. Pharmacology

Primary pharmacodynamics studies

An overview of the RTS,S/AS01 nonclinical pharmacology studies is provided in Table 4, all conducted under non-GLP. The vaccine schedule was 3 intramuscular (i.m.) injections on days 0, 14 and 28, except GSK041 study in monkeys and two mouse studies (LIMS 20110165, LIMS 20130320) designed with immunobridging objectives.

An overview of nonclinical pharmacological studies for ASO1 adjuvant system's MOA, as well as for QS-21 and MPL components is provided in Table 5.

Table 4. An overview of the RTS, S/AS01 nonclinical pharmacology testing program

Study number	Study title	Immunization schedule	Comments
GSK041	Immunogenicity of RTS,S antigen when formulated in several adjuvant systems (AS01 _B , AS02 _A , AS15) in Rhesus macaques	Weeks 0, 4, 12	Characterizing immunogenicity profile of
LIMS 20100112	Evaluation of dose-response relationship for RTS,S antigen formulated with either AS01 $_{\rm E}$ or AS02 $_{\rm D}$ in CB6F1 mouse model	Days 0, 14, 28	RTS,S/AS01 vaccine
LIMS 20100258- 20100259	Justification of the need for the AS01 _E to induce optimal RTS,S antigen-specific immune responses in CB6F1 mouse model	Days 0, 14, 28	
LIMS 20100550	Immunogenicity study in CB6F1 mice comparing one RTS,S commercial lot containing a small percentage of yeast host cell protein (HCP) to one RTS,S phase 3 pilot lot not containing yeast HCP	Days 0, 14, 28	Bridging manufacturing changes
LIMS 20110165	Nonclinical immunogenicity bridging study of RTS,S/AS01 _E phase 3 efficacy lots vs. phase 3 consistency lots in BALB/c mice	Days 0, 14*	
LIMS 20130320	Nonclinical immunogenicity bridging study of RTS,S/AS01 _E phase 3 consistency lots vs. commercial consistency lots in BALB/c mice	Days 0, 14*	

^{*} the study design incl. 2-doses immunization schedule and subcutaneous route and use of BALB/c mouse strain was aligned with in vivo potency assay used for the release and the stability follow-up of RTS,S/AS01E candidate vaccine lots.

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Table 5. Nonclinical pharmacology studies characterizing the adjuvant's Mode of Action

Study number	Study title	Test system	
LIMS20110060 and 20110061	Contribution of MPL and QS-21 in AS01 effect on antibody and T cell response	mouse	For AS01
LIMS20110202 and 20080761	Contribution of MPL and QS-21 in AS01 effect on local innate response	mouse	adjuvant system
LIMS20110310	Local distribution of AS01B at injection site administered alone and combined with gE ¹ antigen	mouse	
LIMS20110226	Characterization of local innate response induced by AS01	mouse	
LIMS20090807 and 20100654	Impact of spatio-temporal injection of AS01B and gE on innate and adaptive responses in mice	mouse	
LIMS20120490 and 20120517	Contribution of MPL and QS-21 in AS01 effect on antigen presentation by activated APC ²	Ex vivo assay ³	
LIMS20080769, 20080771, 20090756	Role of IFN-gamma signalling in AS01 adjuvant effect	mouse	
VR2013QS21-1	In vitro characterization of QS-21 ability to activate human immune cells	In vitro	For QS21
VR2013QS21-2	In vitro evaluation of molecular pathways of QS-21 interaction with immune cells	In vitro	component
VR2013QS21-3	Key role of endocytosis in the immune-stimulatory properties of QS-21	In vitro	
VR2013MPL01	In vivo deficiency in TLR4 abrogates innate and adaptive response induced by MPL	mouse	For MPL
VR2013MPL02	In vitro comparison of MPL and LPS ability to induce pro- inflammatory cytokines and trigger TLR4 downstream pathways	In vitro	component

¹gE, zoster recombinant antigen; 2APC, antigen-presenting cell; 3Ex vivo assay, an in vitro assay using primary cells collected in mice

Nonclinical PD studies assessing immunogenicity profile of the RTS,S/AS01 vaccine

In study GSK041, groups of rhesus macaques (3-9 years-old, 4 males and 4 females per group) were i.m. immunized on Weeks 0, 4, 12, with 50 μ g RTS,S formulated in either PBS, AS01_B (50 μ g QS-21QS21, 50 μ g liposomes MPL), AS02_A (50 μ g QS-21QS21, 50 μ g MPL, 250 μ l SB62 [oil (squalene, a-Tocopherol)-in-water emulsion]), or AS15 (50 μ g QS-21, 50 μ g liposomes MPL, 420 μ g CpG) in 0.5 mL volume. Blood and serum was collected at pre-treatment and Weeks 6, 12, 14, 24, and 34 after the first dose. Vaccine-specific T cell and antibody responses to the circumsporozoite protein (CSP) and hepatitis B surface antigen (HBs) were evaluated by intracellular cytokine staining (ICS) and by ELISA assays, respectively.

In this study, RTS,S/PBS induced very low to undetectable anti-CSP and anti-HBs-specific IgG response and no CSP- or HBs-specific T cell responses. Two weeks post third immunization, CSP-specific CD4+ T cells expressing 2 or 3 cytokines simultaneously (among IL-2, TNF-alpha, IFN-gamma) were detected in more animals in RTS,S/AS01_B group (6/8 responders) than in RTS,S/AS02_A and RTS,S/AS15 groups (2/8 and 1/8 responders, respectively). Similarly, HBs-specific CD4+ T cells could be detected in 6/8 monkeys of RTS,S/AS01_B group, 3/8 monkeys of RTS,S/AS02_A group, and 6/8 monkeys of RTS,S/AS15 group.

When looking at the geometric mean frequencies \pm 95% CI of CSP- and HBs-specific CD4+ T cells expressing at least two cytokines, higher frequencies were observed in RTS,S/AS01_B group than RTS,S/AS02_A and RTS,S/AS15 groups, although these differences were not statistically significant. Of note, no vaccine-specific CD8+ T cell responses were detected in any group.

The kinetics of CSP- and HBs-specific CD4+ T cell and IgG responses was investigated up to Week 34 after the first dose (22 weeks after the third dose). Cytokine-expressing CD4+ T cells were detected after second dose and increased in most animals after the third dose, which were still detectable for some animals 12 weeks and 22 weeks after the third dose. All groups vaccinated with adjuvanted

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formulations induced similar levels of anti-HBs- and anti-CSP-specific IgG responses that were high after the second dose and remained detectable up to 20 weeks after the third injection.

The applicant concluded that the $ASO1_B$ Adjuvant System tend to improve the magnitude of the effector T cell responses to the CSP and HBsAg portions of the RTS,S antigen comparing to $ASO2_A$ and AS15.

Three additional studies in mouse model were conducted to further assess the need for adjuvantation of the RTS,S antigen using $ASO1_E$ adjuvant system, two of them, LIMS20100258 and 20100259, were reported together as they had the same design and the report showed pooled data. Study LIMS20100112 was also designed to explore a dose range of RTS,S antigens, while studies LIMS20100258 and 20100259 were to assess the relative contribution of QS-21 and MPL components in $ASO1_E$.

In LIMS20100112, groups of CB6F1 mice (6-8-weeks-old, female, 30/group) received 3 i.m. injections on days 0, 14, and 28, with either 5 μ g, 2.5 μ g or 1.25 μ g of RTS,S antigen formulated with a fixed amount of either AS01_E or AS02_D adjuvant system in 50 μ l volume (i.e. $1/10^{th}$ of AS final container clinical dose). The dose of 2.5 μ g RTS,S antigen corresponded to $1/10^{th}$ of a human dose. Blood sampling was 7 days after the second and third doses and serum collection was 14 days after the second and third doses for evaluation of CSP- and HBs-specific T cell responses by ICS and CSP- and HBs-specific IgG responses, respectively.

For all RTS,S doses tested, RTS,S/AS01 $_{\rm E}$ vaccine formulation elicited higher levels of CSP and HBs-specific CD4+ T cell responses than RTS,S/AS02 $_{\rm D}$. No statistical differences were observed between doses for both adjuvant systems, however, a trend for higher CSP CD4+ T cell responses was observed with lower dose of RTS,S/AS01 $_{\rm E}$. This was not observed for the HBs CD4+ T cell responses. The vaccine-induced CD8+ T cell responses were mainly specific for HBs and such responses were statistically higher in RTS,S/AS01 $_{\rm E}$ group, compared to RTS,S/AS02 $_{\rm D}$ group.

The cytokine profile of antigen-specific T cell responses induced by RTS,S /AS01 $_{\rm E}$ was mainly IFN $_{\rm Y}$, IFN $_{\rm Y}$ +TNF $_{\rm A}$ and IFN $_{\rm Y}$ +TNF $_{\rm A}$ + IL-2 for CSP- and HBs-specific CD4+ T cells, and IFN $_{\rm Y}$ and IFN $_{\rm Y}$ +TNF $_{\rm A}$ for HBs-specific CD8+ T cells. Whereas RTS,S/AS02 $_{\rm D}$ tended to induce more CD4+ T cells expressing IL-2 alone, TNF- $_{\rm A}$ alone, IL-2+ TNF $_{\rm A}$ and less CD4+ T cells expressing IFN $_{\rm Y}$ +TNF $_{\rm A}$. This suggests that CSP-specific CD4+ T cells induced by RTS,S/AS01 $_{\rm E}$ may be more polyfunctional than the ones induced by RTS,S/AS02 $_{\rm D}$, but their biological significance remains unknown.

Both RTS,S/AS01 $_{\rm E}$ and RTS,S/AS02 $_{\rm D}$ vaccine formulations, at 14 days after 3rd immunization induced similar CSP- and HBs-specific total IgG responses, regardless of the RTS,S dose tested. The applicant concluded that there was no statistically significant dose response relationship for CSP- and HBs-specific CD4+ T cell responses, and a dose response relationship of statistical significance was observed for HBs-specific CD8+ T cell response, but restricted to 2.5 μ g and 1.25 μ g dose groups for RTS,S/AS01 $_{\rm E}$ formulation, and to 5 μ g and 2.5 μ g dose groups for RTS,S/AS02 $_{\rm D}$ formulation. Overall, RTS,S/AS01 $_{\rm E}$, is more immunogenic than RTS,S/AS02 $_{\rm D}$.

To examine synergistic effect of MPL and QS-21 and individual contribution of MPL and QS21 to enhancing RTS,S antigen-specific immunity, in LIMS20100258 and 20100259 studies using the same design, groups of CB6F1 mice (6-8-weeks-old, female, 30/group) received 3 i.m. injections (on Days 0, 14, 28) of either 2.5 μ g RTS,S + 50 μ l AS01_E adjuvant system (i.e. $1/10^{th}$ phase 3 human dose), 2.5 μ g RTS,S + 50 μ l liposomes QS21, 2.5 μ g RTS,S + 50 μ l liposomes MPL, or 2.5 μ g RTS,S + 50 μ l AS01_E buffer. Splenocytes were collected 15 days after the third dose for evaluation of CSP- and HBs-specific T cell responses by ICS. Serum was collected 14 days after second and third doses for

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evaluation of CSP- and HBs-specific IgG response. Results of two studies were combined and data from similar groups pooled.

The RTS,S+AS01 $_{\rm E}$ and RTS,S+liposomes QS-21 groups were the only groups inducing specific T cell responses, with RTS,S+AS01 $_{\rm E}$ inducing significantly more CSP-specific CD4+ T cells (p=0.03) and HBs-specific CD4+ T cells (p=0.05) than RTS,S+liposomes QS-21. Again, CSP-specific CD8+ T cells were low to undetectable in all groups, while HBs-specific CD8+ T cell responses were detected in RTS,S+AS01 $_{\rm E}$ and RTS,S+liposomes QS-21 groups.

All formulations tested induced CSP- and HBs-specific antibody responses 14 days after the second and the third immunization. However, RTS,S+AS01 $_{\rm E}$ induced the highest levels of both anti-CSP and anti-HBs total IgG.

The applicant concluded that two immunostimulants MPL and QS-21 in AS01_E adjuvant system are needed to induce strong CSP- and HBs-specific T cell and antibody responses.

Nonclinical PD studies supporting manufacturing development

Since after scale-up of RTS,S production process from pilot to commercial scale, a small amount of yeast cytosolic catalase (approx.. 1%) was identified in commercial scale lots, that was not observed in pilot scale lots, study LIMS 20100550 was initiated to evaluate the impact of the presence of yeast cytosolic catalase on immunogenicity of the RTS,S/AS01_E vaccine, and to assess the potential induction of cross-reactive responses against human catalase.

Groups of CB6F1 mice (6-8-old, female, 40/group) were immunized i.m. on days 0, 14, 28, with 2.5 μ g, 12.5 μ g or 25 μ g of either RTS,S lots: (1) ARTSAPA017 (commercial scale), (2) DRTSAVA004 (pilot scale), formulated with 50 μ l AS01_E (1/10th of AS01_E final container clinical dose). Control groups (20/group) received either 25 ng or 5 μ g human catalase (obtained commercially) in 50 μ l AS01_E. Blood was collected 7 days after the second and third immunization for evaluation of antigen specific T cell responses by ICS. Serum was collected 14 days after the second and the third immunization for the evaluation of antigen-specific IgG responses.

The conclusions of the experiment were as follows:

- Low human catalase-specific CD4+ T cell responses were detected in 3/8 pools of mice immunized with commercial scale lot at post-dose 3.
- no human catalase-specific IgE antibodies were detected in mice vaccinated with either of the two RTS,S/AS01_E lots: ARTSAPA017/AS01_E and DRTSAVA004/AS01_E
- Anti-human catalase antibody responses were detected in 65 out of 120 mice at post-dose 3 immunized with the commercial scale lot. However, this response was very low as compared to the response induced by the stand-alone injection of an equivalent quantity of human catalase.
- Depending on the dose, commercial scale lot induced lower anti-CS- and anti-HBs specific CD4+ T cell and antibody responses post dose 3 than did pilot scale lot, however, the differences were within the range of inter-experimental variability, suggesting that, overall, the presence of a small amount of catalase did not impact the immunogenicity of RTS,S vaccine candidate.
- The significance of presence of yeast cytosolic catalase in commercial scale lots was also investigated in human in Malaria-061.

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Further two experiments (LIMS 20110165A, LIMS 20130320) were conducted in mouse model to support key manufacturing changes. These experiments were part of the extensive comparability exercise, using a design aligning with the *in vivo* potency assay, and with objectives either to demonstrate the bioequivalence between 3 phase III efficacy Final Container (FC) lots and 3 phase III consistency FC lots of RTS,S antigen regardless of the associated AS01_E adjuvant FC lot (LIMS 20110165A), or to demonstrate the bioequivalence between phase III consistency and commercial consistency lots of RTS,S antigen regardless of the associated AS01_E FC lot (LIMS 20130320). The criterion used to consider bioequivalence between two series of lots was defined as a 95% confidence interval (CI) of the geometric mean antibody titers (GMT) ratio within the range of 0.5 - 2 for both anti-CS and anti-HBs IgG responses measured 21 days after the second immunization.

Results of LIMS 20110165A demonstrated that bioequivalence criteria were met for both anti-CS and anti-HBs antibody responses, when comparing RTS,S phase III efficacy vs. RTS,S consistency lots or, AS01_E Phase III efficacy vs. AS01_E consistency lots. Results of LIMS 20130320 demonstrated that bioequivalence criteria were met for both anti-CS and anti-HBs antibody responses, when comparing RTS,S Phase III consistency vs. RTS,S commercial consistency lots, AS01_E Phase III consistency vs. AS01_E commercial consistency lots, or paired Phase III consistency RTS,S/AS01_E lots vs. paired commercial consistency lots.

Nonclinical PD studies assessing the Mode of Action of the ASO1 adjuvant system, QS-21 and MPL

The underlying mechanism for ASO1 adjuvant system to control antigen-specific adaptive responses was extensively investigated *in vitro* and in mice. Most of these experiments were conducted with the adjuvant alone, and in some instances, model antigens like gE, OVA or HBs were used to perform mechanistic studies.

Collectively, the main characteristics of ASO1 Mode of Action included:

- 1) synergistic effects of MPL and QS-21 in inducing higher number of more functional antigen-specific T cells and antibody response to model antigens (OVA, gE);
- 2) direct impact of MPL (via TLR4) and QS-21 (via NLRP3 inflammasome-including pathway) on dendritic cells and monocytes, respectively;
- 3) ASO1 and the antigen need to be co-localized (time and site) for exerting adjuvant effect;
- 4) MPL and QS-21 combination induces an early and transient cytokine and innate cell recruitment;
- 5) MPL and QS-21 combination induces a transient increase in activated DC number and favors a diversified population of activated APC responsible for T cell priming
- 6) MPL and QS-21 synergise at the innate level leading to IFN γ -mediated modulation of adaptive response

Each step of AS01 mode of action is further detailed below.

1) MPL and QS-21 synergize for the induction of antigen-specific adaptive response

Similar to what was observed in LIMS20100258 and 20100259 studies using the RTS,S antigen discussed above, studies LIMS 20110060-61 using Herpes Zoster antigen gE as a model antigen, a synergistic response to gE was observed for MPL and QS-21 combination. Similar finding was also observed for model antigens OVA and HBs (LIMS 20080769-771 and 20090756, see below #6).

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Therefore, the demonstrated synergistic effect is a general feature of ASO1 Adjuvant System mode of action observed when ASO1 is formulated with different antigens.

2) MPL and QS-21 activate the innate immune system via specific signaling pathways

In vitro studies on human monocytes and dendritic cells have identified MPL as a ligand for Toll-like receptor 4 (TLR4). This was further confirmed in mice in VR2013MPL-01 where MPL specifically interacts with TLR4 to trigger pro-inflammatory cytokines (IL-6, MCP-1) and mount specific IgG response to HBs antigen, whereas both innate and adaptive (humoral) response are abrogated in TLR4-deficient animals.

MPL signaling in human cells does not preferentially induce the IRF-3/TRIF pathways, but triggers a balanced MyD88/TRIF signal. Nonetheless, the absence of type I interferon response in MPL-stimulated primary human PBMC cells indicates that the IRF-3 pathway is not efficiently triggered and that IFN α is unlikely to be induced in humans upon immunization with MPL alone.

Study VR2013QS-21-01 showed that QS-21 induces the production of pro-inflammatory cytokines (IL-6, IL-1beta, TNF-alpha) in monocytes- but not in lymphocytes, and induces cytokine secretion and maturation of dendritic cells.

VR2013QS-21-03 study showed that entry of QS-21 into APCs is via endocytosis and cholesterol plays a major role in the endocytosis of QS-21 and thus QS-21 immune stimulatory property. In study VR2013QS-21-02, QS-21 was found to trigger the NLRP3 inflammasome pathway, leading to caspase-1 activation and to the cleavage of pro-IL-1 β and secretion of mature IL-1 β , in line with the reported observation that QuilA- a complex mixture of saponin fractions- activates the NLRP3 inflammasome *in vitro*. While QS-21 shares the property of activating the NLRP3 inflammasome with Alum, it is believed that QS-21 signals via an additional mechanism.

3) The spatio-temporal co-localization of ASO1 and the antigen is necessary for adjuvant effect

In LIMS20090807 -20100654 studies to investigate AS01 adjuvant properties, it was found that peak production of proinflammatory cytokines occurred after 1 hour for TNF-a and after 6 hours for IFN γ , MCP-1 and IL-6, and all cytokines returned to baseline levels 72 hours after AS01 $_B$ injection.

The maximal antigen-specific CD4+ T cell and IgG responses were obtained when gE antigen was injected at the same injection site no longer than 24 h after AS01_B administration.

4) MPL and QS-21 combination induces an early and transient innate response

Shortly after injection, AS01 induced a rapid production of cytokines both in the injected muscle and in the draining lymph node (dLN), as shortly as 3 hours after immunization (LIMS 20110226). Likewise, AS01-induced inflammatory response was transient, peaking around Day 1-2 and returning to baseline by Day 7.

AS01 induced pro-inflammatory cytokines, such as IL-6, IFN γ , IFN γ -related chemokines including IP-10 and MIG, that are key in activating APCs and T cell response. In the muscle, the early peak of KC, a neutrophil-chemoattractant, correlated with rapid neutrophil recruitment (i.e. within 6 to 16 hours), while the peak of MCP-1, a specific monocyte-chemoattractant, was associated with later monocyte recruitment (i.e. within 24 to 48 hours).

While MPL and QS-21 administered individually also induced the local production of cytokines, the level of some of them was increased when MPL and QS-21 were injected concomitantly, in the AS01 formulation (LIMS 20080761-20110202). This was the case for IL-6, MCP-1, G-CSF, and IFN γ -related

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chemokines. However, for IFN γ , the response was not observed in the absence of either MPL or QS-21, indicative of a synergy between the two components (LIMS 20080769-20080771 -20090756).

MPL contributed to the early cytokine response in the muscle, while QS-21 was more important for the early cytokine response in dLN, consistent with the rapid distribution of QS-21 to dLN (see local distribution study below).

Enhanced cytokine level with AS01 did not translate into a clear synergy for cell recruitment, except for an observed sustained increase in monocytes and neutrophils number at Day 2 in muscle and dLN, respectively. In contrast, some responses induced by MPL and QS-21 were reduced in AS01, such as DC recruitment in the dLN as compared to MPL alone. However, this interference between MPL and QS-21 does not translate into lower immunogenicity as combining MPL and QS-21 into AS01 enhances both antigen-specific antibody and cellular response (Dendouga N, 2012 and LIMS 20090765, 20110060 and 20110061).

5) MPL and QS-21 combination favors a diversified population of activated APC responsible for T cell priming

Using fluorescently-labelled antigens LIMS 20110226 and 20110202 showed that monocytes and neutrophils were the main cells carrying the antigen in the dLN, followed by DCs. In dLN, AS01 not only enhanced the number of DCs and monocytes, but also significantly enhanced the levels of CD86 and CD40 that are key co-stimulatory molecules required for T cell priming. This effect is transient and returns to baseline by Day 7.

Using an ex vivo antigen presentation assay with OVA as a model antigen, LIMS 20120490-20120517 demonstrated that the activated DCs, isolated from the dLN of mice immunized with AS01+OVA were the cells responsible for the efficient priming of cognate CD4 T cells.

The profile of activated APC in dLN is broad: activated DCs comprise 1) sentinel DCs initially present in the muscle and have migrated to dLN upon activation, 2) cells derived blood monocytes and 3) DCs resident in dLN that are directly activated by ASO1 after drainage to dLN. The monocyte-derived DCs (MoDCs) are likely to play an instrumental role in ASO1 adjuvant effect as they have been shown to contribute to the induction of antigen-specific IFN γ -producing T cells.

The large and broad population of activated APCs generated upon AS01 administration ultimately correlates with more efficient antigen presentation to T cells. The combination of MPL and QS-21 seems to be the main factor contributing to this diverse APC repertoire: MPL efficiently recruits activated migratory DCs while QS-21 seems to target monocytes with limited impact on bona fide DCs.

6) IFNγ plays a key role in ASO1 Mode of Action

The significant impact on APC number and activation and production of IFN γ and IFN γ -related cytokines (i.e. CXCL10, CCL9) are the hallmark of the AS01 innate signature. In LIMS 20080769-771 and 20090756 studies using IFN γ -R KO mouse model, reduced polyfunctional CD4+ and CD8+ T cell responses to HBs and to OVA model antigens were observed upon administration of AS01_B-adjuvanted antigens, compared to wild type mice receiving the same formulations. A reduction in local innate responses (reduced cytokine production and activation of APC) was also observed. These data pointed to an unappreciated role of IFN γ in the mechanism of action of the AS01 Adjuvant System, i.e. in stimulating a broader early innate response and its role in APC-T cell interaction. In contrast, no evidence for a role of IFN γ on the induction of antigen-specific-IgG was observed. However, because only total IgG concentrations were measured, it cannot be excluded that IFN γ may have a role in antigen-specific IgG avidity or IgG switching.

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Secondary pharmacodynamics

No secondary pharmacodynamic studies were performed, in accordance with the *Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines* (CPMP/465/95) and *Guideline on Adjuvants in Vaccines for Human Use* (EMEA/CHMP/VEG/134716/2004).

Safety pharmacology

Three dedicated safety pharmacology studies were performed with RTS,S/AS01_B, AS01_B and with MPL (Table 6) all conducted under GLP.

Tested Material	Species or substrate	Route of administration	GLP	Study name
RTS,S/AS01 _B	anesthetized Rat	Im	YES	HLS BVR
RTS,S	anesthetized Rat	Iv		041/013677
AS01 _B	Dog	Im	YES	MDS AA81874
MPL	anaesthetized Dog	Iv	YES	Cov 1729/22

Table 6. Safety Pharmacology program

AS01_BThe objective of this study was to examine the effects of the AS01_B adjuvant system on arterial blood pressure, heart rate, electrocardiogram, body temperature and respiratory parameters following a single intramuscular (I.M.) administration in the conscious beagle dog.

Approximately 3 weeks before initiation of treatment, 4 naïve male beagle dogs (6-10 months-old, 8-9 kg) were surgically implanted with telemetry devices under aseptic conductions, to allow chronic measurement and recording of cardiovascular and respiratory parameters in conscious, freely moving dogs.

Each animal was treated first with saline (0.5 mL/animal) on Day 0 and then with ASO1 $_{\rm B}$ (Batch number: DA01A005A, 0.5mL/animal) on Day 7. Each animal served as its own control with a wash-out period of 7 days in between treatment. Cage-side observations were performed before treatment, immediately after treatment and at least once after dosing. Animals were observed daily, and individual body weights were taken on days -1, 6 and 14. Body temperature, haemodynamic, cardiac and respiratory parameters were recorded in all animals on days 0 and 7, and for 7 days following the ASO1 $_{\rm B}$ treatment.

Results

 ${\rm ASO1_B}$ administered intramuscularly, did not affect the health status and the body weight gain of the animals throughout the study period, and did not relevantly affect the arterial blood pressure, the heart rate and the duration of the RR and PR intervals, of the QRS complex and of the QT and QTc intervals, irrespective of the formula used for QT interval correction, during the 72-hour period following administration. These findings suggest that ${\rm ASO1_B}$ Adjuvant was devoid of any potentially deleterious effect on the atrio ventricular and intra ventricular conduction velocity, and on ventricular repolarization. ${\rm ASO1_B}$ did not induce any disturbances in rhythm or waveform morphology of the ECG during the first 6-hour post-treatment period.

 $ASO1_B$ Adjuvant administered intramuscularly, did not relevantly affect the respiratory rate, the inspiratory and expiratory times, AUC_{ITP} (index of tidal volume) and AUC_{ITP} X Respiratory rate (index of minute volume).

AS01_B Adjuvant, administered intramuscularly, induced a slight increase in body temperature 6 hours after treatment, compared with control (saline).

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Conclusion

 $ASO1_B$ Adjuvant administered intramuscularly, did not affect the health status and the body weight gain of the animals throughout the study period, and did not affect the cardiovascular function and the respiratory function.

MPL

The objective was to determine the cardiovascular and respiratory effects of MPL in the anaesthetised dog following intravenous administration.

Two male and two female adult Beagle dogs (11-13 months, 9.3-11.8 kg) were anaesthetised and received MPL at 1, 10 and 100 μ g/kg body weight intravenously. Control animals received the same dosing regimen. The following haemodynamic and respiratory parameters were measured: systolic, diastolic and mean arterial blood pressure; heart rate, left ventricular pressure and its derivative, mean femoral blood flow, RR, QRS, PR, QT and QT_c-intervals, and heights of the R, P and T-waves of ECG complex, peak inspiratory and expiratory flow, tidal volume, minute volume and rate of respiration.

Results

There was little apparent effect of treatment on cardiovascular paramaters at any dose level of MPL. MPL treated group showed a small and gradual increase in mean heart rate following administration of the highest dose (100 μ g/kg) but this effect was not statistically significant.

There was a dose-dependent decrease in the mean height of the T wave of the ECG complex at 2 minutes post-dose in 2 of 4 animals, however, there was no significant difference between two groups at any time-point.

There was also a small but statistically significant increase in mean respiratory rate (from a baseline mean of 15 brpm to 18 brpm) 10 minutes after administration of the highest dose of MPL. However, this increase was not great enough to be considered as physiologically relevant.

Conclusion

The intravenous administration of escalating MPL doses up to 100 μ g/kg body weight was devoid of major effects on the cardiovascular and respiratory function of anaesthetized Beagle dogs.

RTS,S/AS01_B

The purpose of this study was to evaluate the possible side effects on the cardiovascular and respiratory systems of the RTS,S antigen, the RTS,S/AS01_B or RTS,S/AS02_V (adjuvant containing 50 g MPL, 50 μ g QS-21, holesterol and 50 μ l of o/w emulsion in a total volume of 500 μ l) formulations in anaesthetised male Wistar rats.

Groups of animals (9-11 weeks-old, 4/group) received one of the following treatments: i.v. 1 mL/kg PBS on 4 occasions; i.v. 1 mL/kg PBS then RTS,S antigen at 3 dose levels on 3 subsequent occasions; i.m. RTS,S/ASO1_B vaccine on one occasion, and i.m. RTS,S/ASO2_V vaccine on one occasion. RTS,S/ASO1_B and RTS,S/ASO2_V was given at 0.1 ml/rat, approximately 63-fold the human dose on an adult bodyweight basis. RTS,S antigen was examined at dose levels of 5, 15 and 45 μ g/kg, corresponding to approx. 7, 21 and 63 times the human dose on an adult bodyweight basis. Blood pressure, heart rate, ECG (lead II), and respiration were recorded continuously for at least 30 minutes after each i.v. administrationand for at least 120 minutes after each i.m. administration.

Results and Conclusion

 $RTS_s/ASO1_B$ produced no overt, dose-related or consistent effects on blood pressure (systolic, diastolic, mean), heart rate, respiration depth or respiration rate in the anaesthetised rat. No

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abnormalities were observed in the ECG waveform (lead II). The RTS,S antigen administered intravenously at doses of 5 to 45 μ g/kg body weight did not induce marked or consistent dose-related changes in cardiovascular or respiratory parameters.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were performed according to the *Note for Guidance on Preclinical Pharmacological and Toxicological testing of vaccines* (CPMP/SWP/465/95) and *Guideline on Adjuvants in Vaccines for Human Use* (EMEA/CHMP/VEG/134716/2004).

3.3.3. Pharmacokinetics

Biodistribution studies were conducted with MPL, QS-21 and ASO1 $_{\rm B}$ (Table 7). Of note, the GSK-CH-01-09 study used a test article that was formulated in an ASO1 $_{\rm B}$ -like formulation and was thus most relevant to support RTS,S/ASO1 vaccine formulation, whereas other studies were carried out in an independent context:

- The MPL studies (COV 1990/521 and COV 1990/522) were performed in 2005 as a commitment to EMA in the context of Fendrix MAA. In these studies, the test article ¹⁴C-MPL was presented in an aqueous solution and therefore differs from the ASO1 Adjuvant System as used in RTS,S/ASO1_F. The conclusions therefore strictly apply to MPL as a compound.
- The QS-21 study (TSI MASON 2-R89) was carried out in rabbits and the test article was formulated in phosphate buffered saline (PBS). The conclusions of this study therefore solely apply to QS-21 as a compound.

Table 7. Parameters evaluated in the biodistribution studies conducted with AS01, QS-21 and MPL

Study title	Study number	Test System	Antigen	Test Article	Route of Administration	Parameters studied
Comparison of the in vivo fate in mice of ¹⁴ C-DOPC and ¹⁴ C-QS-21 formulated in ASO1 _B given intramuscularly	GSK-CH-01-09	Mouse	None	¹⁴ C-DOPC ¹⁴ C-QS-21	Intramuscular	Absorption, distribution, excretion
Pilot distribution study of ³ H-labelled QS-21 following intramuscular administration to New Zealand White Rabbits	TSI MASON 2- R89	Rabbit	None	³ H-QS-21	Intramuscular	Absorption, distribution, metabolism, excretion
[14C] -MPL: Pharmacokinetics, distribution and excretion of radioactivity following intramuscular administration to rat	COV 1990/521	Rat	None	¹⁴ C-MPL	Intramuscular	Absorption, distribution, excretion
[14C]-MPL: Pharmacokinetics, distribution and excretion of radioactivity following intravenous administration to rat	COV 1990/522	Rat	None	¹⁴ C-MPL	Intravenous	Absorption, distribution, excretion

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Methods of analysis

GSK-CH-01-09

The study was performed to evaluate differential absorption, distribution and urinary elimination of DOPC and QS-21 formulated in $ASO1_B$, in C57BL/6J female mice (10 weeks old).

The study comprised two phases: the first phase consisted of injecting [14 C]-DOPC formulated with unlabelled QS-21 (and MPL and cholesterol) and the second phase was performed with [14 C]-QS-21 formulated with unlabelled DOPC (and MPL and cholesterol). For both phases, a total dose of 10 μ g of QS-21 mouse and 200 μ g of DOPC/mouse was administrated in muscles (100 μ l/mouse, split 50% into right and left gastrocnemius muscles, corresponding to $1/5^{th}$ human dose (HD) of ASO1_B and $2/5^{th}$ HD of ASO1_E). The tissue distribution and urinary excretion was assessed over 72 hours. Specific tissues (blood, liver, spleen, heart, lungs, kidneys, brain, thymus, gastrocnemius muscles and lymph nodes [iliac, inguinal, brachial, mesenteric]) and urine were analyzed.

TSI MASON 2-R89

The study consisted of two groups of one New Zealand White rabbit/sex/group for a total of four animals. The test article, i.e. QS-21 labelled with [3 H] in PBS (1 ml corresponds to 100 μ g or 50 μ Ci of [3 H]-QS-21), was administered via a single intramuscular injection of 1 mL to each animal. Following collection of the final blood sample, animals were perfused and euthanized and appropriate tissues collected.

The total amount of [³H] activity administered with each dose was calculated. Tissue and feces isotope activity was calculated on a per gram or milligram basis and on a total tissue basis for all samples collected. Total tissue activity is also expressed as the percent of the total dose administered for each tissue/organ collected and analyzed. Plasma, urine and feces samples were analyzed to determine the total activity of the isotope.

COV 1990/521 and COV 1990/522

Following both intramuscular and intravenous administration to rats, samples of blood, faeces, urine, expired air trapping solutions, cage debris, cage wash and a range of tissues, including carcass, were collected up to 56 days post-dose, and analyzed for [¹⁴C] content by Accelerator Mass Spectrometry (AMS). The accelerator mass spectrometer is a sensitive isotope counter. The choice of the trapping solution was based on the use of AMS for analysis, with potassium hydroxide (which has no natural carbon content) being more suitable than other possible reagents.

Absorption

GSK-CH-01-09

For both [¹⁴C]-DOPC and [¹⁴C]-QS-21, levels of radioactivity declined in a biphasic manner from injection sites, with initial rapid decline over the first 3 hours, followed by a much slower rate of decline up to 72 hours. [¹⁴C]-QS-21-related material was more rapidly eliminated from injection site than [¹⁴C]-DOPC-related material, indicating early dissociation of DOPC and QS-21 after i.m. administration.

One hour after the injections, approximately half the injected [¹⁴C]-DOPC dose was cleared from the gastrocnemius muscles since 54 % of injected dose was remaining at the injection sites. For [¹⁴C]-QS-21, 81.5 % of the injected dose was cleared from the injection sites within the first hour.

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Twenty-four hours after the injection, 82 % of [14C]-DOPC and 97 % of [14C]-QS-21 were cleared from the muscles.

TSI MASON 2-R89

In general, the plasma kinetics following I.M. administration of [³H]-QS-21 in the male and female rabbit seemed to follow a multi-phasic profile, with male animal showing very rapid absorption to achieve maximum levels at 5 minutes postdose, compared to females achieving maximum plasma levels at approximately 4 hours postdose. The observed difference in absorption profile appeared to be related to the site of injection and resulted in higher plasma concentrations for the female rabbit as compared to the male rabbit. However, no other pharmacokinetic parameters (the terminal half-life, mean residence time) estimated show any apparent difference between the male and female rabbit, suggesting that the pharmacokinetics of [³H]-QS-21 following absorption is independent of the sex of the animal.

COV 1990/521 and COV 1990/522

Following intramuscular administration, approximately 35% of the dose was absorbed over 56 days post-dose, however, the total absorption is likely to be much higher than this value since only 0.2% of the dose was recovered from the injection site at Day 56.

Absorption after i.m. administration

The highest observed concentration of radioactivity in pooled blood (n=3 animals) occurred at 4 hours post-dose, and was 14.9 ng equivalents of MPL/g. The concentration of radioactivity remained relatively constant up to 168 hours, where it was 11.4 ng equivalents of MPL/g.

Absorption after i.v. administration

The highest observed concentration of radioactivity in pooled blood (n=3 animals) occurred at 5 minutes post-dose, and was 248ng equivalents of MPL/g. The concentration of radioactivity fell, with an elimination half-life of 76.5 hours up to 168 hours post-dose, where the concentration of radioactivity was 15.7 ng equivalents of MPL/g. The area under the curve for the period of sample collection (0 to 168 hours, AUCLast) was 5828 h.ng/mL. The predicted area under the curve extrapolated to infinity concentration (AUC) was 7557 h.ng/mL suggesting that the excretion of the dose was not complete over the period of the analysis.

Distribution

GSK-CH-01-09

The highest radioactive concentrations of [14 C]-DOPC (excluding injection sites) were found in blood [5.4 % of the injected dose (ID)] and iliac lymph nodes (3.3 % ID) 1 hour post-injection, and in the inguinal lymph nodes (0.7 % ID), liver (10.9 % ID) and kidneys (0.7 % ID) 6 hours post-injection. Whereas the highest radioactive concentrations of [14 C]-QS-21 were found in blood (16.0 % ID), liver (14.0 % ID) and kidneys (2.1 % ID) 1 hour post-injection; in the inguinal lymph nodes (0.6 % ID) 3 hours post-injection; and in the iliac lymph nodes between 1 and 6 hours (1.0 % ID) after the injection.

Radioactive concentrations of [¹⁴C]-DOPC and [¹⁴C]-QS-21 in brain, heart, lungs, spleen, thymus, brachial lymph nodes, and mesenteric lymph nodes were very low.

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The lowest accumulation of radioactivity measured in brain seems to show the inability of [14C]-QS-21 to cross the blood-brain barrier.

TSI MASON 2-R89

The liver showed the highest concentration, with recoveries in males increasing from 9.11% at 24 hours to 17.79% at 48 hours. Recoveries in females were similar and increased from 9.88% at 24 hours to 16.02% at 48 hours postdose. Even though the recoveries in iliac LN were less than 1%, the high radioactivity values in iliac LN suggest that these nodes are the draining LN for [³H]-QS-21 from the IM injection site. In male recoveries (counts) in iliac LN increased from 172,000 to 454,770 CPM/g at 24 and 48 hours respectively. Corresponding increases in the female were between 95,604 to 992,749 CPM/g.

The percent of the administered dose recovered in the adrenal glands, brain, oesophagus, gall bladder, heart, lungs, lymph nodes (inguinal, mandibular, mesenteric), pancreas, urinary bladder, stomach, large intestine and fat in the male and female rabbit were very low (<1% of the dose recovered). Similarly, the male and female rabbit did not concentrate [³H]-QS-21 in the reproductive organs, i.e. testes and ovaries. It is important to note that recoveries of less than 1% of the administered dose in tissues are considered insignificant and the changes in recoveries from the 24 to 48 hour period should be considered insignificant. Finally, there was no difference in recoveries in the 24 and 48 hour sampled time-points.

COV 1990/521 and COV 1990/522

Distribution after i.m. administration

Following a single i.m. administration to rats, quantifiable levels of radioactivity were detected in all tissues investigated (fat, lungs, spleen, liver, kidney, adrenals and residual carcass) at 56 days post-dose. The highest mean concentrations were found in the fat and spleen (2.96 and 0.486 ng equivalents of MPL/g tissue, respectively). The liver and kidneys contained means of 0.326 and 0.273 ng equivalents of MPL/g, respectively, with the mean levels of radioactivity in the lungs and adrenals being below 0.2 ng equivalents of MPL/g. The injection site contained a mean concentration of 0.661 ng equivalents of MPL/g.

Distribution after i.v. administration

Quantifiable levels of radioactivity were detected in all tissues investigated at 56 days following a single intravenous administration to rats. As observed after intramuscular administration, the highest mean concentrations were found in the fat and spleen (4.07 and 3.57 ng equivalents of MPL/g tissue, respectively). The liver contained a mean concentration of 1.58 ng equivalents of MPL/g tissue. The lungs, kidneys and adrenals contained 0.3544, 0.350 and 0.336 ng equivalents of MPL/g, respectively.

Metabolism

TSI MASON 2-R89

This study was designed to identify the molecular source of the radioactivity observed in the urine, plasma and tissues. Extraction methods were developed for the plasma, bile, lymph node, urine and liver. Analysis was performed by reverse-phase HPLC with comparison of retention time to known standards.

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The parallel study on the urine, plasma and tissue using HPLC showed that metabolism is not necessary for excretion of QS-21. QS-21H, the hydrolysis product of QS-21, was the major QS-21 metabolite found in the urine samples. The level of QS-21 in the urine decreased with time. Two potential metabolites were observed in one urine sample only (Male; Rabbit #2; 24-48h). Only intact QS-21 was observed in the liver tissue, therefore, the liver carboxylesterases were not responsible for this transformation.

The level of radioactivity in the bile, plasma and in lymph nodes was below the detection limit of the above mentioned HPLC method.

Excretion

GSK-CH-01-09

[14 C]-QS-21 was almost exclusively eliminated via urinary excretion with >99 % of the injected dose recovered in urine by 72 h after dosing. Following intramuscular administration of [14 C]-DOPC, only around 2 % of the injected dose was recovered in urine by 72 hours with about 16 % recovered in the tissues at the end of the collection period. This low recovery could be explained by possible extensive metabolism of DOPC resulting in its excretion as expired CO_2 (not measured) and/or potential reutilization of its metabolites for cholesterol and bile acid synthesis or excretion of compound related material via the faecal route (not measured). Additionally a proportion of the radioactive material may be present in the residual animal carcass which was not assessed for radioactivity during this investigation.

TSI MASON 2-R89

The plasma elimination kinetics of the radioactivity was similar between the male and female rabbit, with terminal elimination of half-lives of 25.06 and 24.44 hours, respectively.

The mean residence time (MRT) that represents the time it takes 63.2% of the administered dose to be eliminated, values ranged from 36.78 hours for the male to 36.79 hours for the female rabbit. However, the total body clearance (35.49 ml/ hr/ kg for the male and 24.56 ml/ hr/ kg for the female) and volume of distribution at steady state (1305.00 ml/ kg for the male and 903.73 ml/ kg for the female) were higher for the male than the female and seemed to be directly related to the higher blood levels observed in the female after pseudo-steady state had been achieved at approximately 4 hours post-dose.

Between 45 to 50% of the administered dose (radioactivity) was excreted in the urine in the male and female rabbit over 48 hours and approximately 1% in the faeces. Since cumulative urinary excretion plots did not reach a plateau at the end of 48 hours, it is hypothesized that significant amounts of radioactivity would still be excreted beyond this time-point, accounting for the remaining radioactivity.

COV 1990/521 and COV 1990/522

Excretion after i.m. administration

At 168 h after a single i.m. administration to rats, faecal and urinary elimination accounted for means of 12.6% and 2.5% of the dose, respectively. Radioactivity continued to be excreted after 168 hours post-dose, as evidenced by radioactivity in excreta, expired air traps and cage wash/debris samples, but to a very limited extent. Extrapolation of the data collected over 8-56 days post-dose resulted in a low mean total recovery of radioactivity from these animals of 35.0% of the dose over the 56 day

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study period: faecal and urinary elimination accounted for mean totals of 23.5% and 3.4% of the dose, respectively, and the mean total in the injection site was 0.2% of the dose.

Excretion after i.v. administration

At 168 hours following a single i.v. administration to rats, faecal and urinary elimination accounted for means of 16.4% and 4% of the dose, respectively. Radioactivity continued to be excreted after 168 hours post-dose as evidenced by radioactivity in excreta, expired air traps and cage wash/debris samples, but to a very limited extent. Extrapolation of the data collected over 8-56 days post-dose resulted in a low mean total recovery of radioactivity from these animals of 40.1% of the dose: faecal and urinary elimination accounted for mean totals of 25.4% and 5.2% of the dose, respectively, and the mean total in the tissues and residual carcass at 56 days was 6.7% of the dose.

3.3.4. Toxicology

Nonclinical toxicology studies were undertaken with RTS,S/ASO1_B, the ASO1_B Adjuvant System and its individual immunoenhancers QS-21 and MPL, in accordance with the CPMP Note for Guidance on preclinical, pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95), the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004) and the WHO Guideline on Nonclinical Evaluation of Vaccines (WHO, 2005).

All studies were conducted according to Good Laboratory Practice (GLP) requirements.

As given in the WHO and EMA guidance, rat and rabbit are considered acceptable species for vaccine nonclinical safety studies and therefore generally chosen for the single dose/local tolerance and repeat dose toxicology studies.

An overview of all toxicological studies performed is shown in Tables 8-11.

Route of GLP Study type Species or substrate Study name administration Repeat-dose Toxicity 4 injections, at 2 week intervals Rabbit lm YES HLS BVR 033 **Local Tolerance** Rabbit lm YES HLS BVR 051 Rabbit YES SLI 3566.4 lm

Table 8.RTS,S/AS01 $_{\rm B}$ Toxicology program

Table 9. AS01_B Toxicology program

Study type	Species or substrate	Route of administration	GLP	Study name
Repeat-dose Toxicity				
7 injections, at 2 weeks intervals	Rats	lm	YES	TNO V 20165
5 injections, at 2 weeks intervals	Rabbit	lm	YES	HLS BVR 045/0022412
4 injections, at 2 weeks intervals	Rabbit	lm	YES	TNO V 20094
Genotoxicity				
Micronucleus assay	Rat	lm	YES	HLS BVR 317/032657
AS01 _B effect on blood cells and bone marrow	Rat	lm	YES	HLS BVR 681/043748
Consolidated report				HLS GVB 0026/070209
Local Tolerance				
	Rabbit	lm	YES	HLS BVR 051
	Rabbit	lm	YES	SLI 3566.4
	Rabbit	lm	YES	TNO V 6212/04

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Table 10. QS-21 (DQ) Toxicology program

Study type	Species or substrate	Route of administration	GLP	Study name
Repeat-dose Toxicity				
6 injections, at 2 occasions/week	Rat	lm	YES	TNO V 20154
6 injections, at 2 occasions/week	Rabbit	lm	YES	TNO V 20155
Genotoxicity				
Reverse induction assay	S. typhimurium, E. coli	In vitro	YES	TNO V 20205/04 TNO V 20205/05
Mouse Lymphoma assay	Mouse lymphoma cell line	In vitro	YES	TNO V 20203/04
Micronucleus assay	Rat	lv	YES	TNO V 20204/04
Local Tolerance				
	Rats	lm	YES	TNO V 20212/01
	Rabbits	lm	YES	TNO V 20212/02

In accordance with the CHMP scientific advice, nonclinical toxicology studies with the QS-21 immunoenhancer were performed using a liposomal formulation of QS-21 (i.e. DQ), rather than solutions of QS-21, since DQ corresponds to the physical form of QS-21 in the RTS,S/AS01_E vaccine formulation. This liposomal formulation has the specific feature of quenching the lytic effects of QS-21.

Table 11. MPL Toxicology program

Study type	Species or substrate	Route of administration	GLP	Study name
Single-dose toxicity				
	Rat	lp	YES	BAX DT127
Repeat-dose Toxicity				
7 injections, daily	Rat	lv	YES	SLS 3262.4
8 injections, daily	Rat	lv	YES	SLS 3262.2
14 injections, daily	Dog	lv	YES	SLS 3262.1
Genotoxicity				
Reverse induction assay	S. typhimurium, E. coli	In vitro	YES	Cov 1729/3
Chromosome aberration assay	Cultured Chinese Hamster Ovary Cells	In vitro	YES	Cov 1729/4
Micronucleus assay	Rat	lm	YES	HLS BVR 730/052198

Single dose toxicity

RTS, S/ASO1_B

The single-dose toxicity of RTS,S/AS01_B was assessed as part of the local tolerance toxicity studies.

Repeat-dose toxicity

 $RTS, S/ASO1_B$

The objective of study HLS BVR 033 was to evaluate potential local and/or systemic reactions after vaccination following four intramuscular injections of two malaria candidate vaccines, RTS,S/AS01 $_{\rm B}$ and RTS,S/AS02 $_{\rm V}$, at two week intervals in the rabbit. The RTS,S/AS01 $_{\rm B}$ formulation contains twice the amounts of RTS,S; MPL; QS-21 and liposomes than the Mosquirix candidate vaccine. Only the data from the relevant RTS,S/AS01 $_{\rm B}$ formulation will be described here.

Two groups of 20 New Zealand White rabbits (10 males + 10 females) were administered 4 intramuscular (IM) injections at 2 weeks interval (Day 0, 14, 28 and 42) of the candidate vaccine. One group of animals received a full human dose (500 μ L) and the other received ¼ of a human dose (125

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 μ L) of RTS,S/AS01_B. A similarly constituted group served as a control and received saline (500 μ L). The IM injections were administered into the gastrocnemius muscle, alternating sides at each dosing. Five males and five females from each group were killed on Day 45 (3 days after the last injection) and the remainder on Day 70 of study (28 days after the last injection) in order to study recovery.

A number of animals, including controls, presented very slight to moderate erythema and very slight oedema at the injection sites shortly after dosing. An unwillingness to use hindlimbs was apparent in some animals shortly after dosing, the incidence of which was generally highest in the group receiving the 500 μ L dose. One male in the saline control group and one male in the 125 μ L RTS,S/AS01_B dose group were found dead during the course of the study. These deaths were attributed to dehydration and digestive problems (occasionally seen in laboratory rabbits) and were not considered to be treatment related.

Food consumption and bodyweight gain were unaffected during thethe study, as was body temperature before and after the 1st and the 4th dose. There were no treatment-related ophthalmoscopic findings. Haematological investigations revealed increases in neutrophil count, with a concomitant increase in total leucocyte count, one day after dose administration in animals treated with 500 µL of the vaccine. After the fourth dose, animals given 125 µL RTS,S/AS01_B were similarly affected. Fibrinogen concentrations were high one day after administration in animals given 500 µL of the vaccine; animals given 125 µL RTS,S/AS01_B were similarly affected one day after the fourth administration. The neutrophil counts and fibrinogen concentrations showed evidence of a return to levels similar to those recorded before treatment commenced within three days after dosing. Transient decreases in the albumin to globulin ratios were mainly noted for animals receiving the 500 µL doses and were attributed to the increased globulin levels. Popliteal lymph node weights were slightly higher than control, 3 days after the final administration in animals receiving the 500 µL vaccine dose. This change in draining lymph node weight was not evident 28 days after the final administration. Histopathology revealed no evidence of systemic toxicity in the 34 tissues examined. Microscopic examination on Day 45 revealed increased incidences of inflammation with fibrosis at the injection sites of animals given either the full or a 1/4 of the full human dose that resolved at recovery. Acinar cell vacuolation and apoptosis in the pancreas was evident in a few treated animals, although this was considered an exacerbation of a background finding and was not seen on Day 70. Slight inflammatory infiltration of the choroid plexus of the brain was seen on study Day 45 in three out of five males given RTS,S/AS01_B at 500 µL per occasion and at study Day 70 in one male out of five males given RTS,S/AS01_B at 125 µL per occasion. The pathology report stated that although this finding is uncommon, this finding was considered unlikely to be a direct effect of treatment and was of no toxicological significance since inflammatory lesions are occasionally seen as spontaneous background changes in laboratory rabbits, were not seen in females given RTS, S/AS01_B and were not seen in males given RTS, S/AS01_B at 500 µL on Day 70 of study. As this finding was nevertheless considered as uncommon, an additional review of the brain histopathology slides was performed by pathologists, to get a better understanding of the nature of these histopathological changes. This additional review confirmed the conclusions of the study report that the presence of slight inflammatory infiltration of the choroid plexus only in three out of five males given 500 µL of RTS,S/AS01_B on Study Day 45 together with the concomitant absence of findings in the five males given 125 µL of RTS,S/AS01_B and in the ten females given RTS,S/AS01_B at 125 µL or 500 µL per occasion, and the presence of this acute inflammation in a single male given 125 μL of RTS,S/AS01_B on Study Day 70 while it was not seen in the five males given 500 μL of RTS,S/AS01_B or in the ten females given RTS,S/AS01_B at 125 µL or 500 µL per occasion supports the interpretation that this finding is unlikely to be a direct effect of treatment and thus of no toxicological significance.

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Serological analysis demonstrated seroconversion in all vaccine treated animals and none in the saline-treated controls, confirming that the rabbit is an immunological responder species for the RTS, $S/ASO1_B$ candidate vaccine formulation.

In conclusion, intramuscular administration of RTS,S/AS01_B on four occasions at 2-week intervals triggered an acute inflammatory response at injection sites that increased with the vaccine dose and volume administered. Nonetheless, full recovery was observed 28 days after the last administration. No systemic toxicity was apparent with RTS,S/AS01_B.

Genotoxicity

RTS,S/ASO1_B

As noted in the WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO, 2005), the EMA Note for Guidance on Preclinical Pharmacological and Toxicological testing of Vaccines (CPMP/SWP/465/95) and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004), genotoxicity studies are not required for final vaccine formulations.

Vaccines are not used in the long term and this reduces risk from consequences of genotoxicity. However, the genotoxic potential of the Adjuvant System and its components should be understood. The applicant conducted genotoxicity studies with these components in compliance with Good Laboratory Practice.

MPL

MPL was tested for mutagenic activity in the bacterial reverse mutation test in *Salmonella typhimurium* strains TA1535, TA 1537, TA 98 and TA 100 and in *Escherichia coli* WP2 uvrA in both the presence and absence of rat liver S9 homogenates mixed with an NADPH generating system. The testing method included testing with and without S9. The methods were in accordance with OECD Test Guideline 471 on Genetic Toxicology: Bacterial Reverse Mutation test, of July 1997. Positive controls were used for the respective 5 strains of bacteria used and confirmed the possibility of detecting increases in number of revertants. MPL was supplied as a suspension in aqueous formulation at 1 mg/ml. MPL was tested over the range 0.0016- 1 mg/ml (in the absence of S9) and at 0.0012-20.750 mg/ml (in thepresence of S9). The top dose was set based on evidence of toxicity at 1 mg/ml in the absence of S9, indicated by a slight thinning of the background bacterial lawn in TA100, TA-98 and TA-1537 strains. The diluent was shown not to have any detectable adverse effects.

MPL did not induce mutations in any of the five tester strains, when tested under the conditions employed in this study. The applicant concluded that MPL was not mutagenic.

MPL was tested in the in vitro chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the presence and absence of rat liver S9 homogenates mixed with an NADPH generating system up to the maximum feasible concentration of 475 μ g/ml. This concentration was judged to induce negligible reductions in cell number in either the absence or presence of S9. Exposure of cells to MPL was for 3 hours and for 20 hours in different experiments. Chromosomal aberrations were scored. Positive controls were used (4-nitroquinoline 1-oxide in the absence of S9 and cyclophosphamide with S9) and confirmed the possibility of detecting increases in the proportion of cells with structural aberrations.

MPL did not increase the proportion of cells with structural aberrations. The applicant concluded that MPL did not induce chromosomal aberrations in this experiment.

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The in vivo genotoxic potential of MPL was tested by assessing the potential induction of micronuclei in bone marrow cells of Sprague Dawley CD rats. Male and female rats were given two intramuscular doses of MPL (1.05 mg/ml) or saline, as 2 intramuscular administrations of 0.1 ml per occasion (ie ~200 µL/rat per day), 24 hours apart and were killed 24 hours later. Bone marrow smears were prepared to allow examination of the presence of micronuclei in 2000 immature erythrocytes from each animal. The proportion of immature erythrocytes was examined in 1000 erythrocytes from each animal. As a positive control, further rats were given cyclophosphamide orally at 10 mL/kg: this induced an increase in the number of micronucleated immature erythrocytes and confirmed the possibility of detecting genotoxicity. Initial testing in 2 male and 2 female rats indicated that the intended dose was clinically tolerable.

MPL did not result in any increases in micronucleated immature erythrocytes and did not decrease the proportion of immature erythrocytes.

QS-21

QS-21 was examined for mutagenic activity in the bacterial reverse mutation test in *Salmonella typhimurium* strains TA1535, TA 1537, TA 98 and TA 100 and in *Escherichia coli* WP2 uvrA in both the presence and absence of rat liver S9 homogenates mixed with an NADPH generating system. The testing method was similar to the one described for MPL and also included positive controls to validate the experiment. QS-21 was supplied at a concentration of 9090 μ g/ml and tested at concentrations of 56-4545 μ g/plate.

QS-21 was not toxic when incubated with bacteria up to this top concentration. A 2-fold decrease in the mean number of revertants was noted at the concentration of 1515 μ g/plate in the absence of S9 but as this was not seen at any other concentration, this was judged to be an artefact. QS-21 did not induce a more than 2-fold and/or dose related increase in the mean number of revertant colonies compared to the background spontaneous reversion rate observed with the negative control. Consequently, the applicant concluded that QS-21 was not mutagenic in this study.

In a second bacterial reverse mutation assay, the testing was performed with DQ (see 1.1 Type of application and aspects on development, where DQ is explained to be what the applicant has termed detoxified QS-21). The testing method was the same as that described for QS-21. DQ was supplied at 200 μ g/ml QS-21, 4000 μ g/ml DOPC and 1000 μ g/ml cholesterol and was used at concentrations of 6.25, 12.5, 25, 50 and 100% with dilution in water, corresponding to 6.25 to 100 μ g QS-21. In one test, the negative controls for TA 98 and WP2 uvrA were outside the acceptable range so this testing was repeated.

DQ did not induce any increases in numbers of revertant colonies and was therefore concluded by the applicant not to be mutagenic in this experiment.

DQ was tested for its potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells in both the presence and absence of rat liver S9 homogenates mixed with an NADPH generating system with incubations of 4 and 24 hours respectively. The test item contained a maximum of 200 μ g/ml QS-21, 4000 μ g/ml DOPC and 1000 μ g/ml cholesterol resulting in a maximum possible concentration of QS-21 in this experiment of 10 μ g/ml. The study was conducted in accordance with OECD Test Guideline 476 on Genetic Toxicology: In vitro mammalian cell gene mutation tests, of July 1997. Positive controls were used (methyl methanesulphonate in the absence of S9 and 3-methylcholanthrene in the presence of S9) and confirmed the possibility of detecting increases in mutation frequencies. No initial dose range study was performed, because the highest concentration of 10 μ g QS-21/ml was expected not to be cytotoxic. QS-21 was tested at 0.43-10 μ g/ml

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in the absence of S9 and at 0.11-10 μ g/ml in the presence of S9. However, QS-21 was found to be toxic to the test cells with relative total growth reduced by >10% at and above 1.7 μ g/ml QS-21 in the absence of S9. In both the absence and presence of S9-mix no indication for a mutagenic potential was observed at any dose level. The applicant concluded that DQ was not mutagenic in this experiment.

The in vivo genotoxic potential of DQ was tested in male rats. Prior to the bone marrow micronucleus test, a dose range finding study was performed in rats to define the maximum tolerable dose of the test substance after intravenous administration. A DQ dose of 160 μ g/kg of QS-21 per day for two consecutive days with an interval of approximately 24h between doses was selected, as higher doses caused piloerection, other clinical signs of toxicity and >10% body weight loss. In the main test, rats were given DQ at doses of 40, 80 and 160 μ g/kg, or saline, intravenously twice, 24 hours apart and were killed 24 hours later and bone marrow smears were prepared to allow examination of the presence of micronuclei in polychromatic erythrocytes. As a positive control, further rats were given mitomycin C intraperitoneally, once at 1.5 mg/kg. This induced an increase in the number of micronucleated polychromatic erythrocytes and confirmed the possibility of detecting genotoxic effects.

In this experiment, DQ did not result in any increases in micronucleated polychromatic erythrocytes, indicating that DQ did not result in damage to the chromosomes and/or to the spindle apparatus of the bone marrow cells of male rats. There was no statistically significant decrease in the mean number of polychromatic erythrocytes, when comparing the animals that received DQ to the negative control animals, which reflects a lack of toxic effects on erythropoiesis by the test substance. Systemic availability of the test substance was achieved by intravenous administration and demonstrated by a reduction in body weight in the highest dose group, indicating that the negative response observed in this bone marrow micronucleus test was not due to lack of systemic availability of the test substance or its metabolites. The applicant concluded that DQ did not induce chromosomal damage and/or damage to the mitotic spindle apparatus of the bone marrow target cells.

AS01

The study was designed to evaluate the potential induction of micronuclei in bone marrow cells in rats by ASO1_B and 2,4 dichlorobenzoic acid (2,4 DCBA). The treatment was administered on two occasions, 24 hours apart. The negative control (saline) was administered by intravenous injection at a dosage volume of 0.5 mL on consecutive days. The ASO1_B formulation and the spiked formulation with 2,4 DCBA was administered by intramuscular injection at a dosage volume of 0.1 mL in each hind limb on consecutive days. Bone marrow smears were obtained from five male and five female animals in the vehicle control, ASO1_B and 2,4 DCBA spiked formulation treated groups approximately 24 hours after the second dose. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes.

No statistically significant increases in the frequency of micronucleated immature erythrocytes were observed in rats treated with $ASO1_B$ or 2,4 DCBA spiked formulation. No statistically significant decreases in the proportion of immature erythrocytes were observed in rats treated with the 2,4 DCBA spiked formulation. However, there was a statistically significant reduction in the proportion of immature erythrocytes in animals treated with $ASO1_B$. The conclusion of the applicant was that $ASO1_B$ showed no evidence of causing micronuclei but did show evidence of causing bone marrow cell toxicity.

Previous testing indicated that there may be an effect of $ASO1_B$ on erythroid cell line production. A study was done to investigate this further, in particular to determine replicability of the findings on the erythroid cell line and to determine recovery by assessing effects at day 13. In this study, male rats were given either one intramuscular injection or two intramuscular injections 24 hours apart, of $ASO1_B$

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or saline and were killed either on day 3 (ie 48 hours after the single dose or 24 hours after the second dose) or on day 13. All injections were of 0.1 ml given into each hind limb thigh muscle ie a dose of 0.2 ml/rat. Peripheral blood was taken under light isoflurane anaesthesia from rats who were alive on days 3, 6, 9 and 12 and subject to a complete blood count. Bone marrow samples were prepared post-mortem for all rats using 2 different methods: the standard method for myelogram; and the method used the previous rat micronucleus study above.

Clinical findings were unremarkable except that there was a suppression of body weight gain, and this was dose-dependent, being of greater magnitude in those animals dosed on two consecutive days as compared to those dosed only once with ASO1_B. In the haematological analyses, on day 3, there was a reduction in haematocrit, haemoglobin, mean cell haemoglobin, mean cell volume, lymphocytes and monocytes for rats dosed on two consecutive days with ASO1_B. On day 6, these animals had significantly low haematocrit, haemoglobin, mean cell volume, monocyte counts and significantly high white blood cell count, neutrophils, lymphocytes, eosinophils and platelets when compared to controls. On day 9, these rats showed low haemoglobin, high neutrophils and platelets and a marginal increase in % reticulocytes with increases in anisocytosis, hypochromasia and macrocytosis. However, there was no decrease in the proportion of immature erythrocytes and this finding from the earlier study was therefore not replicated. In rats given only a single dose of ASO1_B, there were similar changes of reductions in haematocrit, haemoglobin, mean cell haemoglobin, mean cell volume but again, no increase in immature erythrocytes. These changes in haemoglobin occurred despite no change to red cell number. Based on this result, AS01B was concluded not to affect the ability of bone marrow to produce red blood cells. Information on the cause of the reduction in haemoglobin concentrations is not provided by this study, but the applicant considers it possible that a direct effect of ASO1_B on circulating red cells could be occurring. However, the recovery of haemoglobin concentration by Day 12 indicates that this is a very short-term effect.

Overall, the applicant described that in rats gelatinous clumping was noted in preparing blood smears from rats given $ASO1_B$: this may have been a physical effect. Haematocrit and haemoglobin were reduced to between 0.94 and 0.97-fold control values between 1 and 7 days after 1 or two doses with recovery from day 10. The applicant concluded that in rats, there was no evidence that the lower proportion of immature erythrocytes in the bone marrow was a real effect of $ASO1_B$.

In rabbits, data were reviewed from general toxicity studies. The applicant summarised that, reviewing the totality of data over several studies, marginally lower mean haematocrit, haemoglobin and red blood cell count was associated with $ASO1_B$. In 4 of 6 studies, the conclusion was that haematology parameters were not affected or that the degree of effect was not of toxicological significance and effects were inconsistent across different studies in respect of the time of occurrence after dosing.

Carcinogenicity

According to the WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO, 2005), the EMEA Note for Guidance on Preclinical Pharmacological and Toxicological testing of Vaccines (CPMP/SWP/465/95) and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004), carcinogenicity studies are not required for final vaccine formulations, Adjuvants Systems and/or immunoenhancers.

Reproductive and developmental toxicity

In line with the WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO, 2005), the EMEA Note for Guidance on Preclinical Pharmacological and Toxicological testing of Vaccines (CPMP/SWP/465/95) and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004), reproductive

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and developmental toxicity studies were not included in this Application since the RTS,S/ AS01E candidate vaccine containing, AS01_E, MPL, QS-21 is not intended for administration to women of childbearing potential.

Toxicokinetic data

According to the WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO, 2005) and the Guideline on Adjuvants in Vaccines for Human Use (EMEA/CHMP/VEG/134716/2004), toxicokinetic studies were not performed with RTS,S/ASO1_B, MPL, QS-21 or ASO1_B. No toxicokinetic data were generated since determination of circulating levels of antigens is not requested according to the Note for Guidance on Preclinical Pharmacological and Toxicological testing of vaccines (CPMP/SWP/465/95) and the WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO, 2005).

Local tolerance

The applicant conducted testing with RTS,S/AS01_B, and with DQ (representing QS-21).

RTS,S/AS01_B

Local irritation of RTS,S/ $ASO1_B$ was tested in a study reported in 2002, in which it was given to New Zealand white rabbits as a single intramuscular injection. The dose volume was 0.5 ml and rabbits were given two injections, either saline plus saline, or RTS,S/ $ASO1_B$ or with $ASO1_B$ only. The dose was justified as being 23-28 times the human dose for an adult of 70 kg bodyweight and the dose was stated as being known to induce an immune response. Vaccines were supplied in separate syringes and vials containing antigen and adjuvant which, where appropriate, were mixed and gently shaken shortly prior to dosing. Male and female rabbits were used with the site of dosing shaved one day before dosing with injection into the paravertebral muscles. Rabbits were monitored for 3 days after dosing for clinical signs and on two separate 5-point rating scales for erythema and oedema; they were killed \sim 72 hours after dosing and injections sites examined.

There were no unscheduled deaths in this study. Local reactions were limited to the site of injection of RTS,S/AS01_B in one male and was described as purple discolouration with very slight oedema. Of 16 injections sites given saline in this study, 14 were judged normal at post-mortem on microscopic examination on day 3 after dosing. The remaining two showed minimal chronic-to-chronic/active inflammation with myofibre degeneration with only the occasional involvement of muscle fibre. Minimal regenerative processes were also evident. Of 12 injection sites given AS01_B, 5 were judged normal at post-mortem and 7 were judged to show minimal chronic-to-chronic/active inflammation with minimal myofibre degeneration, regeneration and haemorrhage. Of 12 injection sites given RTS,S/AS01_B, 7 were judged normal and 5 were judged to show minimal chronic-to-chronic/active inflammation that infiltrated the endomysial and perimysial connective tissue, with no-to-minimal disruption of fibres. The applicant concluded that injection of RTS,S/AS01_B and of AS01_B alone each produced lesions more frequently than did saline but the changes seen were slight and not considered to be toxicologically significant. In particular, changes were not noted clinically or macroscopically and were only detected on post-mortem histopathological examinations.

A further study was conducted to assess the intramuscular tolerance of vaccine injected once into rabbits. Two vaccines were used in this study: RTS,S/ AS01_B and RTS,S/ AS02_V. Vaccines were supplied in separate syringes and vials containing antigen and adjuvant which, where appropriate, were mixed and gently shaken shortly prior to dosing. Methods were based on CHMP/SWP/2145/00 Note for guidance on non-clinical local tolerance testing of medicinal products. Male and female rabbits were injected with one of the two vaccines or with each adjuvant; each rabbit was given 4 injections,

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each of 0.5 ml at the same instance of dosing with further rabbits being given only saline; the site of dosing was shaved one day before dosing. Rabbits were monitored for 4 days after dosing for clinical signs and on two separate 5-point rating scales for erythema and oedema; they were killed ~72 hours after dosing and injections sites examined.

There were no unscheduled deaths in this study. Local reactions were limited to one instance of bruising in one female one day at the site of dosing with ASO1_B. Haemorrhage was seen at injections sites with all treatments including saline. At histopathological examination, changes seen at the site of injection of saline were of myofibre necrosis with or without mineralisation, inflammation, fibroblast proliferation and haemorrhage. With RTS,S/ ASO1_B, these findings were also seen with the only additional finding being of slight interstitial oedema with inflammation at the site of injection of the ASO1_B adjuvant only; this was unremarkable and the applicant concluded that there was no difference between saline and RST,S/ ASO1_B. With RTS,S/ ASO2_V, the same finding of slight interstitial oedema with inflammation at the site of injection was seen at one injection site and it was also noted in one adjuvant site in one rabbit. The applicant concluded that the changes seen were due to the injection procedures with an additional degree of oedema, inflammation and necrosis in rabbits given ASO2_V.

The applicant also provided results from a study with vaccine containing ASO1 $_{\rm B}$ with antigens unrelated to malaria: the use of the adjuvant in potential vaccines for streptococcal disease was investigated. In this testing, male and female rabbits were dosed by intramuscular injection with saline, ASO1 $_{\rm B}$ adjuvant, ASO2 $_{\rm V}$ adjuvant, or with vaccine containing antigen plus either ASO1 $_{\rm B}$ or ASO2 $_{\rm V}$ in a dose volume of 0.5 ml into the calf muscles. The antigens used in this vaccine were not described in any detail but it can be identified that these relate to a product used in treating pneumonia. Results indicated that responses were the same as in rabbits that had been given adjuvanted malaria vaccine with no clinical signs or effects on body weight and nothing of note on macroscopic examination. Microscopic examination of injection sites indicated an inflammatory response to adjuvant and to the adjuvanted vaccine: this was described as very slight.

QS-21

A study was conducted and reported in 2012 to assess acute toxicity and intramuscular tolerance of DQ injected once in Sprague Dawley rats. Testing was in accordance with EMA/CHMP/VEG/134716/2004, Guideline on adjuvants in vaccines for human use and WHO Guideline 927, Annex 1 of 2005 on Nonclinical evaluation of vaccines. Male and female rats were injected with one of three different doses of DQ (4, 20 or 40 μ g/dose; 10, 50 or 100 μ g/kg) or were given saline in a dose volume of 0.1 ml per site of injection with dosing given as two separate injections into left and right anterior thigh muscles. Dose selection was based on the use of the highest concentration of QS-21 that could be obtained while maintaining the liposomal formulation as similar to that intended for use in humans. The injection sites were shaved one day before dosing. Rats were monitored over 3 days after dosing for clinical signs and any changes in body weight. Blood was taken for haematological and clinical chemistry analyses on days 0 and 3. Assessment of local tolerance was also assessed using a 5-point rating scales for erythema and oedema; on day 3 rats were killed and injections sites and organs (heart, kidneys, liver, lungs) examined.

There were no unscheduled deaths in this study and no changes noted in clinical signs or body weights following dosing. Changes consistent with an inflammatory reaction were seen. Fibrinogen was raised in all groups given DQ, compared to controls; changes were seen in reticulocytes but this finding was attributed to blood sampling. Higher absolute neutrophil counts and lower absolute lymphocyte counts were noted. Changes over time were noted in some clinical chemistry but typically did not deviate from normal ranges: none were judged by the applicant to be toxicologically significant. Macroscopic

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examination of injection sites showed white discoloration in a minority (3 of 10) rats at the top dose of DQ with no other macroscopic findings noted. Microscopic examination of injection sites showed minimal localised mononuclear cell inflammatory responses in most rats, including in 6 of 10 controls. This increase was of lymphocytes and macrophages and was widespread (extending along the epimysium and diffusely between muscle fibres, but not severe, being graded mild-to-moderate).

The applicant concluded that the changes described reflected expected effects following intramuscular injection of an immunostimulant with local reactions at the site of injection of inflammatory responses and, systemically, increases in fibrinogen, neutrophils and decrease in lymphocytes. The applicant judged that a NOEL was <4 μ g/dose; noting that all the effects seen were expected with an immunostimulant, these were considered not adverse by the applicant, giving a NOAEL of >40 μ g/dose.

Another study was conducted and reported in 2012 to assess acute toxicity and intramuscular tolerance of DQ injected once into New Zealand rabbits. Testing was in accordance with EMA/CHMP/VEG/134716/2004, Guideline on adjuvants in vaccines for human use and WHO Guideline 927, Annex 1 of 2005 on Nonclinical evaluation of vaccines. Male and female rabbits were injected with one of three different doses of DQ (20, 100 or 200 µg/dose; ~7, 35 or 67 µg/kg) or were given saline in a dose volume of 0.5 ml per site of injection with dosing given as two separate injections into left and right anterior thigh muscles. The injection sites were shaved one day before dosing. Rabbits were monitored over 3 days after dosing for clinical signs and any changes in body weight. Assessment of local tolerance was also assessed using a 5-point rating scales for erythema and oedema; on day 3 rabbits were killed and injections sites and organs (heart, kidneys, liver, lungs) examined.

There were no unscheduled deaths in this study and no changes noted in clinical signs or body weights following dosing. Macroscopic examination indicated discolouration (white area) at one injection site of one male and one female rabbit, each from the middle dose-group of those given DQ. Microscopic examination indicated a minimal to mild localised mononuclear inflammatory cell response in all rabbits, including the controls; however this was exacerbated by DQ, in a dose-related manner, with evidence of wider distribution of inflammatory cells.

3.3.5. Ecotoxicity/environmental risk assessment

The applicant stated that an environmental risk assessment is not applicable for this vaccine due to the nature of their constituents and in line with the *Guideline on the environmental risk assessment of medicinal products for human use* (EMA/CHMP/SWP/4447/00). The applicant's position was endorsed by the CHMP.

3.3.6. Discussion on non-clinical aspects

The pharmacological testing program proposed for RTS,S/AS01 $_{\rm E}$ candidate vaccine is considered adequate and no additional studies are required.

Pharmacokinetic studies are normally not required for a vaccine. Pharmacokinetic study of the vaccine antigen was not studied which is considered acceptable by the CHMP. The applicant provided 4 biodistribution studies to support understanding of the mode of action of the novel ASO1 adjuvant system.

All toxicological studies have been conducted according to GLP requirements and according to the relevant WHO and EMA guidelines to demonstrate the vaccine's nonclinical safety and tolerability.

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The non clinical toxicology studies were performed with the RTS,S/AS01 $_{\rm B}$ candidate vaccine, the Adjuvant System AS01B and its immunoenhancers QS-21 and MPL in adequate animal models. The tested RTS,S/AS01 $_{\rm B}$ formulation consists of 50 μg of RTS,S antigen combined with AS01 $_{\rm B}$ containing 50 μg of MPL and 50 μg of QS-21 in liposomes and therefore contains twice the amounts of these constituents than the RTS,S/AS01 $_{\rm E}$ (Mosquirix) candidate vaccine.

The results from the toxicity studies indicate that RTS,S/AS01_B, AS01_B, QS-21 and MPL are well tolerated by the animals and support the safe use of Mosquirix in human subjects.

3.3.7. Conclusion on the non-clinical aspects

The non-clinical testing program proposed for RTS,S/AS01_E candidate vaccine is considered adequate and supportive of the safe use of Mosquirix in human subjects.

3.4. Clinical aspects

3.4.1. Introduction

GCP

The applicant claimed that the clinical trials were performed in accordance with GCP.

The applicant has provided a statement to the effect that clinical trials conducted outside the Union were carried out in accordance with the ethical standards of Directive 2001/20/EC.

3.5. Clinical efficacy

3.5.1. Rationale for dose, adjuvant and schedule of RTS,S/AS01E

Human challenge studies

Clinical development was initiated in studies in malaria-naïve adults in collaboration with the Walter Reed Army Institute of Research (WRAIR) using a controlled human malaria infection (CHMI) model with *P. falciparum* sporozoite challenge. The first CHMI study compared RTS,S antigen adsorbed on aluminium salts or on aluminium salts combined with MPL (ASO4 Adjuvant System). Both formulations were immunogenic but after sporozoite challenge 0/6 in the RTS,S/alum group and only 2/8 in the RTS,S/ASO4 group were protected from patent parasitaemia.

RTS,S/AS04, RTS,S in an oil-in-water emulsion (AS03) or in an oil-in-water emulsion+MPL+QS-21 (AS02) were tested in the CHMI model using a 0, 1, 7-month schedule in which the last dose was reduced to 1/5 (0.1 mL) for RTS,S/AS03 and RTS,S/AS02 $_{\rm A}$ groups. While 1/8 and 2/7 were protected in the RTS,S/AS04 and RTS,S/AS03 groups there were 6/7 protected in the RTS,S/AS02 group but on re-challenge 6 months later only 1/5 was still protected.

All subsequent studies have used either AS02 or AS01. In consideration of the acceptable reactogenicity in subsequent studies with RTS,S/AS02, a reduced third dose has not been used. However, the high observed VE (6/7) was not observed in any other CHMI study with either RTS,S/AS02 or RTS,S/AS01; two or three doses of RTS,S/AS02_A were protective in ~40 to 50% whereas one dose protected 3/10 subjects. A later study compared accelerated schedules (0, 1, 3-month and 0, 7, 21-day) in which 9/20 vs. 7/18 were protected [Malaria-012]. Malaria-071 is ongoing in 51 malaria-naïve adults to re-assess efficacy after a delayed fractional dose (0.1 mL at month 7). The lyophilised antigen preparation was developed because the liquid formulation of RTS,S slowly

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degraded in the presence of AS02. A WRAIR study showed similar safety and immunogenicity of 2 doses of the liquid and lyophilised formulations and the lyophilised formulation provided 42% protection against experimental challenge so subsequent studies with RTS,S/AS02 $_{\rm A}$ used the lyophilised formulation.

Immunologic analyses in these early studies were consistent with the hypothesis that the functional antibody response and elicitation of CD4+ T-cells expressing interferon- γ (IFN- γ) play an important role in protection. Further characterisation showed that RTS,S-specific lymphoproliferation responses and antibodies to CS (NANP repeat and flanking regions) were strongly induced in a vast majority of volunteers. The CS-specific CD4+ T-cell responses were directed against several epitopes but were predominantly focussed on the Th2R immunodominant polymorphic C-terminal region of the CS protein. CS-specific CD8+ cytotoxic lymphocytes were not detected. RTS,S/AS02_A was a potent inducer of Th-1 cellular and humoral immunity [Malaria-002].

Dose of RTS,S

Following CHMI studies RTS,S/AS02 $_A$ was studied in African children. A dose was selected from Phase I studies in children aged 6 to 11 years (Malaria-015 in which $1/5^{th}$, ½ and full doses of RTS,S AS02 were used) and 1 to 5 years (Malaria-020 in which $1/5^{th}$, ½ and full doses of RTS,S AS02 were used) in which vaccine was given at a 0, 1, 3-month schedule. In both studies all doses of RTS,S/AS02 were immunogenic for anti-CS. At one month post-dose 3 similar GMCs were observed in the 25 μ g and 50 μ g RTS,S/AS02 $_A$ groups with a trend to higher GMCs in the 25 μ g group. RTS,S/AS02 $_A$ was highly immunogenic for anti-HBsAg with all seroprotected at one month post-dose 3.

Taking into account also the safety profile the 25 μ g RTS,S/AS02 dose (0.25 ml, or half the adult dose) was selected for paediatric development. This same dose formulated in 0.5 ml was called RTS,S/AS02_D.

Adjuvant

RTS,S/AS01 formulations were compared with RTS,S/AS02 formulations in three adult studies (Malaria-027, -044 and -048) and two paediatric studies in endemic areas (Malaria-046 and -047).

In malaria-naïve adults (Malaria-048) RTS,S/AS01_B and RTS,S/AS02_A elicited significantly superior anti-CS concentrations vs. non-adjuvanted RTS,S/saline (p<0.0001 and p=0.0011, respectively). In malaria-naïve adults (Malaria-027 and -048) and semi-immune adults (Malaria-044) anti-CS responses were (often significantly) higher in for RTS,S/AS01_B vs. RTS,S/AS02_A.

In children (Malaria-046) non-inferiority of RTS,S/AS01 $_{\rm E}$ (i.e. containing half the antigen and adjuvant amounts compared to AS01 $_{\rm B}$) vs. RTS,S/AS02 $_{\rm D}$ was shown for anti-CS at one month post-dose 3 (GMR 0.88; 95% CI: 0.68-1.15). Anti-CS responses were higher with RTS,S/AS01 $_{\rm E}$ vs. RTS,S/AS02 $_{\rm D}$ and there was a marked further increase in anti-CS response after the third dose.

In adults both adjuvanted formulations were immunogenic for anti-HBs.

In children (Malaria-046), non-inferiority of RTS,S/AS01_E to RTS,S/AS02_D with respect to anti-HBs immunogenicity was demonstrated at one month post-dose 3 (GMR 0.76; 95% CI: 0.42-1.39).

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Formulations of RTS,S/AS vaccine used in clinical studies

	Freeze-dried fraction	L	iquid fr	action		Studies
Formulation	RTS,S		MPL	QS21	Dose	
	(µg)		(µg)	(µg)	Volume	
RTS,S/AS02 _A	50	Oil-in-water	50	50	0.5 mL	Early studies in naïve adults,
(0.5 mL dose)		emulsion				including challenge studies, and in
						semi-immune adults (Malaria-005)
RTS,S/AS02 _A	25	Oil-in-water	25	25	0.25 mL	Dose selected in Malaria-015/020,
(0.25 mL dose)		emulsion				used in Malaria-025, Malaria-026
						and Malaria-034
RTS,S/AS02 _D	25	Oil-in-water	25	25	0.5 mL	Formulation used in Malaria -034,
		emulsion				Malaria-038, Malaria-040, Malaria-
						046, Malaria-047
RTS,S/AS01 _B	50	Liposomes	50	50	0.5 mL	Studies in naïve adults (Malaria-
						027, Malaria-048) and in semi-
						immune adults (Malaria-044)
RTS,S/AS01 _E	25	Liposomes	25	25	0.5 mL	Final formulation (Malaria-046,
						Malaria-047, Malaria-049, Malaria-
						050, Malaria-055, Malaria-057,
						Malaria-058, Malaria-061, Malaria-
						063)

The letter in subscript refers to the amount of the immunoenhancers MPL and QS-21

Schedule selection

Following adult data and study 046, 047 and 050 in children (0, 1 and 2 month vaccination schedule), which showed a further increase in anti-CS after the third dose vs. the second dose, the 0, 1 and 2 month vaccination schedule was selected.

Study Malaria-047

Title: A Phase II randomized, controlled, partially-blind study of the safety and immunogenicity of the candidate *Plasmodium falciparum* vaccines RTS,S/AS02_D and RTS,S/AS01_E, when administered IM according to one of three dose schedules in children aged 5 to 17 months living in Ghana.

Study	Objective	Study Design	Study population	Ctudy groups	TVC	ATP
-	Objective		1	Study groups		immuno
Malaria- 047	1°: Safety 2°: Safety and immunogenicity	Phase II, partially blind randomized controlled 0-1 months 0-1-2 months 0-1-7 months	Healthy male and female children 5 - 17 months <i>Ghana</i>	RTS,S/AS01 _E , 0-1, 25µg/0.5ml RTS,S/AS02 _D , 0-1, 25µg/0.5ml RTS,S/AS01 _E , 0-1-2, 25µg/0.5ml Rabies vaccine, 0-1-2 RTS,S/AS02 _D , 0-1-2, 25µg/0.5ml ^b RTS,S/AS01 _E , 0-1-7,	90 90 90 45 45 90 90	86 87 86 43 44 88 88
				25µg/0.5ml RTS,S/AS02 _D , 0-1-7, 25µg/0.5ml		

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Healthy subjects aged between 5 and 17 months at randomisation (mean 10.7 months) had completed a 3-dose regimen of a licensed HBV vaccine in early infancy. Data were collected to 19 months post-dose 1.

Anti-CS antibody

Pre-vaccination seropositivity rates were < 20% per group with low GMTs. All subjects were seropositive after 2 doses of RTS,S/AS01_E or RTS,S/AS02_D. Low background levels of anti-CS antibodies were found in the rabies vaccine control group (<25% at M19). Within each vaccination schedule, the RTS,S/AS01_E formulation consistently yielded higher peak anti-CS responses as compared to RTS,S/AS02_D. For all schedules AUCs were consistently higher for the AS01_E groups.

The highest GMTs were seen with RTS,S/AS01 $_{\rm E}$ at M3 in the 0, 1, 2 schedule group (631.8 EU/mL vs. 366.9 EU/mL for RTS,S/AS02 $_{\rm D}$ at the same schedule). With the 0, 1, 7 schedules the M8 GMTs were 373.0 EU/mL for RTS,S/AS01 $_{\rm E}$ vs. 272.1 EU/mL for RTS,S/AS02 $_{\rm D}$. With the 0, 1 schedule the M2 GMTs were 483.4 EU/mL vs. 318.4 EU/mL, respectively. However, at M7, 10 and 19 the 2-dose groups had lower GMTs vs. the 3-dose groups.

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Table 12. Seropositivity rates and GMTs for anti-CS antibodies (ATP cohort for immunogenicity)

				≥0.5	ELU/N	1L		GMT			
					95	% CI		959	% CI		
Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
2D(01)	SCREENING	87	12	13.8	7.3	22.9	0.3	0.3	0.4	<0.5	4.9
	PII(M2)	87	87	100	95.8	100	318.4	269.1	376.8	41.0	2799.3
	PII(M7)	85	85	100	95.8	100	34.5	26.1	45.5	2.1	850.4
	PII(M10)	84	84	100	95.7	100	19.9	14.8	26.8	8.0	532.7
	PII(M19)	80	78	97.5	91.3	99.7	10.2	7.4	14.2	< 0.5	214.6
1E(01)	SCREENING	86	12	14.0	7.4	23.1	0.3	0.3	0.4	< 0.5	4.7
	PII(M2)	85	85	100	95.8	100	483.4	395.4	590.9	27.0	3321.6
	PII(M7)	82	82	100	95.6	100	52.7	40.7	68.2	4.9	1337.7
	PII(M10)	84	84	100	95.7	100	30.5	23.2	40.1	1.6	825.3
	PII(M19)	83	82	98.8	93.5	100	15.3	11.3	20.9	<0.5	565.1
2D(012)	SCREENING	44	4	9.1	2.5	21.7	0.3	0.2	0.3	<0.5	1.6
	PIII(M3)	43	43	100	91.8	100	366.9	293.2	459.2	59.6	1753.0
	PIII(M7)	43	43	100	91.8	100	78.2	57.9	105.6	9.5	611.5
	PIII(M10)	42	42	100	91.6	100	43.4	31.6	59.6	5.3	385.5
	PIII(M19)	41	41	100	91.4	100	20.4	14.4	28.7	1.9	232.8
1E(012)	SCREENING	86	16	18.6	11.0	28.4	0.3	0.3	0.4	< 0.5	2.2
	PIII(M3)	85	85	100	95.8	100	631.8	554.3	720.2	135.9	2827.7
	PIII(M7)	86	86	100	95.8	100	162.3	134.2	196.3	10.6	1061.0
	PIII(M10)	84	84	100	95.7	100	102.1	83.4	125.1	9.0	1020.7
	PIII(M19)	85	85	100	95.8	100	45.9	36.8	57.2	3.1	458.3
2D(017)	SCREENING	88	14	15.9	9.0	25.2	0.3	0.3	0.3	<0.5	3.0
	PII(M7)	87	87	100	95.8	100	25.9	19.7	34.1	0.7	390.4
	PIII(M8)	81	81	100	95.5	100	272.1	218.5	338.8	28.1	3753.3
	PIII(M10)	83	83	100	95.7	100	120.0	92.2	156.1	3.9	1327.0
	PIII(M19)	82	82	100	95.6	100	43.6	32.8	58.0	2.3	726.5
1E(017)	SCREENING	88	13	14.8	8.1	23.9	0.3	0.3	0.4	<0.5	12.5
	PII(M7)	84	84	100	95.7	100	51.0	40.4	64.4	1.6	551.4
	PIII(M8)	83	83	100	95.7	100	373.0	311.1	447.2	50.8	3632.9
	PIII(M10)	84	84	100	95.7	100	166.5	140.1	198.0	14.5	958.0
	PIII(M19)	84	84	100	95.7	100	50.9	39.6	65.5	0.7	333.3
RABIES	SCREENING	43	7	16.3	6.8	30.7	0.3	0.3	0.4	<0.5	3.7
	PIII(M3)	43	9	20.9	10.0	36.0	0.4	0.3	0.6	<0.5	97.8
	PIII(M7)	43	5	11.6	3.9	25.1	0.3	0.3	0.4	<0.5	5.8
	PIII(M10)	43	8	18.6	8.4	33.4	0.3	0.3	0.4	<0.5	5.9
	PIII(M19)	42	10	23.8	12.1	39.5	0.5	0.3	0.7	< 0.5	390.8

2D(01) = RTS,S/AS02D (01) 1E(01) = RTS,S/AS01E (01) 2D(012) = RTS,S/AS02D (012) 1E(012) = RTS,S/AS01E (012) 2D(017) = RTS,S/AS02D (017) 1E(017) = RTS,S/AS01E (017) RABIES = RABIES

 $\label{eq:GMT} \textbf{GMT} = \textbf{geometric mean antibody titer calculated on all subjects}$

N = number of subjects with available results

n/% = number/percentage of subjects with titer within the specified range

95% CI = 95% confidence interval; LL = Lower Limit

MIN/MAX = Minimum/Maximum

PII(M10) = post dose 2; Month 10
PII(M19) = post dose 2; Month 19
PII(M2) = post dose 2; Month 2
PII(M7) = post dose 2; Month 7
PIII(M10) = post dose 3; Month 10

PIII(M19) = post dose 3; Month 19 PIII(M3) = post dose 3; Month 3

PIII(M3) = post dose 3; Month 3
PIII(M7) = post dose 3; Month 7

PIII(M8) = post dose 3; Month 8

UL = Upper Limit

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СМІ

CMI was evaluated at one month after dose 2 (M2) or 3 (M3) and at M19. RTS,S/AS01E induced a significantly higher frequency of CS-specific cellular immune response characterised by CD4+ T cells producing at least IL-2 or TNF- γ or IFN- α vs. controls. No CS-specific CD8+ T cell responses were detected after vaccination. RTS,S/AS01_E also induced a slightly higher frequency of CS-specific CD4+ T cell responses (IL-2, IFN- γ or TNF- α) than RTS,S/AS02.

For both vaccine formulations at one month post final dose the frequencies of CS-specific CD4+ T cells (CD40L, IL-2, IFN- γ or TNF-a) were significantly higher in the 0,1,7-month schedule vs. other schedules. The CS-specific IL-2+ CD4+ T cell response at one month post final dose in the 0,1,2-month schedule was significantly higher compared to the 0,1-month schedule. Correlations between the frequency of CS-specific CD4+ T cells expressing at least IL-2 or TNF- γ and the amounts of serum anti-CS antibodies were observed with both vaccine formulations at peak and at M19.

Anti-HBs

All except one who received at least 2 RTS,S vaccine doses had seroprotective levels of anti-HBs antibodies. Seropositivity rates in the control (rabies) group were 83.7% at M3 and 85.7% at M19.

Within each vaccination schedule there was no statistically significant difference in GMTs between RTS,S/AS01_E and RTS,S/AS02_D. The 3-dose schedules gave higher GMTs than the 2-dose schedule.

It was concluded that 3-dose schedules were more immunogenic than 2-dose schedules and that $RTS_1S_1AS_2$ was consistently more immunogenic for anti-CS than $RTS_1S_1AS_2$.

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Table 13. Seropositivity rates and GMTs for anti-HBs antibodies (ATP cohort for immunogenicity)

			≥ 10 MIU/ML				GMT				
					95% C	l		95% CI			
Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
2D(01)	SCREENING	87	77	88.5	79.9	94.3	100.8	68.3	148.8	<10.0	11059.8
	PII(M2)	87	86	98.9	93.8	100	17042.9	10466.7	27750.8	<10.0	1360865
	PII(M19)	80	79	98.8	93.2	100	3509.9	2398.3	5136.5	<10.0	161989.5
1E(01)	SCREENING	86	78	90.7	82.5	95.9	107.6	73.5	157.6	<10.0	10791.0
	PII(M2)	85	85	100	95.8	100	15106.5	9508.4	24000.6	199.9	1249069
	PII(M19)	83	83	100	95.7	100	4478.3	3154.9	6357.0	169.4	148143.8
2D(012)	SCREENING	44	37	84.1	69.9	93.4	108.7	60.6	194.8	<10.0	9456.5
	PIII(M3)	43	43	100	91.8	100	29999.7	18798.9	47874.2	3228.1	933712.1
	PIII(M19)	41	41	100	91.4	100	5112.4	3349.8	7802.6	464.4	65608.5
1E(012)	SCREENING	86	81	94.2	87.0	98.1	82.1	61.0	110.5	<10.0	1503.5
	PIII(M3)	85	85	100	95.8	100	34935.1	25177.9	48473.6	709.5	983322.3
	PIII(M19)	85	85	100	95.8	100	7105.5	5160.5	9783.7	15.1	273570.1
2D(017)	SCREENING	88	78	88.6	80.1	94.4	88.1	60.7	128.0	<10.0	6900.2
	PIII(M8)	81	81	100	95.5	100	96754.4	72062.0	129907.7	977.2	1284437
	PIII(M19)	82	82	100	95.6	100	17191.2	12528.8	23588.5	449.9	412510.0
1E(017)	SCREENING	88	75	85.2	76.1	91.9	89.9	60.8	132.7	<10.0	7150.3
	PIII(M8)	83	83	100	95.7	100	103224.8	83034.7	128324.1	6260.6	1063712
	PIII(M19)	84	83	98.8	93.5	100	13386.4	9661.4	18547.7	<10.0	212048.6
RABIES	SCREENING	43	39	90.7	77.9	97.4	107.9	63.5	183.3	<10.0	4117.2
	PIII(M3)	43	36	83.7	69.3	93.2	121.7	63.2	234.5	<10.0	104754.5
	PIII(M19)	42	36	85.7	71.5	94.6	114.3	57.6	226.8	<10.0	86149.6

2D(01) = RTS,S/AS02D (01) MIN/MAX = Minimum/Maximum

1E(01) = RTS,S/AS01E (01) PII(M2) = post-dose 2 (Month 2)

2D(012) = RTS,S/AS02D (012) PII(M19) = post-dose 2 (Month 19)

1E(012) = RTS,S/AS01E (012) PIII(M3) = post-dose 3 (Month 3)

2D(017) = RTS,S/AS02D (017) PIII(M19) = post-dose 3 (Month 19)

1E(017) = RTS,S/AS01E (017) PIII(M8) = post-dose 3 (Month 8)

RABIES = RABIES

GMT = geometric mean antibody titer calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with titer within the specified range

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Study Malaria-050

Ctudu		Study Doolan	Ctudy nanulation		Number of Subjects			
Study	Objective(s) Study Design Study population		Study groups	HVC:	ATP efficacy	ATP immuno		
Malaria- 050	1°: Safety 2°: Safety and immunogenicity Exploratory: Efficacy against clinical disease		infants 6 - 10 weeks Gabon, Ghana, Tanzania	Tritanrix-HepB™/Hib (DTPw- HepB/Hib) at 6, 10, 14 weeks; measles and yellow fever at 9	170 171	159 154 156	148 139 147	
				months	511	469	434	

Infants were to have received one previous dose of OPV and BCG. The mean age at baseline was 7.0 weeks and at M7 it was 8.4 months. The mean baseline weight was 4.9 kg and 51% of subjects were male.

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Anti-CS antibody responses

At pre-vaccination seropositivity rates were 26%-30% but GMTs were very low. At M3 99% in the RTS,S/AS01E groups were seropositive. The highest GMT occurred at M3 in the 0, 1, 2-month group. At M19 seropositivity rates were 94% and 85% in the RTS,S/AS01_E groups vs. 5% in the control group but GMTs had fallen to < 99. Anti-CS titres remained low in the control group.

Table 14. Seropositivity rates and GMTs for anti-CS antibodies (ATP Cohort for Immunogenicity)

				Serop	ositive			GM [*]	Γs (EU/mL))	
Group	Timing	N	n	%	95% (i i	value	95% CI	95% CI		Max
RTS,S/AS01E	PRE	153	46	30.1	22.9	38.0	0.4	0.3	0.4	<0.5	2.9
(0,1,2-month)	PII(D60)	137	135	98.5	94.8	99.8	86.6	66.5	112.7	<0.5	929.7
	PIII(M3)	131	130	99.2	95.8	100	190.3	154.3	234.7	<0.5	2593.2
	PIII(M7)	137	136	99.3	96.0	100	35.3	28.5	43.8	<0.5	388.1
	PIII(M19)	126	118	93.7	87.9	97.2	6.6	5.1	8.4	<0.5	93.6
RTS,S/AS01 _E	PRE	141	37	26.2	19.2	34.3	0.4	0.3	0.4	<0.5	10.0
(0,1,7-month)	PIII(M3)	121	120	99.2	95.5	100	57.7	43.7	76.2	<0.5	1117.3
	PIII(M7)	127	118	92.9	87.0	96.7	6.1	4.6	7.9	<0.5	122.7
	PIII(M8)	127	125	98.4	94.4	99.8	107.8	81.1	143.4	<0.5	2188.0
	PIII(M19)	123	104	84.6	76.9	90.4	8.3	5.8	11.7	<0.5	573.0
Control	PRE	156	43	27.6	20.7	35.3	0.4	0.3	0.4	<0.5	7.9
	PIII(M3)	129	14	10.9	6.1	17.5	0.3	0.3	0.3	<0.5	82.7
	PIII(M7)	132	8	6.1	2.7	11.6	0.3	0.3	0.3	<0.5	1.7
	PIII(M8)	135	12	8.9	4.7	15.0	0.3	0.3	0.3	<0.5	49.0
	PIII(M19)	120	6	5.0	1.9	10.6	0.3	0.3	0.3	<0.5	8.1

Seropositive ≥ 0.5 EU/mL

RTS,S/AS01E in combination with DTPwHepB/Hib + OPV

Control = DTPwHepB/Hib + OPV

GMT = geometric mean antibody titre calculated on all subjects

 $N = number\ of\ subjects\ with\ available\ results$

n/% = number/percentage of subjects with titre within the specified range

95% CI = 95% confidence interval

MIN/MAX = Minimum/Maximum

PRE = Pre-vaccination

PII = Post Dose 2, PIII(M3) = Post Dose 3

M3/M7/M8/M19 = Month 3/Month 7/Month 8/Month 19

The post-dose 3 anti-CS GMTs were highest in Gabon (318 for 0,1,2 vs. 239 for 0,1,7 month schedules) and lowest in Ghana (75 vs. 53 for respective schedules).

The avidity of anti-CS antibodies elicited in the RTS,S/AS01 $_{\rm E}$ groups was assessed in a post hoc analysis based on samples at pre-vaccination and after doses 2 (M2; Day 60) and 3 (either M3; Day 90 or M8 according to schedule group). For the total vaccinated cohort there was no difference between the two schedule groups in the mean anti-CS avidity index after 2 or 3 doses.

Anti-HBs

Pre-vaccination, 25%-38% were seroprotected but GMTs were low. At M3 all recipients of RTS,S/AS01 $_{\rm E}$ and 98% of controls were seroprotected. GMTs for both RTS,S/AS01 $_{\rm E}$ groups were very much higher vs. the control group while the highest GMT occurred at M8 in the 0, 1, 7-month group. At M19 all in the RTS,S/AS01 $_{\rm E}$ groups and 97% of controls were still seroprotected but the GMTs remained much higher for RTS,S/AS01 $_{\rm E}$ groups and highest for the 0, 1, 7-month group.

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Table 15. Seroprotective rates and GMTs for anti-HBs antibodies (ATP Cohort for Immunogenicity at Month 19)

				Seroprotected					Ts (mIU/i	mL)	
Group	Timing	N	n	%	95% C		value	95% CI		Min	Max
RTS,S/AS01E	PRE	145	55	37.9	30.0	46.4	12.5	9.9	15.7	<10.0	1247.2
(0,1,2-month)	PII(D60)	133	128	96.2	91.4	98.8	173.4	131.9	228.0	<10.0	13796.3
	PIII(M3)	130	130	100	97.2	100	1355.7	1100.6	1669.9	16.1	16307.2
	PIII(M7)	137	137	100	97.3	100	1555.5	1315.8	1839.0	115.8	18900.2
	PIII(M19)	126	126	100	97.1	100	1844.7	1524.9	2231.6	150.0	21849.7
RTS,S/AS01 _E	PRE	131	37	28.2	20.7	36.8	9.6	7.8	11.8	<10.0	572.3
(0,1,7-month)	PIII(M3)	119	119	100	96.9	100	651.2	541.1	783.8	45.5	12152.5
	PIII(M7)	126	126	100	97.1	100	1133.1	972.3	1320.6	64.1	16985.3
	PIII(M8)	125	125	100	97.1	100	59813.5	47050.5	76038.6	357.6	748793.1
	PIII(M19)	123	123	100	97.0	100	8748.2	6735.2	11362.8	69.0	153563.8
Control	PRE	143	35	24.5	17.7	32.4	8.7	7.3	10.5	<10.0	899.4
	PIII(M3)	126	123	97.6	93.2	99.5	338.0	266.3	429.0	<10.0	12428.3
	PIII(M7)	131	125	95.4	90.3	98.3	159.9	127.0	201.3	<10.0	5041.7
	PIII(M8)	133	128	96.2	91.4	98.8	162.4	127.9	206.3	<10.0	147082.1
	PIII(M19)	120	116	96.7	91.7	99.1	139.6	106.3	183.3	<10.0	11356.8

Seroprotected ≥ 10 mIU/mL

RTS,S/AS01E in combination with DTPwHepB/Hib + OPV

Control = DTPwHepB/Hib + OPV

GMT = geometric mean antibody titre calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with titre within the specified range

95% CI = 95% confidence interval

MIN/MAX = Minimum/Maximum

PII = Post Dose 2, PIII(M3) = Post Dose 3

M3/M7/M8/M19 = Month 3/Month 7/Month 8/Month 19

The post-dose 3 GMTs were lowest in Ghana (514 for 0,1,2 and 26465 for 0,1,7) and highest in Gabon (1966 and 113907 for respective schedules). The control group showed the same pattern.

At Month 3, 96% and 86% in the two RTS,S/AS01_E groups but 66% of controls tested (50 tested per group) were seropositive for anti-RF1. The highest GMT was observed in the 0, 1, 2-months group.

High anti-CS and anti-HBs responses were induced. Highest peak anti-CS responses were observed with the RTS,S/AS01E 0, 1, 2-month schedule.

Antibody against co-administered antigens

Non-inferiority of the immune response to D, T, BPT, PRP, polio 1, polio 2, Me and YF when RTS,S/AS01E was co-administered with DTPwHepB/Hib,OPV, measles and yellow fever vaccines compared to DTPwHepB/Hib,OPV, measles and yellow fever vaccine given alone was demonstrated. The non-inferiority criteria was not passed for anti-polio 3 in subjects receiving RTS,S/AS01E at 0, 1, 2-months plus DTPwHepB/Hib + OPV, but titres at screening were also lower in this group and seroconversion rates were equivalent across the groups. There was a tendency towards lower mean responses in antibody GMTs to the EPI antigens, with the exception of polio 1 and polio 2, in the RTS,S/AS01E co-administration groups compared to recipients of DTPwHepB/Hib + OPV alone. Nevertheless, seroprotective/seropositive levels were high.

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Vaccine efficacy

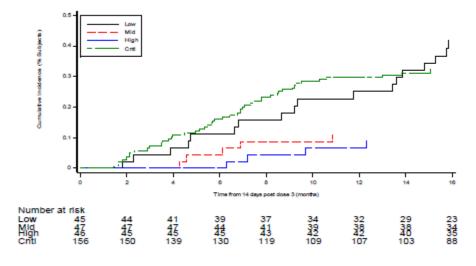
There was passive case detection (PCD) of malaria cases and efficacy was an exploratory endpoint. In the ATP Efficacy:

- VE from M2½ to 19 for RTS,S/AS01_E (0, 1, 2) against first or only episode meeting the primary case definition (PDef) was 52.5% (95% CI: 25.5 to 69.7, p=0.001) and 41.5% (95% CI: 11.3 to 61.5, p=0.012) using the secondary case definition (SDef).
- VE over one year post Dose 3 against first or only episode (PDef) was 61.6% (95% CI: 35.6 to 77.1, p<0.001; M2½ to 14 for RTS,S/ASO1_E (0, 1, 2)) and 63.8% (95% CI: 40.4 to 78.0, p<0.001; M7½ to 19 for RTS,S/ASO1_E (0, 1, 7)).))
- VE against first or only episode from M2½ to 8 for RTS,S/AS01_E (0, 1, 2) was 66.7% (95% CI: 27.2 to 84.8, p=0.006) or from M1½ to 7 for RTS,S/AS01_E (0, 1, 7) was 15.2% (95% CI: 70.2 to 57.7, p=0.643).
- VE in the TVC against multiple episodes from M0 to 19 for the PDef was 57.2% (95% CI: 33.1 to 72.7, p<0.001) for RTS,S/AS01 $_{\rm E}$ (0, 1, 2) and 32.0% (95% CI: 16.4 to 44.7, p<0.001) for RTS,S/AS01 $_{\rm E}$ (0, 1, 7) .

Thus, RTS,S/AS01 $_{\rm E}$ at 0, 1, 2-months protected against malaria over 19 months. The 0, 1, 7 month schedule provided similar efficacy when considering the one year post Dose 3 period, but was less protective than the 0, 1, 2-month schedule when considering the whole study period.

In the RTS,S/AS01E 0, 1, 2-month group, a 10-fold increase at M3 in anti-CS titres was associated with a reduction in the risk of a new episode of 13.5% (p=0.595). The HR per higher (T66=314.4 EU/mL) vs. lower tertile (T33=143.6 EU/mL) in M3 anti-CS titres was 0.265 (p=0.019) corresponding to a reduction in risk of malaria disease of 73.5%.

Figure 1. Analysis per tertile of anti-CS antibodies in recipients of RTS,S/AS01 $_{\rm E}$ (0, 1, 2-month) (ATP Cohort for Efficacy [Months 2.5- 19])

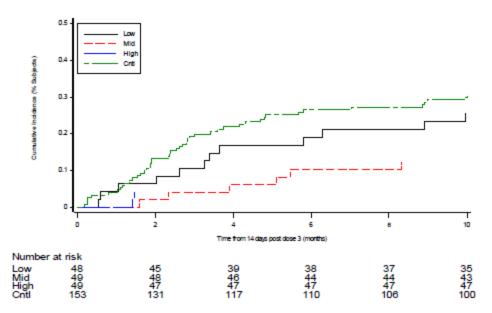


Note: timepoints represent time to episode

In the RTS,S/AS01 $_{\rm E}$ 0, 1, 7-month group, a 10-fold increase at M8 in anti-CS titres was associated with a reduction in the risk of a new episode of 26.1% (p=0.270). The HR per higher (T66=214.4 EU/mL) vs. lower tertile (T33=92.0 EU/mL) in M8 anti-CS titres was 0.58 (p=0.445) corresponding to a reduction in risk of clinical malaria disease of 42%.

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Figure 2. Analysis per tertile of anti-CS antibodies in recipients of RTS,S/AS01_E (0, 1, 7-month) (ATP Cohort for Efficacy [Months 7.5- 19])



Note: timepoints represent time to episode

In both schedule groups a significant difference in anti-CS antibody GMTs at screening was observed between subjects that did not have an episode of *P. falciparum* malaria disease compared to those who did but at least 50% of the values were <LLOD and given an arbitrary value, thereby reducing the variance.

The avidity index post-dose 3 means for the No case and the Case groups differed significantly (p=0.0127). Comparison of the highest vs. the lowest tertile showed a 63% reduction of the risk of subsequent clinical malaria for the highest tertile (p=0.0143). Univariate analysis showed that a 2-fold increase in avidity index resulted in 40% reduction of the risk of subsequent clinical malaria (p=0.0215).

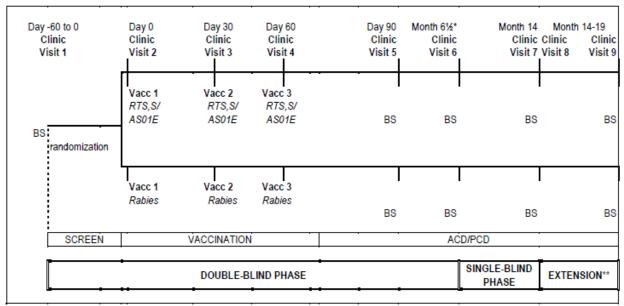
Phase 2b study Malaria-049 in children aged 5-17 months

Following selection of the 0,1,2-month schedule applied to the ASO1_E formulation a Phase 2b efficacy study was conducted as follows:

Study	Objective(s)	Study Design	Ctudy nanulation		Number of Subjects		
			Study population	Study groups	HVC:		ATP immuno
049	2°: Safety and		,	RTS,S/AS01ε, 25μg/0.5ml Rabies vaccine		415 420	414 423
					894	835	837

Data from **Malaria-049** are reported to 12 months post-dose 3 (i.e. M14) at both sites and up to M16 in Kenya only. Further data come from the extension study **Malaria-059**.

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KEY: BS; Blood Sample. Vacc; Vaccination. ACD; Active Case Detection. PCD; Passive Case Detection. Extension; Extension of the single-blind phase

Active case detection (ACD) of malaria commenced at 2 weeks post-dose 3. General features of the study design, case definitions, diagnostic and methodological issues, were as for Malaria-055 (see below).

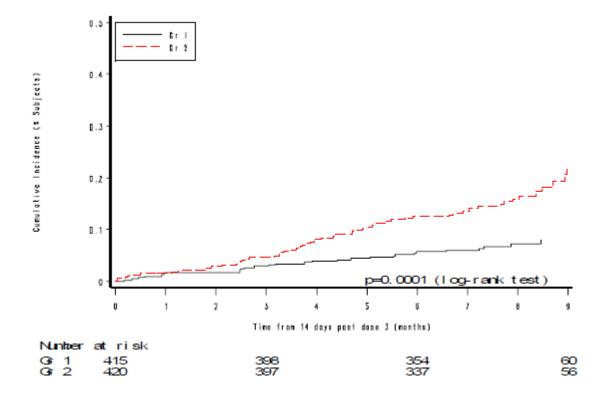
Of the 894 eligible infants enrolled 89 withdrew prior to Clinic Visit 6. The main reason for withdrawal was migration from study area. No subject withdrew due to an AE. The RTS,S/ASO1 $_{\rm E}$ and rabies control group were balanced in both cohorts for the covariates assessed (age, gender, area, distance of residence from nearest health centre, bedbed net usage [about 80%], indoor residual spraying (IRS) and altitude). The mean age overall at randomisation was 11.4 months and ~50% were male. The most important protocol deviation was that ACD was not commenced after dose 3 at Korogwe although PCD occurred.

Vaccine efficacy up to the cross-sectional visit (V6) for both study sites

Covariate-adjusted VE against first or only PDef (ACD/PCD) was 52.9% (95% CI: 28.1 to 69.1, p<0.001).

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Figure 3. Kaplan-Meier survival curves showing the cumulative incidence of *P. falciparum* disease (Case Definition 1) (ATP Cohort for Efficacy [Month 2.5-Xsec])



X-sec = Cross-sectional visit (Clinic Visit 6). The cross-sectional visit, scheduled for $4\frac{1}{2}$ months post Dose 3, took place between 7 and 13 months post Dose 1 (mean 10 months, SD 1.29); the efficacy follow-up was between $4\frac{1}{2}$ and $10\frac{1}{2}$ months (mean 8 months, SD 1.14)

Gr 1 = RTS,S/ASO1E; Gr 2 = Rabies vaccine

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Table 16. Vaccine efficacy against *P. falciparum* (ATP Cohort for Efficacy [Month 2.5-Xsec])

	RTS,S/AS01E				Rabies				Point estimate of VE adjusted			
	Subjects	No. of events	PYAR	Rate	Subjects (N)	No. of events	PYAR	Rate	for covariates ¹			
	(N)								(%)	95% CI		P value
Disease 1º	402	32	244.77	0.13	407	66	238.51	0.28	52.9	28.1	69.1	<0.001
Disease 2 ^b	402	33	244.72	0.14	407	70	237.21	0.30	54.6	31.2	70.0	<0.001
Disease 3c	402	33	244.72	0.14	407	72	236.42	0.31	56.0	33.4	70.9	<0.001
Disease 1d	402	38	253.69	0.15	407	86	255.36	0.34	55.8	31.0	71.7	<0.001
Disease 2e	402	40	253.62	0.16	407	94	255.06	0.37	58.0	34.8	73.0	<0.001
Disease 3f	402	40	253.62	0.16	407	96	254.99	0.38	59.3	36.9	73.7	<0.001
	RTS,S/AS01E				Rabies				Point estimate of VE unadjusted			
	Subjects No. of		PYAR Rate	Subjects	No. of	PYAR	Rate	for covariates				
	(N)	events			(N)	events			(%)	95% CI		P value
Disease 1º	415	32	249.23	0.13	420	68	242.06	0.28	55.0	31.4	70.4	<0.001
Disease 2 ^b	415	33	249.17	0.13	420	72	240.76	0.30	56.5	34.2	71.2	<0.001
Disease 3º	415	33	249.17	0.13	420	74	239.98	0.31	57.7	36.3	72.0	<0.001
Disease 1d	415	38	258.15	0.15	420	89	259.61	0.34	57.9	34.3	73.0	<0.001
Disease 2e	415	40	258.07	0.15	420	97	259.31	0.37	59.5	37.1	73.9	<.0001
Disease 3f	415	40	258.07	0.15	420	99	259.24	0.38	60.4	38.8	74.4	<.0001

X-sec = Cross-sectional visit (Clinic Visit 6). The cross-sectional visit, scheduled for 4½ months post Dose 3, took place between 7 and 13 months post Dose 1 (mean 10 months, SD 1.29); the efficacy follow-up was between 4½ and 10½ months (mean 8 months, SD 1.14)

PYAR: Episodes/Person Years at Risk; VE: Vaccine Efficacy (1-HR); CI: Confidence Interval; p value from Cox PH model; Poisson regression for multiple episodes a first or only episodes; the presence of P. falciparum asexual parasitemia > 2500 per μ L and the presence of fever \geq 37.5°C detected by ACD or PCD

b first or only episodes; the presence of P. falciparum asexual parasitemia > 0 per μ L and the presence of fever \geq 37.5°C detected by ACD or PCD c first or only episodes; any level of P. falciparum asexual parasitemia > 0 per μ L and (the presence of fever \geq 37.5°C or a clinical diagnosis of malaria) detected by ACD or PCD

d multiple episodes; the presence of P. falciparum asexual parasitemia > 2500 per μ L and the presence of fever \geq 37.5°C detected by ACD or PCD e multiple episodes; any level of P. falciparum asexual parasitemia > 0 per μ L and the presence of fever \geq 37.5°C detected by ACD or PCD f multiple episodes; any level of P. falciparum asexual parasitemia > 0 per μ L and (the presence of fever \geq 37.5°C or a clinical diagnosis of malaria) detected by ACD or PCD

VE adjusted for covariates was similar between Kilifi (54.6% [95% CI: 24.7 to 72.6, p=0.002]) and Korogwe (56.4% [95% CI: 4.0 to 80.2, p=0.039]). VE was comparable using the second and third case definitions and for multiple events. Few children ($\leq 2.1\%$) had second or third episodes of malaria complying with the PDef. At V6 the rates for parasitaemia were low (RTS,S/AS01_E 1.8% vs. control 2.8%) but parasite density tended to be higher in the control group (3486/µL vs. 1020/µL).

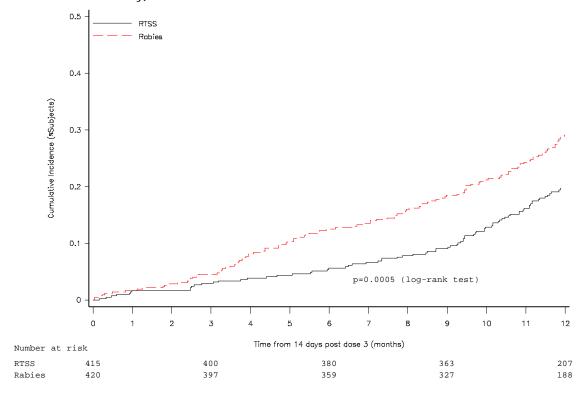
Vaccine efficacy up to month 14 (visit V7) for both study sites

At month 14 VE against first or only episode meeting the PDef adjusted for covariates was 39.2% (95% CI: 19.5 to 54.1, p=0.0005). VE unadjusted for covariates was 38.5% (95% CI: 18.7 to 53.4, p=0.0006). VE adjusted for covariates was similar at both sites (Kilifi 43.4% [95% CI: 20.2 to 59.9, p=0.001; Korogwe 38.6% [95% CI: -03 to 62.3, p=0.05]). VE based on the secondary case definition, adjusted and unadjusted for covariates was similar to that using the PDef.

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^{1.} Adjusted for site age bednet use area and distance from health center

Figure 4. Kaplan-Meier survival curves showing the cumulative incidence of *P. falciparum* disease meeting the primary case definition over a follow-up of 12 months post Dose 3 [M2.5 - M14] (ATP Cohort for Efficacy)



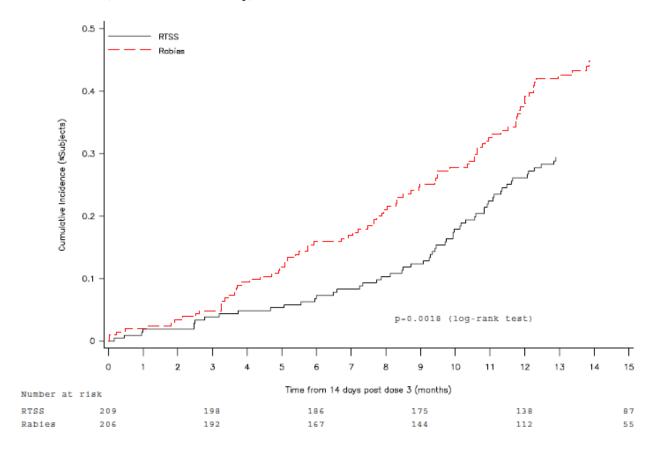
Vaccine efficacy up to month 18 for Kilifi

Among the 447 enrolled in Kilifi there were 349 subjects in the extension phase up to V9, which was conducted at a mean of 17.8 months on study, corresponding to a mean of 15.4 months after dose 3.

- VE against first or only episode meeting the PDef adjusted for covariates was 45.8% (95% CI: 24.1 to 61.3, p=0.0004) and VE unadjusted was 41.0% (95% CI: 17.7 to 57.8, p=0.002).
- VE of RTS,S/ASO1_E against first or only episode meeting the secondary case definition adjusted for covariates was 46.1% (95% CI: 25.4 to 61.0, p=0.0004) and VE unadjusted was 41.1% (95% CI: 18.8 to 57.3, p=0.001).
- VE against multiple events meeting the PDef adjusted for covariates was 50.8% (95% CI: 28.6 to 66.1, p=0.0002) and VE unadjusted was 46.5% (95% CI: 21.8 to 63.4, p=0.001).

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Figure 5. Kaplan-Meier survival curves showing the cumulative incidence of *P. falciparum* disease meeting the primary case definition over a mean follow-up of 15 months post Dose 3 [M2.5 - XsecExt] (ATP Cohort for Efficacy)



Malaria-059 extended follow-up in Kilifi to 7 years as an investigator-initiated study. Over the first 4 years of FU, adjusted VE against all episodes of clinical malaria (defined as *P. falciparum* parasitaemia $\geq 2500/\mu l$ and temperature $\geq 37.5^{\circ}C$) was 23.5% (95% CI: -0.7 to 41.9, p=0.06). Efficacy data up to 7 years of follow-up are awaited.

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Comparative incidence of clinical malaria in children 5-17 months in Malaria-059 over 6 years (ITT cohort)

	RTS,S/A	S01			Rabies	control vaccine		Vaccine efficacy			
Year	N	PYAR	cases	cases /PYAR	N	PYAR	cases	cases /PYAR	%	95% CI	
1	223	203.20	98	0.48	224	202.91	151	0.74	37.6	8.8	57.2
2	204	197.00	125	0.63	202	187.40	160	0.85	19.9	-19.9	46.5
3	178	172.18	132	0.77	158	154.39	145	0.94	19.9	-17.5	45.4
4	175	174.90	163	0.93	158	154.42	142	0.92	-2.3	-45.7	28.2
5	172	168.42	174	1.03	157	153.23	123	0.80	-33.6	-88.8	5.5
6	171	164.84	85	0.52	154	147.79	68	0.46	-11.5	-72.9	28.1

Personal communication from the investigator

N= number of children, PYAR= per year at risk

Cases= cases of clinical malaria (P. falciparum asexual parasitaemia > 2,500 per μ L and the presence of fever \geq 37.5°C)

Information on severe malaria cases was not collected as an efficacy endpoint but there were 25 SAEs of *P. falciparum* infection, cerebral malaria and malaria over 6 years in the control group vs. 10 in the RTS,S/AS01E group with no fatal cases reported.

Anti-CS and efficacy

Pre-vaccination seropositivity rates were < 5% and GMTs were < 0.5 EU/mL. At M3 99.7% in the RTS,S/AS01 $_{\rm E}$ group vs. 4.5% of controls were seropositive with GMTs of 539.6 vs. <0.5 EU/mL. At the cross sectional visit, there was no decrease in seropositivity rate in the RTS,S/AS01 $_{\rm E}$ group and no change for controls. There was no correlation between GMTs at screening and anti-CS GMTs after vaccination (r=0.06, p=0.21).

At M14 the GMTs were 41.6 EU/mL for RTS,S/AS01 $_{\rm E}$ and <0.5 EU/mL for controls with all vs. 4.9% seropositive. At the end of the extension (V9), the tGMTs were 31.3 EU/mL vs. <0.5 EU/mL and seropositivity rates of 98.1% vs. 4.2%.

At M3, V6 and M14, anti-CS antibody GMTs were similar in non-diseased and diseased subjects. The HR per 10-fold increase in anti-CS titre at M3 was 0.94, which results in a 5.9% (p=0.9; non-significant) reduction in the risk of malaria [M2.5 - M14]. HR per 10-fold increase in anti-CS titre at cross-sectional visit (V6) was 0.43, which results in a 56.8% (p=0.006) reduction in the risk of malaria during the period from cross-sectional visit to month 14.

Anti-HBs

All subjects had received HBsAg vaccine at age 6, 10 and 14 weeks. At pre-vaccination there were \sim 95% seroprotected with similar GMTs between groups. However, at M3 the GMT was significantly higher for the RTS,S/AS01_E group (46776 vs. 168168) and only one subject was not seroprotected. There was a strong correlation between GMTs at screening and the titre after vaccination (r=0.48, p<0.001). At M14 seroprotection rates were 99.7% vs. 91.6% for controls but with a large difference in the GMTs 12356 vs. 108).

T-cell immune responses assessed by ICS (Kenya)

Similarly low CS-specific CD4+ T-cell responses were observed in both groups before vaccination.

 At M3 the frequency (GM) of CS-specific CD4+ T-cells expressing at least IL-2 in the RTS,S/AS01_E group and control group was 681/106 cells and 212/106, respectively (p<0.0001). At M14 the respective GMs were 102/106 and 1/106 (p<0.0001).

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- At M3 the frequency of CS-specific CD4+ T-cells expressing at least TNF- α was 426/106 cells in the RTS,S/AS01_E group and 182/106 cells in the control group. Corresponding values at M14 were 48/106 and 8/106 cells (p<0.0001).
- At M3 the frequency of CS-specific CD4+ T-cells expressing at least IFN- γ was 26/106 cells in the RTS,S/AS01_E group and 14/106 cells in the control group with M14 values of 20/106 and 10/106 cells (p<0.05).
- At M14 the frequency of CS-specific CD8+ T-cells expressing at least IL-2 was 20 per 106 cells in the RTS,S/AS01_E group and 9 per 106 in the control group (p<0.01). Significant differences were not seen at M3 or for other cell types.

T-cell immune responses assessed by ELISPOT (Kenya)

The assessment of CMI responses with ELISPOT was performed on blood samples from Kenya.

- For IFN-γ cultured ELISPOT results were significantly higher in the RTS,S/AS01_E group at M3 and V6 but not at M14. For both the ex vivo IL-2 ELISPOT and cultured IFN-γ ELISPOT, the vaccine induced cellular responses were limited to two peptide pools (i.e. TH2R and TH3R/CS.T3T pools).
- For IFN-γ ex vivo ELISPOT results did not vary by vaccination group at any time point. IL-2 ex vivo ELISPOT responses were significantly greater in the RTS,S/ASO1_E group at M3 but not at V6.

Humoral immune response to malaria blood stage antigens up to M14

These assessments were performed for both study centres.

- Antibody concentrations to AMA-1, EBA-175 and MSP-142 decreased with age during the first year of life then increased to 32 months of age.
- Anti–MSP-3 antibody concentrations gradually increased, and GIA gradually decreased up to 32 months.
- Vaccination with RTS,S/AS01E resulted in modest reductions in AMA-1, EBA-175, MSP-142 and MSP-3 antibody concentrations and no significant change in GIA.
- Increasing anti-merozoite antibody concentrations and GIA were prospectively associated with increased risk of clinical malaria.

It was concluded that antibodies to blood stage antigens appeared to reflect past exposure to malaria parasite rather than the vaccination status.

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3.5.2. Main study

Phase 3 Pivotal efficacy study in two age groups

Ctudu		Study Doolan	Ctudy population		Number of	Subjects	
Study	Objective(s)	Study Design	Study population	Study groups	TVC	ATP efficacy	ATP immuno
Malaria- 055	1°: Efficacy against clinical disease 2°: Efficacy against severe disease; Role of booster; Efficacy against hospitalization and mortality	Phase 3, double-blind, randomized (1:1:1), controlled, multi-centre, multi-country study with three groups in two cohorts 0-1-2-20 months	infants and children 6-12 weeks and 5- 17 months Burkina Faso, Gabon,	children 5-17 months of age: RTS,S/AS01ε(R3R), 25μg/0.5ml RTS,S/AS01ε(R3C), 25μg/0.5ml Rabies vaccine(C3C)	R3R+R3C = 5949 C3C=2974	R3R+R3C = 2830; C3C = 1466	R3R+R3C = 1060; C3C = 540
					8923	4296	1600
				infants 6-12 weeks of age*: RTS,S/AS01ε(R3R), 25μg/0.5ml RTS,S/AS01ε(R3C), 25μg/0.5ml MCC (C3C)	R3R+R3C = 4358 C3C=2179	R3R+R3C = 3995 C3C = 2008	R3R+R3C = 1280 C3C = 658
				* Tritanrix-HepB™/Hib (DTPw- HepB/Hib) + OPV to all groups at 6, 10, 14 weeks of age	6537	6003	1938

The study was initiated in March 2009 at 11 centres and data have been reported in stages.

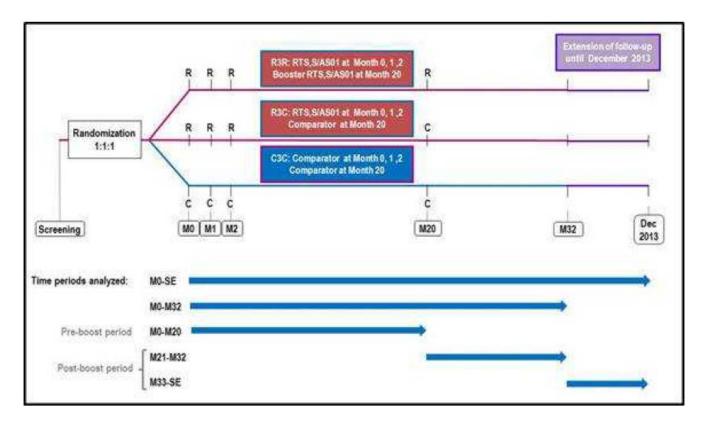
- The analysis at month 20 (18 months post-dose 3, all results before the 4th dose) was submitted in the initial Application.
- The final analysis at month 32 (month 30 post-dose 3) including the 4th dose data, and the analysis of the extension phase (from month 32 up to month 55), was made available during the procedure

The description of the results after the primary series is structured in the following order:

- Efficacy data up to month 14 and month 20 in subjects aged 5-17 months at enrolment
- Efficacy data after the 4th dose (post-boost as defined in the protocol) in subjects aged 5-17 months at enrolment
- Immunogenicity for the first 200 per study site aged 5-17 months at enrolment
- Efficacy data up to month 14 and 20 in subjects aged 6-14 weeks at enrolment
- Efficacy data after the 4th dose (post-boost as defined in the protocol) in subjects aged 6-12 weeks at enrolment
- Immunogenicity for the first 200 per study site aged 6-14 weeks at enrolment
- Efficacy against severe malaria
- Immunogenicity in HIV positive subjects

The study was planned to include up to 16,000 subjects across the three treatment groups and two age categories 6-12 weeks and 5-17 months, including at least 6000 in each age category. The overall study design was as follows:

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Eligible subjects were aged 5-17 months or 6-12 weeks and at least 28 days post-natal at the screening visit. Subjects aged 6-12 weeks were not to have received any vaccine against diphtheria, tetanus or pertussis or *Haemophilus influenzae* type b. Subjects were excluded if they had:

- An acute disease at the time of enrolment (moderate or severe illness with or without fever)
- Clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality
- Haemoglobin < 5.0 g/dL or < 8 g/dL associated with heart failure or severe respiratory distress
- Major congenital defects
- History of allergic reactions, significant IgE-mediated events or anaphylaxis to previous immunizations
- A history of a neurological disorder or atypical febrile seizure
- Malnutrition requiring hospital admission
- HIV disease of Stage III or Stage IV [WHO, 2005]

In children 5-17 months of age at enrolment

The control vaccines were a cell culture rabies vaccine (Verorab, Sanofi-Pasteur) on a 0, 1, 2-month schedule and a meningococcal C conjugate vaccine (MCC; Menjugate, Novartis) at Month 20.

In children 6-12 weeks of age at enrolment

The test and control groups were vaccinated as shown below.

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Vaccine	Sch	edule	Route of	Site of injection		
	Primary (0, 1, 2 months)	Booster (Month 20)	administration			
RTS,S/AS01 _E	X	X	Intramuscular	Left anterolateral thigh for primary course Left deltoid for booster		
MCC vaccine*	Х	Х	Intramuscular	Left anterolateral thigh in children < 1 year, into left deltoid in children > 1 year		
Tritanrix HepB/Hib	X	-	Intramuscular	Right antero-lateral thigh		
Polio Sabin	X	X	Oral	-		

BCG, a neonatal dose of OPV, measles and yellow fever vaccines were given according to local policy.

- Use of immune modifying drugs or blood transfusions was captured through the hospital surveillance system for severe disease.
- Use of antimalarial agents was captured through the surveillance system for clinical malaria.
- Use of antipyretics, analgesics or systemic antibacterial agents was captured for children who were assessed for reactogenicity in the 6-day period following each vaccine dose (i.e. the first 200 children vaccinated in each age category at each study centre).
- There was no routine testing for HIV. HAART and PMCT were available at all study centres according to national policies.
- Research teams at study sites were to ensure that insecticide treated bednet (ITN) use was optimised.

The co-primary objective in each age group at the time of the first dose was to evaluate the protective efficacy of RTS,S/AS01E against clinical malaria (primary case definition) caused by *Plasmodium falciparum*. Secondary objectives included:

- To evaluate the protective efficacy of RTS,S/AS01E on a primary schedule with and without booster dose against secondary case definitions of clinical malaria
- To evaluate the protective efficacy of RTS,S/AS01E on a primary schedule with and without booster dose against severe malaria
- Other secondary objectives included assessments of safety and immunogenicity (anti-CS and anti-HBsAq antibodies)

Two methods were used for the determination of P. falciparum density in blood samples:

- Method 1 counts against a known white blood cell concentration and follows the principles described by Greenwood and Armstrong (1991)
- Method 2 counts against an assumed known blood volume and follows the principles described by Planche et al. (2001)

Blood was collected by venipuncture or finger or heel prick and transferred to the slide directly or to an EDTA tube. Two slides per subject were prepared and all were read twice by two independent microscopists. If the initial two readings gave concordant results, the final parasite density was considered to be the geometric mean of the two readings. A third independent microscopist read the slide if:

- 1. Initial readings gave discrepant positive and negative readings
- 2. Both microscopists recorded parasitaemia >400 parasites/ μ L but with a ratio between values >2

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3. at least one microscopist recorded parasitaemia ≤400 parasites/µL but the higher reading was > 10 times the lower reading

If the readings were discordant, then the following principles were applied:

- 1. If one reading was positive and the other negative, the majority decision obtained following the reading by the third microscopist was adopted. If this was positive, the final result was the geometric mean of the two positive results
- 2. Where all three readings were positive, the final result was the geometric mean of the two closest readings.

All parasite species were identified.

An internal QC was performed on one negative and one positive slide for each batch of stain. The EQA process comprised species identification and parasite quantification with 3 assessments per year including 20 samples per microscopist. The "true value" was the median of the values obtained from the Parasitology Reference Unit of the NICD, two WHO reference laboratories and the laboratories of the study centres.

The case detection methodology was PCD at health facilities within the study area. A blood sample was taken for evaluation of malaria parasites in all children reported to have had a fever within 24 h or with an axillary temperature \geq 37.50C. There was training of clinicians in the assessment of clinical signs and the standardisation of equipment used for laboratory investigations.

The clinical study report (CSR) states definition of clinical malaria had a minimum specificity of 80% at each site based on substantial previous research. Three secondary case definitions of clinical malaria were also evaluated. The approach was in accordance with the recommendation of the WHO on measures of malaria vaccine efficacy (2007).

Case definition for clinical mala	ıria
1° Definition	P. falciparum asexual parasitemia > 5000 parasites/µL AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility OR a case of malaria meeting the primary case definition of severe malaria disease
2° Definition 1	P. falciparum asexual parasitemia > 0 AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation or history of fever within 24 hours of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility
2° Definition 2	P. falciparum asexual parasitemia > 500 parasites/µL AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility
2° Definition 3	P. falciparum asexual parasitemia > 20 000 parasites/µL AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility

Cases meeting the case definition of severe malaria were all included in the analysis of clinical malaria whether or not they meet the case definition for clinical malaria.

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Primary case definition of severe malaria is detailed in the Table below:

P falciparum >5000 parasites per μL	
AND with one or more marker of disease severity	 Prostration Respiratory distress Blantyre score ≤ 2 Seizures 2 or more Hypoglycemia < 2.2 mmol/L Acidosis BE ≤-10.0 mmol/L Lactate ≥ 5.0 mmol/L Anemia < 5.0 g/dL
AND without diagnosis of a co-morbidity	 Radiographically proven pneumonia Meningitis on CSF examination Positive blood culture Gastroenteritis and dehydration

Prostration is defined as in an acutely sick child, the inability to perform previously-acquired motor function in a child previously able to stand, inability to stand, in a child previously able to sit, inability to sit in a very young child, inability to suck.

Respiratory distress is defined as lower chest wall indrawing or abnormality deep breathing

2 or more seizures occurring in the total time period including 24h prior to admission, the emergency room and the hospitalisation

Radiographically proven pneumonia is a consolidation or pleural effusion as defined in the protocol on a chest X-ray taken within 72h of admission Meningitis on CSF examination is defined as WC \geq 50 x106/L or positive culture of compatible organism or latex agglutination positive for Hib, pneumococci or meningococci [Berkley 2001]

Gastroenteritis with dehydration is defined as a history of 3 or more loose or watery stools in previous 24h and an observed watery stool with decreased skin turgor (> 2 seconds for skin to return following skin pinch)

Positive blood culture as defined in the protocol on a blood culture taken within 72h of admission

Secondary case definitions of severe malaria are:

2° definition 1	P. falciparum > 5000 parasites per µL
"With co-morbidity"	AND with one or more marker of disease severity
2° definition 2	P. falciparum > 0
"Without a density threshold"	AND with one or more marker of disease severity
	AND without diagnosis of a co-morbidity
2° definition 3	P. falciparum > 0
"Without HIV"	AND with one or more marker of disease severity
	AND without diagnosis of a co-morbidity

All children were sampled for anti-HBs and anti-CS testing. The immune response to a primary course was evaluated pre vaccination and one month post primary course in the first 200 subjects in each age category enrolled at each centre and assayed as follows:

Assay	Marker	Assay method	Test kit/ Manufacturer	Assay unit	Assay cut-off	Laboratory
Anti-CS antibodies	R32LR	ELISA	In-house ELISA	EU/mL	0.5	CEVAC
Anti-HBs antibodies		ELISA	In-house ELISA	mIU/mL	10*	CEVAC

^{*:} seroprotective level

CEVAC: Center for Vaccinology, Ghent University ELISA: Enzyme-linked Immunosorbent Assay

EU/mL: ELISA Unit per milliliter

The monitoring strategy was implemented by one Regional Operational Manager and was insourced or outsourced at different sites. All critical data for endpoint evaluation were fully verified. Data was collected by electronic data capturing (RDE). During monitoring visit 20% of the subjects were full source document verified. The remaining 80% of subjects had selected data fully verified. The frequency of monitoring was defined as one initial monitoring visit performed at each site within 1-2 weeks of the first subject enrolled. Thereafter monitoring visits were performed a minimum of every 4 weeks per site. On completion of enrolment monitoring frequency could be reduced to 6-weekly visits. The blood slides and FTA cards collected during this study were centrally stored at Quintiles Laboratory

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located in Pretoria, South Africa. This study was subject to audits by the sponsor's Worldwide Regulatory Compliance-GCP (WRC-GCP).

Assuming at least 5400 evaluable subjects (randomised 2:1), an attack rate in controls of 10/100 children years at risk (cyr) over the follow-up period from 2 weeks to one year post Dose 3 and a true vaccine efficacy of 30%, the study had 90% power to detect a lower limit of the 97.5% CI around estimated VE above 0%. The analysis was to be conducted when 450 cases had been accumulated or until the boost Visit at 22 months (~18 months post Dose 3) had been completed, whichever occurred earlier.

The secondary analysis of efficacy against severe malaria was performed when approximately 250 episodes of severe malaria met the primary case definition. This was a total pooled over the study centres and age categories and gave 80% power to detect 30% VE with a lower limit of the 95% CI above 0%. Assuming 50% VE 250 episodes gave 90% power to detect a lower limit of the 95% CI above 25%.

Data pertaining to RTS,S/AS01E or control vaccines was collected in a double blinded (observer blind) manner, i.e. families of the vaccinees and those evaluating study endpoint data were unaware of treatment assignments. The contents of the syringes were masked with an opaque label to avoid unblinding of parent/guardian but staff administering the vaccines were aware of the vaccine assignment.

Modified Intention to treat population (ITT)

For operational reasons randomised subjects who did not receive study vaccine were not followed-up further. Therefore, the modified ITT population included all subjects that received at least one dose of study vaccine and cases were counted from the time of the first dose onwards.

According to protocol (ATP) population for efficacy

The ATP population for efficacy contains all subjects included in the ITT who received all vaccinations according to protocol procedures within specified intervals that contributed to the time at risk in the follow-up period starting 14 days post Dose 3.

The primary analysis was carried out after 6000 subjects had been enrolled in the age category under evaluation and followed for 14 months. Since enrolment into the 5-17 months age category was faster the co-primary analyses were conducted at different times. In order to control the overall alpha-level (5%) each was performed at a 2.5% alpha-level (Bonferroni correction), leading to 97.5% CI. The co-primary analyses were based on first or only episodes of *P. falciparum* malaria (primary case definition, site-adjusted) over a follow-up period [2½-14] in the ATP population.

<u>For analyses of first or only episodes</u> of *P. falciparum* malaria the distribution of the survival time was compared with Log-rank tests. VE was assessed using Cox regression models. The primary analysis was stratified for study site but unadjusted for other covariates. Adjusted and unadjusted estimates were presented for the ATP analyses. Cox regression assumes proportional hazards throughout the follow-up period. This assumption was checked by a test based on the Schoenfeld residuals and AIC and SBC of models with time-varying covariates.

<u>For analysis of all episodes</u> of *P. falciparum* malaria negative binomial regression allowing for interdependence between episodes within the same subject was used. The 95% CI and p-values of VE estimates were calculated from this model.

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Risk Period

For each endpoint, the time at risk was calculated separately. The time at risk was counted in days and expressed as person years at risk (days/365.25).

In order to avoid mathematical problems because of time equals zero when an event occurred the same day that the time at risk started, the first day counted as 1 so that the duration was calculated as (date of event or censoring – date of start follow up +1).

For endpoints evaluating first or only malaria episodes, time at risk was counted in days and expressed as child years at risk (days/365.25). Time at risk ended whenever one of the following conditions occurred first: meets the case definition under evaluation, lost to follow-up, emigration from the study area, consent withdrawal, death or end of follow-up period.

For endpoints evaluating all malaria episodes where time at risk did not end when the episode met the case definition, 14 days following the episode were subtracted from the time at risk (day of episode + 14). If an episode was detected during a period of time not counting for the time at risk it was not included.

Covariates

All analyses of clinical malaria were adjusted for study site. For the Cox model, this was done by using site as a stratification factor, allowing different baseline hazards between study sites. ATP analyses evaluating first or only episodes or all episodes of clinical malaria were also performed adjusted for other covariates. Covariates were:

- Study site (as strata for Cox model)
- Age at first vaccination (2 levels [5-11] months, [12-17] months)
- Distance to outpatient facility (2 levels: [0-5] and [6-] km)

Bednet use was not a covariate as this was to be optimised in all study sites at baseline.

Important protocol amendments after study initiation

- Based on a theoretical concern that the use of new adjuvanted vaccines could impact on immunological self-tolerance, regulatory authorities required optimizing the data collection on immune-mediated diseases (IMD). The sponsor defined IMD as AEs of interest.
- The follow-up period of the study was extended. Based on the enrolment at the time of the protocol amendamendement, ment, the mean follow-up time was planned to be 49 months post Dose 1 (range: 41-55) for the 5 to 17 months age category and 41 months post Dose 1 (range: 32-48) for the 6 to 12 weeks age category.
- The protocol was amended to collect during this extension study data on severe malaria, malaria hospitalisation and parasite prevalence in the 11 participating centres using the same methodologies and case definitions as in the primary phase. Occurrence of SAEs was to be monitored in all centres. Surveillance for clinical malaria was to take place in at least 3 centres with varying transmission levels. Immunogenicity endpoints were to be collected on a subset of individuals from both age categories in at least these 3 centres.

Protocol deviations

Some of the more notable deviations included the following:

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Manhiça - several study vaccines (RTS,S/AS01E or VeroRab) had been exposed to temperatures outside of the acceptable storage range. Among the 136 children that had not yet received the third dose, 24 did not receive the third administration dose and the rest had a delay from 3.5 to 4.5 months. The 996 subjects from the 5-17 months age category impacted by this deviation were excluded from the ATP population. This site failed to report as SAEs 9 deaths that were detected only during the cleaning process.

Lilongwe - failed to provide bednets to all screened children and gave them only to enrolled subjects.

Kombewa - failed to provide bednets at study start until February 2010.

Korogwe - failed to provide bednets until June 2010.

Kilifi – failed to provide bednets directly to screened subjects because the site was advised by the Ministry of Health representative that at age 6-12 weeks children received a net at the BCG visit 2 and all 5-17 months children received bednets from the PLAN program.

GCP inspection

As part of the assessment a routine GCP inspection was requested for the clinical trial 110021 (Malaria-055). The inspection was carried out at three investigator sites in Gabon, Tanzania and Malawi. The inspection team concluded that the data generated at the investigator sites were acceptable and could be used for the evaluation and assessment of the application. Although falsification of data at one site was detected this was limited to a small portion of the trial at that site and the sensitivities analyses performed by the sponsor demonstrate that this has minimal impact on data quality.

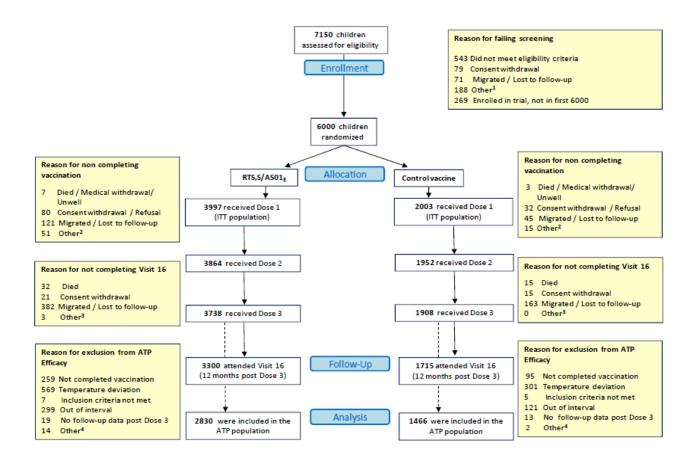
Based on the inspection findings reported it was concluded that the trial has been conducted in compliance with GCP at this investigator site.

3.5.2.1. Efficacy data in subjects aged 5-17 months at enrolment

Efficacy data up to Month 14 post-dose 1

Disposition of the first 6000 to be enrolled was as follows:

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Groups were balanced for age (mean age at first dose was 10.9 months) and gender (49% males) as well as for important covariates. At 12 months post-dose 3 the coverage of ITNs was 75.4% for the RTS,S/AS01E groups vs. 74.4% for controls but up to 33% in both groups were reported to be using ITNs with holes.

Unadjusted and adjusted VE against first or only episodes meeting the PDef over 12 months follow-up post-dose 3 was 55.8% (LL 97.5% CI 50.6%). VE did not show a significant interaction by study site (p=0.455). Proportionality of hazard over 12 months follow-up post-dose 3 was not demonstrated; the Schoenfeld residual was -0.19 (p<0.0001) (ATP population).

Table 17. Vaccine efficacy: First or only episodes of clinical malaria (Primary case definition) (97.5% CI) (ATP population for efficacy)

							VE		
_							97.5% CI		
Event Type	Group	N	n	T (year)	n/T	%	LL	UL	p-value
Any	R3R+R3C	2830	932	2144.0	0.435	55.8	50.6	60.4	< 0.0001
	C3C	1466	752	902.8	0.833	-	-	-	

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

n = number of subjects reporting at least one event(s) in each group

T (year) = sum of follow-up period expressed in years censored at the first occurrence of event in each group

n/T = person-year rate in each group

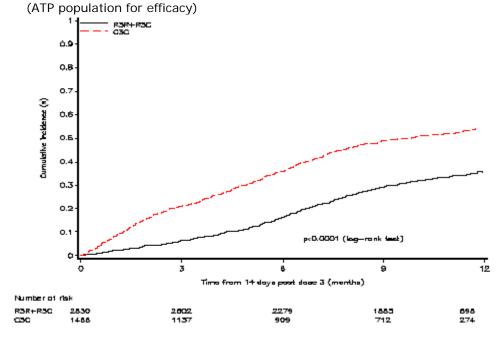
LL, UL = 97.5% Lower and Upper confidence limits

VE (%) = Vaccine efficacy (Cox regression model stratified by study site)

P-value from Cox regression model stratified by study site to test H0 = (Y = (start, stop))

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Figure 6. Cumulative incidence of first or only episodes of clinical malaria (Primary case definition)



Unadjusted and adjusted estimates of VE against first or only episodes of clinical *P. falciparum* malaria meeting SDefs were similar to estimates based on the PDef.

Table 18. Vaccine Efficacy: First or only episodes of clinical malaria (ATP population for efficacy)

	R3R+	R3R+R3C							unadjusted for covariates				Point estimate of VE adjusted for covariates			
	N	n	(year)		N	n	(year)		` '	95%		P value				P value
Primary Case Definition	2830	932	2144.0	0.435	1466	752	902.8	0.833	55.8	51.3	59.9	<0.0001	55.8	51.3	59.8	<0.0001
Secondary Case Definition 1												<0.0001				
Secondary Case Definition 2	2830											<0.0001				
Secondary Case Definition 3	2830	838	2195.8	0.382	1466	686	947.1	0.724	55.1	50.3	59.5	<0.0001	55.0	50.2	59.4	<0.0001

 $R3R+R3C = RTS_{r}S$ primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

n = number of subjects reporting at least one event(s) in each group

T (year) = sum of follow-up period expressed in years censored at the first occurrence of event in each group

n/T = person-year rate in each group

95% CI = Lower and Upper confidence limits of 95% CI

VE (%) = Vaccine efficacy (Cox regression model stratified by study site)

P-value from Cox regression model stratified by study site to test H0 = [VE=0%] (Y = (start, stop))

Adjusted for covariates: age at first vaccination and distance to outpatient health facility

Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/µL AND fever (axillary temperature ≥ 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility, or a case of malaria meeting the primary case definition of severe malaria disease

Secondary Case Definition 1: P. falciparum asexual parasitemia > 0 parasites/ μ L AND fever (axillary temperature $\ge 37.5^{\circ}$ C) or history of fever within the last 24h occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 2: P. falciparum asexual parasitemia > 500 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 3: P. falciparum asexual parasitemia > 20000 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who

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Unadjusted VE against all episodes meeting the PDef was 55.1% (95% CI: 50.5 to 59.3, p<0.0001).

Table 19. Vaccine Efficacy: All episodes of clinical malaria (ATP population for efficacy)

	R3R+	R3C			C3C	C3C			Point estimate of VE				Point estimate of VE				
										adjusted for site				adjusted for			
													covariates				
	N	n	pyr	rate	N	n	pyr	rate	(%)	95%	CI	P	(%)	95%	CI	Р	
												value				value	
Primary Case	2830	1834	2495.3	0.735	1466	1854	1263.2	1.468	55.1	50.5	59.3	<.0001	55.1	50.5	59.2	<.0001	
Definition																	
Secondary Case	2830	2999	2450.2	1.224	1466	2833	1225.5	2.312	53.7	49.5	57.6	<.0001	53.6	49.4	57.5	<.0001	
Definition 1																	
Secondary Case	2830	2105	2485.0	0.847	1466	2045	1255.8	1.628	53.4	48.9	57.5	<.0001	53.3	48.9	57.4	<.0001	
Definition 2																	
Secondary Case	2830	1567	2505.5	0.625	1466	1584	1273.6	1.244	54.7	49.8	59.1	<.0001	54.6	49.6	59.0	<.0001	
Definition 3																	

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

N = number of subjects included in each group

n = number of episodes included in each group

pyr = child years at risk

Rate = n /pyr

VE (%) = Vaccine efficacy (Negative binomial model)

95% CI = Lower and Upper confidence limits of 95% CI

P-value from Negative binomial model

Adjusted for covariates: age at first vaccination, distance to outpatient health facility and site

Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/µL AND fever (axillary temperature ≥ 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility, or a case of malaria meeting the primary case definition of severe malaria disease

Secondary Case Definition 1: P. falciparum asexual parasitemia > 0 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) or history of fever within the last 24h occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 2: P. falciparum asexual parasitemia > 500 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 3: P. falciparum asexual parasitemia > 20000 parasites/µL AND fever (axillary temperature ≥ 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility

The number of episodes of clinical malaria within and outside risk period (14 days following an episode) and the distribution of numbers of episodes of clinical malaria per subject for the PDef (ATP population) are provided below. The ITT results showed a similar pattern.

Table 20. Number of episodes of clinical malaria within and outside risk period (14 days following episode) (Primary case definition) (all episodes) (ATP population for efficacy)

	Episodes outside risk period	Episodes within risk period	Total
C3C	44	1854	1898
R3R+R3C	51	1834	1885

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

Risk period = ATP Time at risk

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Table 21. Distribution of total number of episodes of clinical malaria per subject (Primary case definition) (ATP population for efficacy)

		+R3C 2830		3C 1466		
Characteristics	Categories	n	%	n	%	P-values
Number of episodes	0	1898	67.1	714	48.7	< 0.0001
	1	458	16.2	288	19.6	-
	2	230	8.1	177	12.1	-
	3	140	4.9	116	7.9	-
	+3	104	3.7	171	11.7	-

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

N = number of subjects

n = number of subjects in a given category

% = n / Number of subjects with available results x 100

P-values: Chi-square test

Unadjusted VE against severe malaria over 12 months follow-up post-dose 3 (i.e. to month 14 of study, from dose 1) meeting the PDef was 47.3% (95% CI: 22.4 to 64.2, p=0.0008), as mentioned in Table 22.

Table 22. Vaccine Efficacy: Children affected by severe malaria (ATP population for efficacy)

HISTOR TOP CO	ovariates
	P value
	0.0008

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

N = number of subjects included in each group

n = number of subjects reporting at least one event in each group

Proportion affected (%) = percentage of subjects reporting at least one event

VE (%) = Vaccine Efficacy (Conditional Method)

95% CI = Lower and Upper confidence limits of 95% CI

P-value = Two-sided Fisher Exact test

Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/µL AND with one or more marker of disease severity AND without diagnosis of a co-morbidity

Secondary Case Definition 1: P. falciparum asexual parasitemia > 5000 parasites/µL AND with one or more marker of disease severity

Estimates of VE in the ITT population were very similar to those obtained in the ATP population for efficacy. For example, unadjusted VE of RTS,S/AS01E against first or only episodes of clinical malaria meeting the PDef was 50.4% (95% CI: 45.8 to 54.6, p<0.0001) over 14 months follow-up post-dose 1. The cumulative incidence curves diverged after about 2 weeks from the first dose onwards.

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Table 23. Vaccine Efficacy: First or only episodes of clinical malaria (ITT population)

	R3R+	R3C			C3C				un-a	djust riate	ed fo	
	N	n	(year)		N	n	(year)		` '			P value
Primary Case Definition	3997	1155	3633.4	0.318	2003	879	1587.5	0.554	50.4	45.8	54.6	<0.0001
Secondary Case Definition 1												
Secondary Case Definition 2	3997	1287	3517.6	0.366	2003	922	1538.9	0.599	47.0	42.3	51.4	<0.0001
Secondary Case Definition 3								0.480	50.5	45.6	54.9	<0.0001

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

n = number of subjects reporting at least one event(s) in each group

T (year) = sum of follow-up period expressed in years censored at the first occurrence of event in each group

n/T = person-year rate in each group

95% CI = Lower and Upper confidence limits of 95% CI

VE (%) = Vaccine efficacy (Cox regression model stratified by study site)

P-value from Cox regression model stratified by study site to test H0 = [VE=0%] (Y = (start, stop))

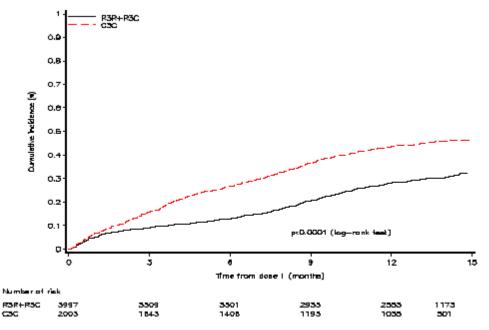
Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/µL AND fever (axillary temperature ≥ 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility, or a case of malaria meeting the primary case definition of severe malaria disease

Secondary Case Definition 1: P. falciparum asexual parasitemia > 0 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) or history of fever within the last 24h occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 2: P. falciparum asexual parasitemia > 500 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 3: P. falciparum asexual parasitemia > 20000 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility

Figure 7. Cumulative incidence of first or only episodes of clinical malaria (Primary case definition) (ITT population)



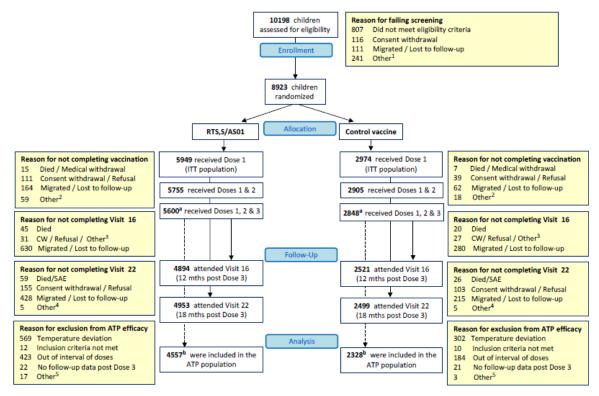
In the ITT population unadjusted VE against all episodes of clinical malaria meeting the PDef was 53.9% (95% CI: 49.6 to 57.8, p<0.0001) and unadjusted VE against severe malaria meeting the PDef

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was 45.1% (95% CI: 23.8 to 60.5, p=0.0002).

Efficacy data up to month 20 post-dose 1

The study report on efficacy data for all 8923 subjects aged 5-17 months at enrolment for up to 18 months post-dose 3 (20 months post-dose 1) with a cut-off date in April 2013 was provided. Patient disposition is summarised in the study flowchart.



Up to 18 months post-dose 3 overall VE against clinical malaria was 45.7% (95% CI: 41.7 to 49.5, p<0.0001). In contrast to the analysis at 12 months post-dose 3 the VE by site ranged from 40% to 77% with a significant interaction (p-value=0.0006). However, VE was statistically significant at all study sites.

Vaccine efficacy against clinical malaria up to Month 20: Overview ([5-17] months)

vaccine enicac	y against chincar n	iaia	ı ıa c	ip to i	/101111	20.	Ovi	CI VICV	, (LJ-	1711110	<i>/</i> 11113	,	
			R	3R+R3C				C3C		Point estima covariates	ate of VE	unadjust	ed for
ATP population for efficacy	(18 months post Dose 3)												
All episodes of clinical mala	aria (ATP population for efficacy)	N	n	T (year)	n/T	N	n	T (year)	n/T	(%)	95	% CI	P-value
Agogo	Primary Case Definition	371	288	511.66	0.56	192	296	256.16	1.16	53.55	40.34	63.84	<.0001
Bagamoyo	Primary Case Definition	462	68	648.40	0.1	235	89	321.70	0.28	65.37	46.16	77.73	<.0001
Kilifi	Primary Case Definition	336	4	450.65	0.01	171	9	229.30	0.04	77.39	26.36	93.06	0.0137
Kintampo	Primary Case Definition	602	801	793.29	1.01	296	702	379.18	1.85	47.19	39.05	54.23	<.0001
Kombewa	Primary Case Definition	609	978	811.12	1.21	311	762	407.72	1.87	40.17	28.51	49.92	<.0001
Korogwe	Primary Case Definition	568	33	817.29	0.04	293	44	418.72	0.11	61.09	34.83	76.77	0.0003
Lambarene	Primary Case Definition	380	63	550.95	0.11	196	57	283.39	0.2	42.48	11.22	62.73	0.0126
Lilongwe	Primary Case Definition	359	99	498.49	0.2	183	82	253.64	0.32	42.41	12.88	61.93	0.0091
Nanoro	Primary Case Definition	389	707	498.38	1.42	198	596	247.91	2.4	41.13	33.63	47.79	<.0001
Siaya	Primary Case Definition	481	1216	605.76	2.01	253	1002	302.63	3.31	43.25	33.06	51.89	<.0001
OVERALL	Primary Case Definition	4557	4257	6185.97	0.69	2328	3639	3100.35	1.17	45.72	41.71	49.46	<.0001

VE against first or only episodes using the PDef was 49% (95% CI 45, 52.6).

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Table 24. Vaccine efficacy against first or only episodes of clinical malaria (primary case definition) ([5-17] months) by site and overall (FU: M2.5-M20) (ATP population for efficacy)

									Point estin	nate of VE	unadjusted	for covariates
		R3F	R+R3C			. (C3C			95%	6 CI	
Site	N	n	T (year)	n/T	N	n	T (year)	n/T	(%)	LL	UL	p-value
Agogo	371	158	417.06	0.38	192	119	159.20	0.75	51.53	38.47	61.81	<0.0001
Bagamoyo	462	49	619.45	0.08	235	57	273.80	0.21	61.83	44.07	73.95	<0.0001
Kilifi	336	4	447.71	0.01	171	9	220.47	0.04	78.06	28.76	93.24	0.0116
Kintampo	602	368	544.79	0.68	296	244	175.72	1.39	52.80	44.47	59.89	<0.0001
Kombewa	609	357	553.00	0.65	311	231	202.46	1.14	42.72	32.38	51.48	<0.0001
Korogwe	568	31	802.32	0.04	293	34	402.68	0.08	53.98	25.10	71.73	0.0018
Lambarene	380	53	525.89	0.1	196	42	254.40	0.17	39.31	9.02	59.52	0.0156
Lilongwe	359	65	468.05	0.14	183	53	219.19	0.24	43.20	18.35	60.48	0.0023
Nanoro	389	317	279.22	1.14	198	178	110.38	1.61	48.04	37.46	56.83	<0.0001
Siaya	481	351	349.44	1	253	217	109.90	1.97	48.63	39.08	56.69	<0.0001
OVERALL	4557	1753	5006.93	0.35	2328	1184	2128.20	0.56	48.98	45.05	52.64	<0.0001

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

n = number of episodes included in each group

T(year) = person years at risk

n/T = person year rate in each group

LL, UL = 95% Lower and Upper confidence limits

VE (%) = Vaccine efficacy (Cox regression model)

P-value of the Wald test from a Cox regression model to test HO = [VE=0%]

VE waned over the 18-months follow-up period.

Among children enrolled in the 5-17 months age category, the incidence comparison of all episodes of clinical malaria (primary case definition) assessed over 6 month breakdown periods in the ATP population for efficacy was:

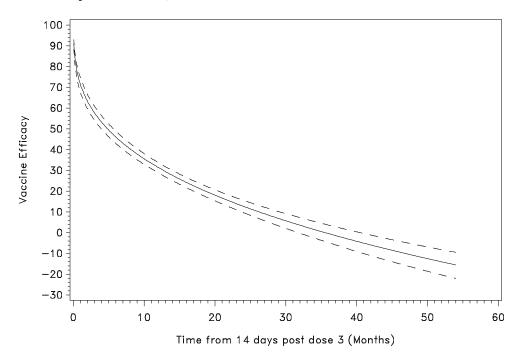
[Month 2.5 – Month 8]: 68.3 % (95% CI; 64.3 to 71.8 p < 0.0001)

]Month 8 – Month 14]: 41.1 % (95% CI; 35.8 to 46.0 p < 0.0001)

]Month 14 – Month 20]: 26.3 % (95% CI; 18.5 to 33.4 p < 0.0001)

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Figure 8. Vaccine efficacy against all episodes of clinical malaria (primary case definition) over time of a primary schedule without 4th dose in the 5-17 months age category (M2.5-SE) (ATP cohort for efficacy, Malaria-055)



RTS,S/AS01E showed significant efficacy overall against severe malaria (ATP 35.5% [95% CI: 14.6 to 51.1, p=0.0016]; ITT 33.9% [95% CI: 15.3 to 48. 3, p=0.0007]). Efficacy was also shown against malaria hospitalisation (41.5% [95% CI: 29.1 to 51.7, p<0.0001]), severe anaemia (51.0% [95% CI: 10.5 to 73.2, p=0.0146]) and all-cause hospitalisation (19.0% [95% CI: 8.5 to 28.2, p=0.0002]) over 18 months post-dose 3 (ATP population for efficacy).

Vaccine efficacy against severe malaria up to Month 20: Overview ([5-17] months)

			R3R	+R3C		C	3C	Point estimat	te of VE una	adjusted fo	or covariates
ATP population t	for efficacy (18 months post Dose 3)	•		•	•	•	•				•
Children affected efficacy)	d by severe malaria (ATP population for	N	n	Proportion affected	N	n	Proportion affected	(%)	95	% CI	P-value
Agogo	Primary Case Definition	371	13	0.04	192	8	0.04	15.90	-134	67.70	0.8150
Bagamoyo	Primary Case Definition	462	1	0	235	4	0.02	87.30	-28.5	99.70	0.0466
Kilifi	Primary Case Definition	336	0	0	171	0	0				-
Kintampo	Primary Case Definition	602	34	0.06	296	21	0.07	20.40	-44.3	55.10	0.4592
Kombewa	Primary Case Definition	609	19	0.03	311	18	0.06	46.10	-8.90	73.20	0.0743
Korogwe	Primary Case Definition	568	2	0	293	2	0.01	48.40	-612	96.30	0.6085
Lambarene	Primary Case Definition	380	2	0.01	196	6	0.03	82.80	3.80	98.30	0.0211
Lilongwe	Primary Case Definition	359	5	0.01	183	3	0.02	15.00	-447	83.50	1.0000
Nanoro	Primary Case Definition	389	9	0.02	198	6	0.03	23.70	-161	75.70	0.5904
Siaya	Primary Case Definition	481	35	0.07	253	27	0.11	31.80	-17.1	59.90	0.1254
OVERALL	Primary Case Definition	4557	120	0.03	2328	95	0.04	35.50	14.60	51.10	0.0016

Over the 18 months of follow-up post-dose 3, there were six cases of fatal malaria meeting the SDef for severe malaria [P. falciparum >5000 parasite per μL and one or more marker of disease severity]. VE against prevalent parasitaemia assessed at the cross-sectional survey at 18 months post-dose 3 was 30.7% (95% CI: 17.3 to 41.9, p<0.0001) (ATP population for efficacy).

Over the 18 months period post-dose 3, vaccination with RTS,S/AS01E averted overall:

 941 cases of clinical malaria per 1000 vaccinees, ranging between 47 and 2356 across study sites

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• 21 cases of severe malaria per 1000 vaccinees ranging from -4 to 44 across sites (clinical and severe malaria secondary case definition 1, ATP population for efficacy).

The exploratory model of determinants of incidence of clinical malaria showed that body weight and age at vaccination had borderline significance in the full model. In the final model malaria incidence was lower in those aged 5-11 months vs. 12-17 months at enrolment and for those with moderate anaemia vs. those with no anaemia.

Efficacy during follow-up and after the 4th dose in children aged 5-17 months at enrolment

Over the whole FU period (median FU of 46 months post Dose 3) VE against all episodes of clinical malaria was 26.2% (95% CI: 20.8 to 31.2) in children who received a RTS,S/AS01E primary vaccination course without a 4th dose. Protection against clinical malaria was enhanced when a 4th dose was administered, i.e., VE against clinical malaria was 39.0% (95% CI: 34.3 to 43.3), with an incremental efficacy of the 4th dose of 21% over the FU period up to SE, after the 4th dose. Without a4th dose, efficacy waned over time and was no longer significant during the last FU period (M31-SE). In contrast, significant VE persisted over all successive FU periods up to SE in children who received a RTS,S/AS01E 4th dose. Tables 25-26 summarise vaccine efficacy before and after administration of a 4th dose and demonstrate that point estimates were lower after the 4th dose compared to after the primary series.

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Table 25. Comparison of vaccine efficacy against first or only malaria episode and all malaria episodes endpoints in children aged 5-17 months having received RTS,S/AS01E according to the primary schedule during FU periods from Month 2.5 to Month 14 and Month 20

	Post-prir	nary vaccina	tion ana	lysis up	to Mo	nth 14	Post-primary vaccination analysis up to Month 20						
Endpoint	n/T R3	n/T C3C	VE	LL	UL	p-value	n/T R3	n/T C3C	VE	LL	UL	p-value	
Previously reported analysis°													
First or only case* (VE based on hazard ratios)	0.38	0.67	54.6	50.7	58.1	<0.0001	0.35	0.56	49.0	45.1	52.6	<0.0001	
All cases of malaria* (VE based on incidence ratios)	0.63	1.23	51.3	47.5	54.9	<0.0001	0.69	1.17	45.7	41.7	49.5	<0.0001	
New analysis as requested													
First or only case* (VE based on incidence ratios)	0.38	0.67	43.4	38.6	47.9	<0.0001	0.35	0.56	37.1	32.3	41.6	<0.0001	

Table 26. Incremental efficacy against clinical malaria (primary case definition) of a booster dose at Month 20 in children 5-17 months of age having received RTS,S/AS01E according to the primary schedule during FU period from Month 21 to Month 32 (ATP cohort of efficacy)

	R3R				R3C				VE			
	N	n	T (year)	n/T	N	n	T (year)	n/T	(%)	95% C	l	p-value
Post-booster analysis as reported in Malaria	-055 Anr	nex repo	rt 8									
First or only case* (VE based on hazard ratios)	2017	695	1514	0.46	2057	841	1430	0.59	27.2	19.5	34.2	<0.0001
All cases of malaria* (VE based on incidence ratios)	2017	1384	1933	0.72	2057	1872	1956	0.96	29.0	21.6	35.6	<0.0001
New analysis												
First or only case (VE based on incidence ratios)	2017	695	1514	0.46	2057	841	1430	0.59	21.9	13.6	29.5	<0.0001

R3C = RTS,S/AS01E primary schedule without booster

R3R = RTS, S/AS01E primary schedule with booster

Primary case definition used in analysis of clinical malaria case.

N = number of subjects included in each group

n/T = person year rate in each group with:

For first or only episode

n = number of subjects reporting at least one event in each group

T(year) = sum of follow-up period expressed in years censored at the first occurrence of event in each group

For all cases of malaria:

n = number of episodes included in each group

T(year) = person years at risk

Note: For point estimates shown in bold, the p-value was < 0.05.

VE (%) = Vaccine Efficacy unadjusted for covariates, except for study site, LL, UL = 95% Lower and Upper CI limits

Over the 18 months post Dose 3 FU period, VE against all episodes of clinical malaria stratified by age at the time of first dose was similar in children aged 5 to 11 months and 12 to 17 months, i.e., 45.16% (95% CI: 37.61 to 51.80) vs. 48.58% (95% CI: 41.16 to 55.06). There was no evidence for an interaction between age (5-11 months vs. 12-17 months) and vaccine assignment (p=0.4012). Also in the multivariate exploratory model for analysis of the covariates affecting the incidence of clinical malaria, there was no interaction between age (5-11 months vs. 12-17 months) and vaccine assignment, indicating that age was not affecting VE. However, age was a significant covariate for the incidence of clinical malaria (p=0.0217), i.e., the incidence was lower in the younger age group with an incidence rate ratio of 0.918.

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Table 27. Vaccine efficacy against all episodes of clinical malaria (primary case definition) of a primary vaccination schedule by age classification: children aged 5-11 months vs. 12-17 months at first dose (FU: M2.5-M20) (ATP cohort for efficacy)

								VE				
R3R+R	R3C			C3C					95% CI			
N	N	T (year)	n/T	N	n	T (year)	n/T	(%)	LL	UL	p-value	
2593	2314	3523.94	0.66	1333	1906	1773.82	1.07	45.16	37.61	51.80	<.0001	
1989	1972	2699.08	0.73	1003	1772	1333.76	1.33	48.58	41.16	55.06	<.0001	
4582	4286	6223.02	0.69	2336	3678	3107.58	1.18	46.20	42.23	49.89	<.0001	
	N 2593 1989 4582	2593 2314 1989 1972 4582 4286	N N T (year) 2593 2314 3523.94 1989 1972 2699.08 4582 4286 6223.02	N N T (year) n/T 2593 2314 3523.94 0.66 1989 1972 2699.08 0.73 4582 4286 6223.02 0.69	N N T (year) n/T N 2593 2314 3523.94 0.66 1333 1989 1972 2699.08 0.73 1003 4582 4286 6223.02 0.69 2336	N N T (year) n/T N n 2593 2314 3523.94 0.66 1333 1906 1989 1972 2699.08 0.73 1003 1772 4582 4286 6223.02 0.69 2336 3678	N N T (year) n/T N n T (year) 2593 2314 3523.94 0.66 1333 1906 1773.82 1989 1972 2699.08 0.73 1003 1772 1333.76 4582 4286 6223.02 0.69 2336 3678 3107.58	N N T (year) n/T N n T (year) n/T 2593 2314 3523.94 0.66 1333 1906 1773.82 1.07 1989 1972 2699.08 0.73 1003 1772 1333.76 1.33 4582 4286 6223.02 0.69 2336 3678 3107.58 1.18	R3R+R3C C3C N N T (year) n/T N n T (year) n/T (%) 2593 2314 3523.94 0.66 1333 1906 1773.82 1.07 45.16 1989 1972 2699.08 0.73 1003 1772 1333.76 1.33 48.58 4582 4286 6223.02 0.69 2336 3678 3107.58 1.18 46.20	R3R+R3C C3C F (year) N/T V (year) N/T N/T	R3R+R3C C3C 95% CI N N T (year) n/T N n T (year) n/T (%) LL UL 2593 2314 3523.94 0.66 1333 1906 1773.82 1.07 45.16 37.61 51.80 1989 1972 2699.08 0.73 1003 1772 1333.76 1.33 48.58 41.16 55.06 4582 4286 6223.02 0.69 2336 3678 3107.58 1.18 46.20 42.23 49.89	R3R+R3C C3C 95% CI N N T (year) n/T N n T (year) n/T (%) LL UL p-value 2593 2314 3523.94 0.66 1333 1906 1773.82 1.07 45.16 37.61 51.80 <.0001

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

n = number of episodes included in each group

T(year) = person years at risk

n/T = person year rate in each group

VE (%) = Vaccine efficacy unadjusted for covariates, except for study site (Negative binomial random-effects model)

LL, UL = 95% Lower and Upper confidence limits

P-value from Negative binomial random-effects model

No significant VE against severe malaria was shown over the entire FU period in children who did not receive a booster dose. The absence of efficacy during the entire FU period is explained by the higher incidence of severe malaria from Month 21 until SE in children who received a RTS,S/AS01E primary vaccination course without a booster dose compared to the control group. This increased incidence was seen predominantly in sites with a high level of malaria transmission. This contrasts with the statistically significant reduction in severe malaria in children followed until the time of booster administration (i.e., M20 analysis). Efficacy against severe malaria was statistically significant over the entire study period when a booster dose of RTS,S/AS01E was administered (VE=28.5% [95% CI: 6.3 to 45.7]).

Vaccination with RTS,S/AS01E significantly reduced hospital admissions due to malaria and incident severe anaemia in children who received a booster dose (VE=37.2% [95% CI: 23.6 to 48.5] and 61.2% [95% CI: 26.5 to 80.6] over the entire FU period up to SE, respectively), but not in children who did not receive a booster dose.

Over the entire FU period up to SE, 215 to 4,443 cases of clinical malaria were averted across sites per 1,000 children vaccinated with a RTS,S/AS01E primary vaccination schedule without booster. Administration of a RTS,S/AS01E booster dose led to an increased benefit ranging from 205 to 6,565 cases averted per 1,000 children vaccinated. The highest impact was observed in sites with high malaria transmission intensity for both schedules.

Despite the higher incidence of severe malaria observed in the RTS,S/AS01E group without a booster dose as compared to the control group during the FU period from Month 21 up to SE, the number of cases averted tended to remain positive over the entire FU period up to SE (8 [95% CI: -9 to 26] severe cases averted per 1,000 children vaccinated) in the R3C group. For a RTS,S/AS01E schedule with a booster dose, the average number of severe malaria cases averted during the whole FU period was 19 (95% CI: 4 to 35) per 1,000 children vaccinated.

Overall, in children who received a booster dose, the vaccine impact in terms of cases averted over the entire FU period up to SE was significant on clinical and severe malaria, malaria hospitalisation and incident severe anaemia, but not on fatal malaria, probably due to the very low number of fatal cases accrued in any group because of the high level of care in study Malaria-055. In children not having received a booster dose, vaccine impact over the entire FU period up to SE was only significant on clinical malaria and malaria hospitalisation.

For the assessment of vaccine impact on fatal malaria, the more sensitive secondary case definition 4 (taking into account the fatalities in the community) was used. Over the entire study period in the ITT cohort of children, there was a similar number of fatal malaria cases meeting this case definition, i.e.,

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13 in the R3R group, 17 in the R3C group and 12 in the C3C group, for a total of 42 cases. The applicant ascribes the failure to detect an impact of the RTS,S/AS01E vaccine on fatal malaria to the low total fatality rate in this trial, which can be attributed to the facilitated access to high-quality health care provided at study sites. An investigator-initiated case control study was conducted at the Siaya site to quantify the reduction in mortality among children enrolled in study Malaria-055 vs. children not enrolled in Malaria-055, but living in the same area. Children enrolled in study Malaria-055 experienced a marked reduction in all-cause mortality of 70 % as compared to children not enrolled in study Malaria-055 [Hamel, 2014]. As efficacy against clinical and severe malaria has been demonstrated in children aged 5 to 17 months, it can reasonably be expected that the RTS,S/AS01E vaccine will reduce malaria associated mortality in communities where access to a high level of clinical care is less readily available than was the case during the Malaria-055 trial.

Modelling to estimate the potential public health impact (PHI) of the RTS,S/AS01E vaccine when implemented in sub-Saharan Africa in under development. Despite differences between modelling approaches developed by independent expert groups, the PHI estimates were generally well aligned. This increases the confidence in the model estimates.

A consistent observation, irrespective of the modelling approach used to estimate the PHI of RTS,S/AS01E, was that a substantial number of clinical and severe malaria cases and malaria deaths may be prevented by RTS,S/AS01E, especially in moderate and high transmission settings across sub-Saharan Africa. The vaccine impact is estimated to be higher when a booster dose is administered 18 months after completion of the primary vaccination course.

3.5.2.2. Summary of vaccine efficacy in children aged 5-17 months up to study end with and without a 4th dose

Results for VE of a RTS,S/AS01E primary vaccination schedule without and with a 4th dose against the most relevant malaria endpoints in infants over all evaluated FU periods from 2 weeks post Dose 3 up to study end (SE) are summarised in Table 28.

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Table 28. Summary table of vaccine efficacy against malaria endpoints over all evaluated FU periods in children aged 5-17 months having received RTS,S/AS01E according to the primary vaccination schedule without and with a booster dose at Month 20 (ATP cohort of efficacy)

Vaccine efficacy before	the boost	ter										
		Primary	(M14)	analy	sis°			M	20 ana	alysis		
Endpoint	n/T R3	n/T C3C	VE	LL	UL	p-value	n/T R3	n/T C3C	VE	LL	UL	p-value
First or only case*	0.38	0.67	54.6	50.7	58.1	<0.0001	0.35	0.56	49.0	45.1	52.6	<0.0001
All cases of malaria*	0.63	1.23	51.3	47.5	54.9	<0.0001	0.69	1.17	45.7	41.7	49.5	<0.0001
	n/N R3	n/N C3C	VE	LL	UL	p-value	n/N R3	n/N C3C	VE	LL	UL	p-value
Severe malaria*	77/4553	71/2327	44.6	22.4	60.4	0.0004	120/4557	95/2328	35.5	14.6	51.1	0.0016
Incident severe anaemia*	18/4553	20/2327	54.0	8.4	77.1	0.0238	24/4557	25/2328	51.0	10.5	73.2	0.0146
Malaria hospitalisation*	159/4553	156/2327	47.9	34.6	58.5	< 0.0001	236/4557	206/2328	41.5	29.1	51.7	< 0.0001
Fatal malaria*	0/4553	0/2327	-	-	-	-	0/4557	0/2328	-	-	-	-
Vaccine efficacy withou	t a booste	er				•	•					
		Final	(M32)	analys	is			S	E ana	lysis		
Endpoint	n/T R3C	n/T C3C	VE	LĹ	UL	p-value	n/T R3C		VE	LL	UL	p-value
First or only case*	ND	ND	-	-	-	-	ND	ND	-	-	-	-
All cases of malaria*	0.81	1.15	33.9	28.9	38.6	<0.0001	0.9	1.14	26.2	20.8	31.2	< 0.0001
	n/N R3	n/N C3C	VE	LL	UL	p-value	n/N R3	n/N C3C	VE	LL	UL	p-value
Severe malaria*	116/2306	120/2336	2.1	-27.5	24.8	0.8938	141/2306	135/2336	-5.8	-35.0	17.0	0.6640
Incident severe anaemia*	23/2306	32/2336	27.2	-28.4	59.3	0.2783	29/2306	37/2336	20.6	-32.7	52.9	0.3863
Malaria hospitalisation*	202/2306	250/2336	18.1	1.1	32.3	0.0260	237/2306	273/2336	12.1	-5.0	26.4	0.1331
Fatal malaria*	0/2306	0/2336	-	-	-	-	1/2306	1/2336	-1.3	-7852	98.7	1.0000
Vaccine efficacy with a	booster											
		Final	(M32)	analys	is			S	E ana	lysis		
Endpoint	n/T R3C	n/T C3C	VE	LL	UL	p-value	n/T R3C	n/T C3C	VE	LL	UL	p-value
First or only case*	ND	ND	-	-	-		ND	ND	-	-	-	-
All cases of malaria*	0.68	1.15	46.1	41.8	50.1	<0.0001	0.79	1.14	39.0	34.3	43.3	<0.0001
	n/N R3	n/N C3C	VE	LL	UL	p-value	n/N R3	n/N C3C	VE	LL	UL	p-value
Severe malaria*	79/2276	120/2336	32.4	9.5	49.8	0.0058	94/2276	135/2336	28.5	6.3	45.7	0.0100
Incident severe anaemia*	12/2276	32/2336	61.5	23.2	81.9	0.0036	14/2276	37/2336	61.2	26.5	80.6	0.0017
Malaria hospitalisation*	146/2276	250/2336	40.1	26.2	51.5	<0.0001	167/2276	273/2336	37.2	23.6	48.5	<0.0001
Fatal malaria*	1/2276	0/2336	_	-	-	-	1/2276	1/2336	-2.6	-7957	98.7	1.0000

R3C = RTS,S/AS01E primary vaccination schedule without booster

 $n/N = number\ of\ subjects\ reporting\ at\ least\ one\ event\ in\ each\ group/\ number\ of\ subjects\ in\ analysis\ for\ respective\ endpoint.$

n/T = person year rate in each group with n = number of episodes included in each group and T(year) = person years at risk

Note: For point estimates shown in bold, the p-value was < 0.05.

VE (%) = Vaccine Efficacy unadjusted for covariates, except for study site LL, UL = 95% Lower and Upper CI limits

SE = Study end

Additional efficacy analysis provided during the evaluation for children age 5-17 months

Vaccine impact analysis was performed in the ITT cohort and in the ATP cohort for efficacy for the

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 $R3R = RTS, S/AS01E \ primary \ vaccination \ schedule \ with \ booster$

 $R3 = RTS_1S_1AS_01E$ primary schedule vaccination before the booster (R3C + R3R pooled)

C3C = Control

[°] VE assessed in all infants enrolled in the 6-12 weeks age category with 95% CI at the time of the M20 analysis is provided to allow comparison with analyses at other timepoints and with other endpoints at M14 only assessed in the M20 analysis. Note that VE against primary objective as provided in initial Application package was analysed with 97.5%CI as provided in Malaria-055 Annex report 3.

^{*} Primary case definition used in analysis of clinical malaria case, severe malaria case, incident severe anaemia case, malaria hospitalisation case (case definition 1) and fatal malaria case.

same endpoints and case definitions. Of note, the ATP cohort for efficacy includes fewer subjects than the ITT cohort because subjects not compliant to the per-protocol procedures were excluded from the analysis. Also the follow-up periods are shorter (case count starts 14 days after Dose 3 and Dose 4 in the ATP cohort for efficacy, whereas it starts from Day 1 after Dose 1 and Dose 4 in the ITT cohort). In the analysis up to SE, the methodology used to calculate the number of cases averted was different as compared to the one used to present vaccine impact up to Month 20, i.e., upon request from a WHO advisory body on malaria vaccines in phase III and phase IV studies, the follow-up (FU) period was divided into 3-monthly periods.

Overall, the impact of the RTS,S/AS01 $_{\rm E}$ vaccine in terms of the number of cases averted in children in the ATP cohort for efficacy is similar to and consistent with the vaccine impact evaluated in the ITT cohort for all evaluated endpoints (see Table 29)

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Table 29. Number of cases averted in children 5-17 months of age immunised according to a primary vaccination schedule without and with a 4th dose

		Number of cases averted per 10	00 vaccinees in the 5-17 month
	FU period post Dose 1	Schedule without a 4 th dose (R3C)	Schedule with a 4 th dose (R3R)
	post bose i	n (LL ; UL)	n (LL ; UL)
ATP cohort for efficacy (new	l analvsis as requ		11 (22 , 02)
Clinical malaria	M0-M20*		12 ; 1205)
(secondary case definition 1)	M0-M32	1287 (983 ; 1595)	1622 (1333 ; 1919)
	M0-SE	1398 (899 ; 1894)	1984 (1493 ; 2460)
Malaria hospitalisation	M0-M20*		0 ; 54)
(case definition 1)	M0-M32	25 (2 ; 47)	39 (18 ; 59)
	M0-SE	16 (-10 ; 41)	37 (11 ; 60)
Severe malaria	M0-M20*	, , , ,	7; 32)
(secondary case definition 1)	M0-M32	12 (-6 ; 27)	21 (5 ; 36)
	M0-SE	5 (-14 ; 23)	19 (0 ; 35)
Fatal malaria (ICD10 code)	M0-M20*	0 (-2	2;3)
(secondary case definition 4)	M0-M32	-1 (-4 ; 2)	0 (-3;3)
	M0-SE	-1 (-5 ; 2)	0 (-3 ; 4)
Incident severe anaemia	M0-M20*	6 (-2	; 13)
(secondary case definition 3)	M0-M32	7 (-3 ; 16)	9 (-1 ; 19)
	M0-SE	6 (-6 ; 19)	14 (2 ; 25)
ITT cohort (previously reporte	ed analysis in re	sponse to Day 120 Question 109)	
Clinical malaria	M0-M20*	963 (80	7 ; 1133)
(secondary case definition 1)	M0-M32	1221 (973 ; 1483)	1475 (1234 ; 1733)
	M0-SE	1363 (995 ; 1797)	1774 (1387 ; 2186)
Malaria hospitalisation	M0-M20*	42 (2	8 ; 59)
(case definition 1)	M0-M32	32 (13 ; 53)	44 (26 ; 64)
	M0-SE	26 (4 ; 51)	40 (19 ; 64)
Severe malaria	M0-M20*	19 (8	; 32)
(secondary case definition 1)	M0-M32	12 (-2 ; 27)	20 (7 ; 34)
	M0-SE	8 (-9 ; 26)	19 (4 ; 35)
Fatal malaria (ICD10 code)	M0-M20*	0 (-2	2 ; 3)
(secondary case definition 4)	M0-M32	-1 (-4 ; 3)	1 (-2 ; 4)
	M0-SE	-2 (-7 ; 2)	1 (-3 ; 5)
Incident severe anaemia	M0-M20*	8 (1	; 15)
(secondary case definition 3)	M0-M32	9 (0 ; 19)	10 (1 ; 20)
	M0-SE	9 (-3 ; 21)	11 (1 ; 24)

Clinical malaria secondary case definition = illness in a child brought to a study facility with a measured temperature of ≥37.5° C or reported fever within the last 24 hours and P. falciparum asexual parasitaemia at a density of > 0 parasites/µL. This definition was used for this analysis as, during routine clinical practice, these children would normally receive a full course of anti-malarial treatment.

Malaria hospitalisation case definition 1 = a medical hospitalisation with confirmed P. falciparum asexual parasitaemia at a density of > 5000 parasites/ μ L. Severe malaria secondary case definition 1 = P. falciparum asexual parasitaemia at a density of > 5000 parasites/ μ L with one or more markers of disease severity, including cases in which a coexisting illness was present or could not be ruled out. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of \leq 2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia, acidosis, elevated lactate level, or haemoglobin level of < 5 g/dL. Co-existing illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.

Fatal malaria (ICD10 code) = a fatal case associated with International Classification Disease (ICD10) code B50, B53, B54.

Incident severe anaemia secondary case definition 3 = a documented haemoglobin < 5.0 g/dL identified at clinical presentation to morbidity surveillance system.

n = number of cases averted per 1000 vaccinees.

LL, UL = 95% Lower and Upper CI limits

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SE = Study end

* For M0-M20, the schedule without a 4th dose (R3C) and the schedule with a 4th dose (R3R) were pooled (R3R+R3C) to calculate the number of cases averted

Immunogenicity for the first 200 per study site aged 5-17 months at enrolment

In the 2200 subjects in this analysis the seropositivity rates at screening ranged from zero (Kilifi and Korogwe) to 27-30% (Siaya and Nanoro) across the different sites but the GMTs were \leq 0.4. Post-dose 3, the anti-CS seropositivity rate and GMT were significantly different between RTS,S/AS01E and control groups at all sites. Similar findings applied in the ITT population.

Table 30. Seropositivity rates and GMTs for anti-CS antibodies (ATP population for immunogenicity)

				≥	0.5 E	U/mL			GMT			
						95%	95% CI		95% CI			
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
Anti-CS	R3R+R3C	Screening	1034	132	12.8	10.8	15.0	0.3	0.3	0.3	<0.5	7.7
		PIII(M3)	1033	1032	99.9	99.5	100	621.2	591.7	652.1	<0.5	8147.2
	C3C	Screening	524	46	8.8	6.5	11.5	0.3	0.3	0.3	<0.5	16.2
		PIII(M3)	528	31	5.9	4.0	8.2	0.3	0.3	0.3	<0.5	329.9

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

GMT = geometric mean antibody titer calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with concentration within the specified range

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

 $\mathsf{MIN}/\mathsf{MAX} = \mathsf{Minimum}/\mathsf{Maximum}$

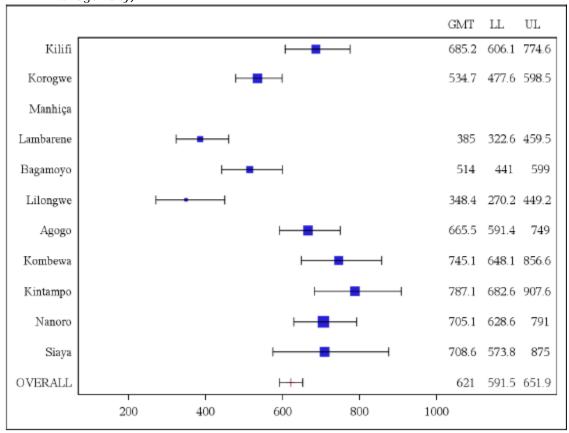
Screening = Screening visit

PIII(M3) = 1 month post dose 3

Despite the high M3 seropositivity rates, the anti-CS GMTs were very variable across study sites as shown in Figure 8. There was no discernible relationship between the baseline seropositivity rates and the M3 GMT.

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Figure 9. Malaria-055: Anti-CS antibody GMCs one month post Dose 3 in RTS,S/AS01E recipients 5-17 months of age by increasing malaria incidence at each study centre (ATP cohort for immunogenicity)



The analysis by very low, low and normal weight for age children showed no effect on seropositivity rates but the GMT was lowest in the very low weight category.

Table 31. Seropositivity rates and GMTs for anti-CS antibodies at baseline and Month 3 by weight for age at baseline ([5-17] months) (ATP population for immunogenicity)

					2	≥ 0.5	EU/n	nl		GMT			
							95%	6 CI		95%	6 CI		
Antibody	Sub-group	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
Anti-CS],-3]	R3R+R3C	SCREENING	40	9	22.5	10.8	38.5	0.3	0.3	0.4	<0.5	3.7
			PIII(M3)	40	40	100	91.2	100	534.0	378.4	753.5	14.4	2186.4
]-3,-2]	R3R+R3C	SCREENING	157	31	19.7	13.8	26.8	0.3	0.3	0.4	<0.5	4.8
			PIII(M3)	156	156	100	97.7	100	727.9	645.1	821.3	52.7	8147.2
]-2,[R3R+R3C	SCREENING	839	92	11.0	8.9	13.3	0.3	0.3	0.3	<0.5	7.7
			PIII(M3)	838	837	99.9	99.3	100	607.3	575.8	640.4	<0.5	5030.9

^{],-3] =} WAZ at baseline less than or equal to -3

]-3,-2] = WAZ at baseline greater than -3 but less than or equal to -2

]-2,[= WAZ at baseline greater than -2

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

GMT= geometric mean antibody titer calculated on all subjects

N = number of subjects with available results

 $\ensuremath{\text{n}}\xspace/\%$ = number/percentage of subjects with titer equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

 $\mathsf{MIN/MAX} = \mathsf{Minimum/Maximum}$

Screening = Screening visit

PIII(M3) = 1 month post Dose 3

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In the analysis of factors influencing the anti-CS response only age (5-11 months vs. 12-17 months) was significant.

Table 32. Determinants of anti-CS response, results from linear regression analysis ([5-17] months) (FU: M2.5-M20) (ATP population for immunogenicity)

Parameter	Parameter Estimate	Std. Error	VIF	p-value
N	1057			
Root MSE	0.34			
Adjusted R-squared	0.04			
Intercept	2.681	0.050	0.000	<.0001
Male vs. female	0.029	0.021	1.016	0.1738
Anti CS Positive at Baseline vs. negative	0.059	0.035	1.100	0.0869
Age 5-11m vs. 12-17m	0.075	0.021	1.032	0.0004
Incidence in controls	0.043	0.010	1.247	<.0001
Vitamin A usage	-0.018	0.023	1.098	0.4253
Low HAZ vs. normal HAZ	0.011	0.027	1.283	0.6862
Low WAZ vs. normal WAZ	0.024	0.030	1.292	0.4257
Hep B priming Yes vs. No	-0.016	0.044	1.113	0.7251

Std. Frror = Standard Frror

VIF = Variance Inflation Factor

P-value from linear regression

Table 33. Effect of anti-CS response on the incidence of clinical malaria (primary case definition) in RTS,S/AS01E recipients ([5-17] months) (FU: M2.5-M20) (ATP population for efficacy)

Parameter	Incident rate ratio	95% CI LL	95% CI UL	p-value
Intercept	0.001	0.000	0.002	<.0001
Male vs. female	0.972	0.792	1.193	0.7872
Anti CS Positive at Baseline	1.498	1.136	1.975	0.0042
Anti-CS titer at M3	0.835	0.618	1.130	0.2426
Age 5-11m vs. 12-17m	0.860	0.700	1.057	0.1520
Incidence in controls	2.324	2.084	2.591	<.0001
Vitamin A usage	0.904	0.721	1.134	0.3841
Low HAZ vs. normal HAZ	1.063	0.824	1.372	0.6363
Low WAZ vs. normal WAZ	0.851	0.641	1.129	0.2627
Hep B priming Yes vs. No	1.052	0.728	1.520	0.7878
Dispersion	3.264	2.599	4.328	_

LL = Lower Limit

UL = Upper Limit

P-value from negative binomial regression

A model evaluated the effect of the anti-CS response one month post Dose 3 on the incidence of clinical malaria in RTS, S/AS01 $_{\rm E}$ recipients (R3R-R3C).

The site average anti-CS antibody had a strong relationship (p < .0001) with efficacy over 14 and over 20 months post-dose 1. Thus, children living in sites with higher anti-CS GMCs experienced more malaria episodes vs. those at sites with lower anti-CS antibody GMCs (p < 0.0001). The applicant hypothesized that this reflected higher rates of natural priming prior to vaccination in sites with more malaria episodes.

In the analysis of the anti-CS antibody response one month after completion of the primary vaccination

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course (Month 3) stratified by age at the time of first dose (5-11 months vs. 12-17 months), all children, except one in the 5-11 months age sub-category, were seropositive for anti-CS antibodies. Anti-CS antibody GMCs were higher in the 5-11 months age sub-category than in the 12-17 months age sub-category, i.e., 674.7 EU/ml (95% CI: 628.6 to 724.2) vs. 568.3 EU/ml (95% CI: 532.2 to 606.9), with non-overlapping 95% CIs (see Table 34). This is in line with the multivariate exploratory model analysis in which RTS,S/AS01 $_E$ recipients (R3R+R3C) who were 5-11 months old had higher individual anti-CS antibody concentrations one month post Dose 3 compared to RTS,S/AS01 $_E$ recipients 12-17 months old based on the interaction found between age and anti-CS antibody response (p=0.0004).

Table 34. Seropositivity rates and GMCs for anti-CS antibodies at Month 0 and Month 3 by age classification: children aged 5-11 months vs. 12-17 months at first dose (ATP cohort for immunogenicity)

	≥ 0.5 EU/ML											
							95% C	l		95% CI		
Antibody	Sub-group	Group	Timing	N	n	%	LL	UL	value	LL	UL	
Anti-CS	[5-11]	R3R+R3C	SCREENING	533	66	12.4	9.7	15.5	0.3	0.3	0.3	
			PIII(M3)	533	532	99.8	99.0	100	674.7	628.6	724.2	
	[12-17]	R3R+R3C	SCREENING	502	66	13.1	10.3	16.4	0.3	0.3	0.3	
İ			PIII(M3)	500	500	100	99.3	100	568.3	532.2	606.9	

[5-11] = 5 to 11 Months at the time of dose 1; [12-17] = 12 to 17 Months at the time of dose 1

 $R3R+R3C = RTS_rS/AS01E$ primary schedule with or without booster

GMC = geometric mean antibody concentration calculated on all subjects

N = number of subjects with available results;

n/% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

SCREENING = Screening visit; PIII(M3) = 1 month post dose 3

Note that in the multivariate exploratory model for analysis of the relationship between individual anti-CS antibody concentrations post Dose 3 and the incidence of clinical malaria reported in age (5-11 months vs. 12-17 months) was not a significant covariate in the correlation between anti-CS antibody response and the incidence of clinical malaria (p=0.1520), suggesting that the age difference in the anti-CS antibody response post Dose 3 does not translate in a difference in VE.

In the analysis of the anti-CS antibody response one month after the booster dose (Month 21) stratified by age at the time of first dose (5-11 months vs. 12-17 months), all children, except one in the 12-17 months age sub-category of RTS,S/AS01 $_{\rm E}$ recipients having received a booster dose (R3R group), were seropositive for anti-CS antibodies. Anti-CS antibody GMCs in both the 5-11 months and 12-17 months age sub-categories in the R3R group at Month 21 were numerically lower than those observed in respective groups after the primary series.

Table 35. Seropositivity rates and GMCs for anti-CS antibodies at Month 20 and Month 21 by age classification: children aged 5-11 months vs. 12-17 months at first dose (ATP cohort for immunogenicity)

					≥ 0.5	EU/ML			GMC		
							95% C			95% CI	
Antibody	Sub-group	Group	Timing	N	n	%	LL	UL	value	LL	UL
Anti-CS	[5-11]	R3R	PIII(M20)	226	224	99.1	96.8	99.9	32.6	27.4	38.9
			PIV(M21)	221	221	100	98.3	100	343.9	313.8	376.9
	[12-17]	R3R	PIII(M20)	216	216	100	98.3	100	36.4	31.4	42.2
			PIV(M21)	205	204	99.5	97.3	100	292.6	259.3	330.1

[5-11] = 5 to 11 Months at the time of dose 1; [12-17] = 12 to 17 Months at the time of dose 1

R3R = RTS,S/AS01E primary schedule with booster; R3C = RTS,S/AS01E primary schedule without booster

GMT = geometric mean antibody concentration calculated on all subjects

N = number of subjects with available results

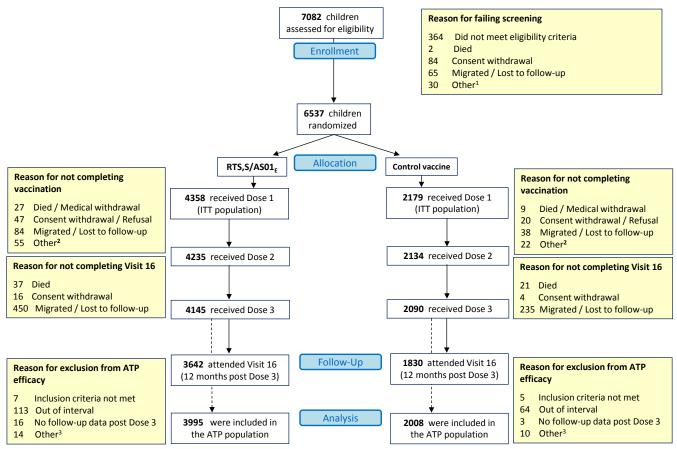
n% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

3.5.2.3. Efficacy data in subjects aged 6-12 weeks

Efficacy data up to Month 14

The report with a data cut-off in August 2012 presents vaccine efficacy for the 6537 subjects enrolled in the 6-12 weeks age category up to 12 months post-dose 3. Subjects who did not receive three co-administered doses of Tritanrix HepB/Hib were excluded from the ATP analysis.



Groups were balanced for age (mean age at first vaccination was 7.1 weeks) and gender (\sim 50% split). At 12 months post-dose 3, ITN coverage was 86.1% in the RTS,S/AS01E group and 85.3% in the control group but about one third of subjects were using ITNs with holes.

Unadjusted VE against first or only episodes of clinical malaria meeting the PDef was 31.3% (97.5% CI: 23.6 to 38.3, p<0.0001) over 12 months post-dose 3. VE differed by site (interaction p-value=0.0238) and was not significant at all sites.

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Table 36. Vaccine efficacy: First or only episodes of clinical malaria (primary case definition) (97.5% CI) (ATP population for efficacy)

							9	7.5% CI	
Event Type	Group	N	n	T (year)	n/T	%	LL	UL	P-Value
Any	R3R+R3C	3995	1161	3162.62	0.367	31.315	23.556	38.286	<0.0001
	C3C	2008	714	1476.38	0.484	-	-	-	-

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

Notes: N = number of subjects included in each group (without missing values)

n = number of subjects reporting at least one event(s) in each group

T (year) = sum of follow-up period expressed in years censored at the first occurrence of event in each group

n/T = person-year rate in each group

LL, UL = 97.5% Lower and Upper confidence limits

VE (%) = Vaccine efficacy (Cox regression model stratified by study site)

P-value from Cox regression model stratified by study site to test H0 = (VE=0%) (Y = (start , stop))

Figure 10. Cumulative incidence of first or only episodes of clinical malaria (primary case definition) (ATP population for efficacy)

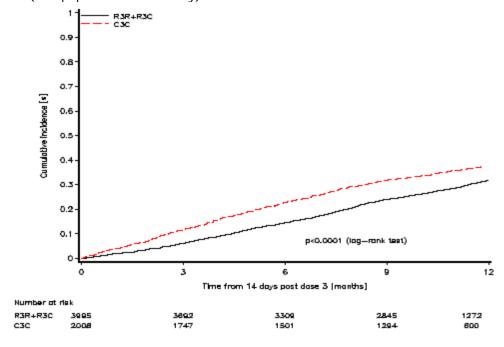


Table 37. Vaccine Efficacy: First or only episodes of clinical malaria (ATP population for efficacy) over 12 months of follow-up

	R3R+R3C C3C								Point estim	ate of VE ur	n-adjusted f	or covariates	Point estimate of VE adjusted for covariates				
	N	n	T (year)	n/T	N	n	T (year)	n/T	(%)	95%	6 CI	P value	(%)	95% CI		P value	
Primary Case Definition	3995	1161	3162.62	0.367	2008	714	1476.38	0.484	31.315	24.576	37.451	<0.0001	31.466	24.740	37.590	<0.0001	
Secondary Case Definition 1	3995	1475	2921.35	0.505	2008	879	1327.61	0.662	32.443	26.526	37.884	<0.0001	32.583	26.678	38.014	<0.0001	
Secondary Case Definition 2	3995	1282	3073.39	0.417	2008	770	1429.03	0.539	30.266	23.723	36.248	<0.0001	30.365	23.830	36.339	<0.0001	
Secondary Case Definition 3	3995	1005	3256.23	0.309	2008	630	1534.68	0.411	31.376	24.167	37.899	<0.0001	31.575	24.385	38.082	<0.0001	

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

n = number of subjects reporting at least one event(s) in each group

T (year) = sum of follow-up period expressed in years censored at the first occurrence of event in each group

n/T = person-year rate in each group

95% CI = Lower and Upper confidence limits of 95% CI

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VE (%) = Vaccine efficacy (Cox regression model stratified by study site)

P-value from Cox regression model stratified by study site to test H0 = [VE=0%] (Y = (start, stop))

Adjusted for covariates: distance to outpatient health facility

Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/µL AND fever (axillary temperature ≥ 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility, or a case of malaria meeting the primary case definition of severe malaria

Secondary Case Definition 1: P. falciparum asexual parasitemia > 0 parasites/ μ L AND fever (axillary temperature $\ge 37.5^{\circ}$ C) or history of fever within the last 24h occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 2: P. falciparum asexual parasitemia > 500 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility

Secondary Case Definition 3: P. falciparum asexual parasitemia > 20000 parasites/µL AND fever (axillary temperature ≥ 37.5°C) occurring in a child who is unwell and brought for treatment to a health facility

Proportionality of hazard over 12 months was not demonstrated (Schoenfeld residual -0.12 (p<0.0001).

The number of episodes of clinical malaria within and outside risk period and the distribution of the total number of episodes of clinical malaria per subject for the primary case definition (ATP population for efficacy) are in Tables 38 and 39.

Table 38. Number of episodes of clinical malaria within and outside risk period (14 days following episode) (primary case definition) (all episodes) (ATP population for efficacy)

	Episodes outside risk period	Episodes within risk period	Total
C3C	36	1626	1662
R3R+R3C	56	2301	2357

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

Risk period = ATP Time at risk

Table 39. Distribution of total number of episodes of clinical malaria per subject (primary case definition) (ATP population for efficacy)

			R+R3C 3995	1	2008	
Characteristics	Categories	n	%	n	%	P. values
Number of episodes	0	2834	70.9	1294	64.4	<0.0001
	1	564	14.1	294	14.6	-
	2	304	7.6	168	8.4	-
	3	160	4.0	125	6.2	-
	+3	133	3.3	127	6.3	-

R3R+R3C = RTS, S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects

 $n = number \ of \ subjects \ in \ a \ given \ category$

% = n / Number of subjects with available results x 100

Pvalues: Chi-square test

 $Unadjusted \ VE \ against \ all \ episodes \ of \ clinical \ malaria \ meeting \ the \ PDef \ was \ 32.9\% \ (95\% \ CI: 26.3 \ to \ 38.8, \ p<0.0001).$

Table 40. Vaccine Efficacy: All episodes of clinical malaria (ATP population for efficacy) over 12 months follow-up

						•										
		R3R+R3C C3C								timate un	-adjusted	for covariates	Point estimate of VE adjusted for covariates			
	N	n	руг	rate	N	n	pyr	rate	(%)	95%	6 CI	P value	(%)	95%	CI	P value
Primary Case Definition	3995	2301	3603.7	0.639	2008	1626	1790.1	0.908	32.9	26.3	38.8	<.0001	33.0	26.4	38.9	<.0001
Secondary Case Definition 1	3995	3517	3556.8	0.989	2008	2465	1757.2	1.403	33.2	27.5	38.5	<.0001	33.3	27.5	38.5	<.0001
Secondary Case Definition 2	3995	2642	3590.5	0.736	2008	1837	1781.8	1.031	31.7	25.5	37.4	<.0001	31.7	25.5	37.5	<.0001
Secondary Case Definition 3	3995	1865	3620.5	0.515	2008	1317	1801.8	0.731	32.9	25.8	39.4	<.0001	33.0	25.9	39.5	<.0001

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects included in each group

 $n = number \ of \ episodes \ included \ in \ each \ group$

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pyr = child years at risk

Rate = n/pyr

VE (%) = Vaccine efficacy (Negative binomial model)

95% CI = Lower and Upper confidence limits of 95% CI

P-value from Negative binomial model

Adjusted for covariates: distance to outpatient health facility

Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/µL AND fever (axillary temperature ≥ 37.5°C) occurring in a child who is unwell and brought for treatment

= to a healthcare facility, or a case of malaria meeting the primary case definition of severe malaria.

Secondary Case Definition 1: P. falciparum asexual parasitemia > 0 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) or history of fever within the last 24h occurring in a child who is unwell and brought for treatment to a healthcare facility.

Secondary Case Definition 2: P. falciparum asexual parasitemia > 500 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility.

Secondary Case Definition 3: P. falciparum asexual parasitemia > 20000 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility.

Unadjusted VE against severe malaria meeting the PDef was 36.6% (95% CI: 4.6 to 57.7, p=0.02).

Table 41. Vaccine Efficacy: Children affected by severe malaria (ATP population for efficacy) over 12 months of follow-up

			R3R+R3C			C3C	Point estin	ted for covariates		
	N n Proportion affected (%)				n	Proportion affected (%)	(%)	95	% CI	P value
Primary Case Definition	3995	58	1.5	2008	46	2.3	36.6	4.6	57.7	0.0211
Secondary Case Definition 1	3995	63	1.6	2008	51	2.5	37.9	8.3	57.8	0.0120

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects included in each group

 $n = number \ of \ subjects \ reporting \ at \ least \ one \ event \ in \ each \ group$

Proportion affected (%) = percentage of subjects reporting at least one event

VE (%° = Vaccine efficacy (Conditional method)

95% CI = Lower and Upper confidence limits of 95% CI

P-value = Tow-sided Fisher Exact test

Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/µL AND with one or more marker of disease severity AND without diagnosis of a co-morbidity.

Secondary Case Definition 1: P. falciparum asexual parasitemia > 5000 parasites/µL AND with one or more marker of disease severity.

In the ITT population unadjusted VE against first or only episodes of clinical malaria meeting the PDef was 30.1% (97.5% CI: 23.6 to 36.1, p<0.0001) over 14 months follow-up post-dose 1. VE based on SDef also gave lower 95% CI at least 27%. Unadjusted VE against all episodes of clinical malaria meeting the PDef was 32.9% (95% CI: 26.7 to 38.5, p<0.0001) while unadjusted VE against severe malaria meeting the PDef was 26.0% (95% CI: -7.4 to 48.6, p=0.09).

Efficacy data up to Month 20

Efficacy data was provided up to 18 months post-dose 3 (20 months post-dose 1) with a cut-off date in April 2013. The overall VE against all episodes of clinical malaria meeting the PDef was 26.6% (95% CI: 20.3 to 32.4, p<0.0001) in the ATP population and 27.0% (95% CI: 21.1 to 32.5, p<0.0001) in the ITT population. VE varied across study sites but there was no significant interaction detected (p value=0.1682). VE did not vary by transmission intensity (p=0.9).

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Table 42. Vaccine efficacy against clinical malaria up to Month 20: Overview ([6-12] weeks)

			R3	R+R3C				C3C		Point es covaria		f VE una	djusted for
ATP population fo	or efficacy (18 months post Dose 3)												
All episodes of cl efficacy)	inical malaria (ATP population for	N	n	T(year)	n/T	N	n	T(year)	n/T	(%)	95	% CI	P-value
Agogo	Primary Case Definition	418	365	573.32	0.64	221	238	302.91	0.79	19.48	-2.67	36.85	0.0805
Bagamoyo	Primary Case Definition	502	52	693.21	0.08	244	48	340.02	0.14	34.84	-21.0	64.91	0.1746
Cilifi	Primary Case Definition	186	9	253.22	0.04	102	3	138.96	0.02	-56.48	-599	64.96	0.5564
(intampo	Primary Case Definition	199	390	255.54	1.53	99	192	128.89	1.49	-2.66	-29.3	18.49	0.8227
Combewa	Primary Case Definition	387	505	534.99	0.94	196	339	256.18	1.32	33.72	14.20	48.80	0.0018
(orogwe	Primary Case Definition	382	15	531.04	0.03	183	14	255.47	0.05	48.46	-6.95	75.16	0.0751
.ambarene	Primary Case Definition	147	23	208.23	0.11	62	11	88.29	0.12	8.73	-113	60.80	0.8314
ilongwe	Primary Case Definition	500	211	698.34	0.3	258	177	355.82	0.5	42.98	21.17	58.76	0.0007
// Anhiça	Primary Case Definition	381	52	524.36	0.1	188	32	259.08	0.12	20.17	-31.8	51.64	0.3776
Vanoro	Primary Case Definition	441	1055	547.21	1.93	225	649	271.60	2.39	19.98	9.64	29.13	0.0003
Siaya	Primary Case Definition	453	1171	577.32	2.03	229	761	276.75	2.75	32.24	19.19	43.18	<.0001
OVERALL	Primary Case Definition	3996	3848	5396.77	0.71	2007	2464	2673.98	0.92	26.55	20.25	32.36	<.0001
\gogo	Secondary case definition 1	418	649	562.43	1.15	221	426	295.59	1.44	21.47	3.03	36.40	0.0248
Bagamoyo	Secondary case definition 1	502	90	691.78	0.13	244	69	339.19	0.2	29.25	-15.8	56.76	0.1681
Cilifi	Secondary case definition 1	186	15	252.97	0.06	102	7	138.81	0.05	-16.34	-246	60.92	0.7850
(intampo	Secondary case definition 1	199	573	248.27	2.31	99	305	124.34	2.45	5.53	-17.7	24.21	0.6115
(ombewa	Secondary case definition 1	387	808	523.13	1.54	196	540	248.23	2.18	36.18	18.51	50.02	0.0003
Corogwe	Secondary case definition 1	382	21	530.81	0.04	183	22	255.20	0.09	54.25	15.14	75.34	0.0132
ambarene	Secondary case definition 1	147	39	207.67	0.19	62	19	87.98	0.22	9.53	-86.8	56.19	0.7858
ilongwe	Secondary case definition 1	500	336	693.61	0.48	258	275	352.19	0.78	43.49	23.36	58.32	0.0003
∕lanhiça	Secondary case definition 1	381	86	523.06	0.16	188	54	258.25	0.21	21.04	-19.0	47.60	0.2585
Nanoro	Secondary case definition 1	441	1372	534.84	2.57	225	850	263.68	3.22	20.87	12.14	28.73	<.0001
Siaya	Secondary case definition 1	453	1792	552.87	3.24	229	1151	261.17	4.41	32.85	20.71	43.13	<.0001
OVERALL	Secondary case definition 1	3996	5781	5321.44	1.09	2007	3718	2624.62	1.42	27.76	22.04	33.06	<.0001
			R3	R+R3C				C3C		Point es		f VE una	djusted for
TT population (2	0 months post Dose 1)	· ·		•			•			Ì			
All episodes of cl	inical malaria (ITT population)	N	n	T(year)	n/T	N	n	T(year)	n/T	(%)	95	% CI	P-value
\gogo	Primary Case Definition	458	402	718.14	0.56	230	247	358.61	0.69	18.78	-2.72	35.78	0.0825
Bagamoyo	Primary Case Definition	533	55	820.49	0.07	269	55	416.61	0.13	37.75	-13.0	65.70	0.1188
Cilifi	Primary Case Definition	199	9	299.52	0.03	105	3	160.19	0.02	-51.91	-580	66.06	0.5834
(intampo	Primary Case Definition	221	438	326.73	1.34	110	215	164.08	1.31	-1.94	-27.0	18.14	0.8631
(ombewa	Primary Case Definition	421	548	640.90	0.86	210	366	305.76	1.2	34.23	15.71	48.69	0.0010
Corogwe	Primary Case Definition	398	16	634.08	0.03	195	15	307.56	0.05	48.26	-4.80	74.46	0.0672
.ambarene	Primary Case Definition	158	23	240.70	0.1	68	11	104.21	0.11	6.66	-118	60.02	0.8729
ilongwe	Primary Case Definition	547	231	838.55	0.28	279	186	437.09	0.43	38.96	17.17	55.03	0.0016
Manhiça	Primary Case Definition	423	54	656.57	80.0	212	35	327.40	0.11	23.60	-23.1	52.58	0.2680
Vanoro	Primary Case Definition	453	1134	649.92	1.74	228	698	322.62	2.16	19.77	9.78	28.65	0.0002
										_			
Siaya	Primary Case Definition	547	1342	758.00	1.77	273	920	369.49	2.49	34.91	23.60	44.54	<.0001

C3C = Control

Primary Case Definition: P. falciparum asexual parasitemia > 5000 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) occurring in a child who is unwell and brought for treatment to a healthcare facility, or a case of malaria meeting the primary case definition of severe malaria

Secondary Case Definition 1: P. falciparum asexual parasitemia > 0 parasites/ μ L AND fever (axillary temperature \geq 37.5°C) or history of fever within the last 24h occurring in a child who is unwell and brought for treatment to a healthcare facility

N = number of subjects included in each group

n = number of episodes included in each group

T(year) = person years at risk

n/T = person year rate in each group

VE (%) = Vaccine efficacy (Negative binomial model)

95% CI = Lower and Upper confidence limits of 95% CI

P-value from Negative binomial model

VE against first or only episodes meeting the PDef was 29.2% overall (95% CI 23, 35) but the measured point estimate varied such that no statistically significant efficacy was observed at some sites.

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Figure 11. Cumulative incidence of first or only episodes of clinical malaria (primary case definition) over all sites ([6-12] weeks) (FU: M2.5-M20) (ATP population for efficacy)

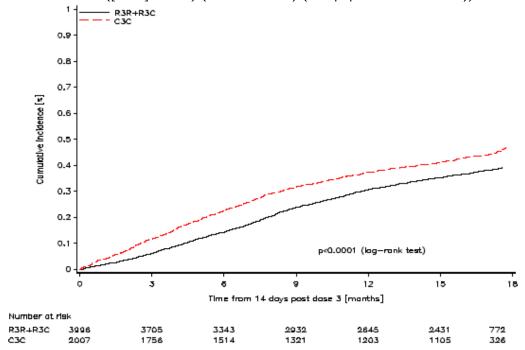


Table 43. Vaccine efficacy against first or only episodes of clinical malaria (primary case definition) ([6-12] weeks) by site and overall (FU: M2.5- M20) (ATP population for efficacy)

									Point estim	ate of VE u	ın-adjusted	for covariates
		R3F	R+R3C				C3C			95%	6 CI	
Site	N	n	T (year)	n/T	N	n	T (year)	n/T	(%)	LL	UL	p-value
Agogo	418	198	450.32	0.44	221	111	223.66	0.5	12.20	-10.8	30.42	0.2724
	502	41	666.04	0.06	244	24	320.62	0.07	18.70	-34.6	50.90	0.4211
Kilifi	186	7	251.90	0.03	102	3	138.27	0.02	-28.85	-398	66.68	0.7134
Kintampo	199	155	152.10	1.02	99	78	78.11	1	-3.29	-35.6	21.32	0.8155
Kombewa	387	201	398.55	0.5	196	126	164.50	0.77	35.04	18.80	48.03	0.0002
Korogwe	382	15	521.66	0.03	183	14	247.27	0.06	49.18	-5.28	75.47	0.0685
Lambarene	147	20	199.06	0.1	62	8	82.80	0.1	-3.68	-135	54.33	0.9311
Lilongwe	500	122	634.66	0.19	258	92	297.58	0.31	38.45	19.31	53.04	0.0004
Manhica	381	44	504.76	0.09	188	26	248.79	0.1	16.68	-35.3	48.69	0.4608
Nanoro	441	360	277.86	1.3	225	200	113.17	1.77	29.61	16.29	40.80	<0.0001
Siaya	453	327	346.70	0.94	229	196	117.96	1.66	44.25	33.35	53.36	<0.0001
OVERALL	3996	1490	4403.60	0.34	2007	878	2032.73	0.43	29.19	23.00	34.88	<0.0001

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

 $n = number \ of \ episodes \ included \ in \ each \ group$

T(year) = person years at risk

n/T = person year rate in each group

LL, UL = 95% Lower and Upper confidence limits

VE (%) = Vaccine efficacy (Cox regression model)

P-value of the Wald test from a Cox regression model to test H0 = [VE=0%]

Proportionality of hazard over 18 months follow-up post Dose 3 was not demonstrated; indicating that VE against all episodes of clinical malaria waned over time (Schoenfeld residual: -0.09 [p<0.0001]). VE based on all episodes of clinical malaria (PDef) in the ATP population was:

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- [Month 2.5-Month 8]: 47.2% (95% CI: 39.4 to 54.1, p<0.0001)
-]Month 8-Month 14]: 23.3% (95% CI: 14.6 to 31.1, p<0.0001)
-]Month 14-Month 20]: 11.5% (95% CI: 0.8 to 21.1, p=0.367)

Similarly the impact of vaccination on severe malaria (PDef) in the ATP population decreased with time:

- [Month 2.5-Month 8]: 0.01 versus 0.03 case/child/year in RTS,S/AS01E vs. controls, respectively.
-]Month 8-Month 14]: 0.02 case/child/year in RTS,S/AS01E recipients and controls.
-]Month 14-Month 20]: 0.03 versus 0.02 case/child/year in RTS,S/AS01E vs. controls, respectively.

Table 44. Vaccine efficacy against severe malaria up to Month 20: Overview ([6-12] weeks)

		R3R+R3C			C3C			Point estimate of VE unadjusted for covariate			
ATP population f	or efficacy (18 months post Dose 3)										
Infants affected befficacy)	y severe malaria (ATP population for	N	n	Proportion affected	N	n	Proportion affected	(%)	95	% CI	P-value
Agogo	Primary Case Definition	418	11	0.03	221	4	0.02	-45.40	-526	56.90	0.5945
Bagamoyo	Primary Case Definition	502	*6*	*_*	244	*6*	*_*				-
Kilifi	Primary Case Definition	186	0	0	102	0	0				-
Kintampo	Primary Case Definition	199	15	0.08	99	9	0.09	17.10	-115	66.00	0.6555
Kombewa	Primary Case Definition	387	12	0.03	196	11	0.06	44.70	-38.2	77.70	0.1759
Korogwe	Primary Case Definition	382	*1*	*_*	183	*1*	*_*				-
Lambarene	Primary Case Definition	147	*2*	*_*	62	*2*	*_*				-
Lilongwe	Primary Case Definition	500	12	0.02	258	5	0.02	-23.80	-349	59.40	0.7998
Manhiça	Primary Case Definition	381	*4*	*_*	188	*4*	*_*				-
Nanoro	Primary Case Definition	441	11	0.02	225	9	0.04	37.60	-70.3	76.50	0.3375
Siaya	Primary Case Definition	453	30	0.07	229	17	0.07	10.80	-72.4	52.40	0.7493
OVERALL	Primary Case Definition	3996	100	0.03	2007	59	0.03	14.90	-19.5	38.90	0.3486

To avoid indirect unblinding before study end, in the event that in one or several sites all events are observed in one treatment group, the results were blinded for the affected site(s) by presenting the total number of events in both treatment groups as *n* indicating there are n such events in one of the treatment groups.

VE against severe malaria, malaria hospitalisation, severe anaemia and all-cause hospitalisation over 18 months post-dose 3 was not demonstrated in the ATP or ITT populations (see below). Similarly, VE against prevalent parasitaemia (12.7% [95% CI: -8.1 to 29.4 p=0.1788]) or moderate anaemia (7.6% [95% CI: -25.6 to 31.5, p=0.5906]) in the cross-sectional survey was not demonstrated

There were four cases of fatal malaria meeting the secondary case definition 1 of fatal malaria. None of the malaria fatalities met the primary case definition of fatal malaria.

The number of cases of clinical malaria with any parasitaemia (SDef 1) averted over the 18 months follow-up post-dose 3 was 444 per 1000 vaccinees, ranging between -12 and 1429 across study sites. Cases averted for each of the consecutive 6-month periods (ATP population for efficacy) were:

- 217 [M2.5-M8]
- 155] 8-M14]
- 71] 14-M20]

The number of cases of clinical malaria meeting the PDef averted over 18 months post-dose 3 was 285 per 1000 vaccinees, ranging between -44 and 932 across study sites. Cases averted overall for each of the consecutive 6-month periods (ATP population for efficacy) were:

• 141 [M2.5-M8]

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- 105]8-M14]
- 39]14-M20]

The number of cases of severe malaria (SDef 1) averted over 18 months post-dose 3 was 8 per 1000 vaccinees, ranging between -14 and 33 across study sites. Cases averted for each of the consecutive 6-month periods (ATP population for efficacy) were:

- 9 [M2.5-M8]
- 3]M8-M14]
- -4]M14-M20]

No demonstrable effect on growth/nutritional parameters was observed in infants vaccinated with RTS,S/AS01 $_{\rm E}$ compared to the control group.

In the full exploratory model of determinants of incidence of clinical malaria (Table 45), the factors with a significant effect on malaria incidence was treatment (p<0.001) and outpatient distance (p=0.006) with borderline significance for anti-CS site average (p=0.052) and for interaction between treatment and age (p=0.071).

In the final exploratory model the significant factors affecting malaria incidence, other than treatment, were outpatient distance, anti-CS site average and gender (higher malaria incidence in male vs. female subjects). The anti-CS GMT at M3 did not significantly interact with treatment, meaning that the vaccine efficacy does not vary by anti-CS antibody GMC at the level of the site.

Table 45. Determinants of incidence of clinical malaria (primary case definition, all episodes) final model ([6-12] weeks) (FU: M2.5-M20) (ATP population for efficacy)

Parameter	Incident rate ratio	95% CI LL	95% CI UL	p-value
Intercept	0.002	0.001	0.002	<.0001
Treatment	0.769	0.706	0.838	<.0001
Male vs. female	1.120	1.031	1.217	0.0071
Anti CS site average	0.417	0.301	0.578	<.0001
IRS	0.820	0.616		0.1743
Bednet use	0.923	0.796	1.069	0.2842
Outpatient distance within 5KM	0.798	0.728	0.876	<.0001
Age 6w vs. 7-12w	0.988	0.909	1.073	0.7680
Low HAZ vs. normal HAZ	1.050	0.945	1.166	0.3658
Low WAZ vs. normal WAZ	1.043	0.889	1.223	0.6055
Moderate anemia	1.184	0.859	1.633	0.3017
Incidence in controls	2.855	2.726	2.990	<.0001
Dispersion	2.497	2.291	2.745	_

LL = Lower Limit

Efficacy during follow-up and after the 4th dose in children aged 6-12 weeks at enrolment

It was observed that the waning of efficacy continued in infants who did not receive a RTS,S/AS01 $_{\rm E}$ booster dose. Nevertheless, VE against clinical malaria over the whole FU period in these infants was still 18.2% (95% CI: 11.4 to 24.5). Protection against clinical malaria in infants was enhanced by administration of a booster dose of RTS,S/AS01E, but the point estimates were lower after the 4 $^{\rm th}$ dose than after the primary series.

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UL = Upper Limit

P-value from negative binomial regression

Table 46. Comparison of vaccine efficacy against first or only malaria episode and all malaria episodes endpoints in infants aged 6-12 weeks having received RTS,S/AS01 $_{\rm E}$ according to the primary schedule during FU periods from Month 2.5 to Month 14 and

Month 20 (ATP cohort for efficacy)

	Post-pri	mary vaccin	ation ana	alysis u	p to Mo	onth 14	Post-primary vaccination analysis up to Month 20						
Endpoint	n/T R3	n/T C3C	VE	LL	UL	p-value	n/T R3	n/T C3C	VE	LL	UL	p-value	
Previously reported analysis°													
First or only case* (VE based on hazard ratios)	0.37	0.48	31.2	24.5	37.4	<0.0001	0.34	0.43	29.2	23.0	34.9	<0.0001	
All cases of malaria* (VE based on incidence ratios)	0.64	0.91	32.9	26.4	38.9	<0.0001	0.71	0.92	26.6	20.3	32.4	<0.0001	
New analysis as requested													
First or only case* (VE based on incidence ratios)	0.37	0.48	24.0	16.4	30.8	<0.0001	0.34	0.43	21.8	14.9	28.1	<0.0001	

Table 47. Incremental efficacy against clinical malaria (primary case definition) of a booster dose at Month 20 in infants aged 6-12 weeks having received RTS,S/AS01_E according to the primary schedule during FU period from Month 21 to Month 32 (ATP cohort of efficacy)

	R3R				R3C				VE			
						l n	T (year)	n/T	(%) 95% CI			n value
	N	n		11/1	IN	n	T (year)	11/1	(%)	93% C	!	p-value
Post-booster analysis as reported in Malaria	1-055 Anr	nex repoi	t 9									
First or only case* (VE based on hazard ratios)	1743	621	1360.82	0.46	1788	723	1320.39	0.55	19.7	10.5	27.8	<.0001
All cases of malaria* (VE based on incidence ratios)	1743	1520	1662.25	0.91	1788	1942	1686.98	1.15	24.0	15.7	31.5	<.0001
New analysis as requested												
First or only case (VE based on incidence ratios)	1743	621	1360.82	0.46	1788	723	1320.39	0.55	16.7	7.1	25.3	0.0036

R3C = RTS,S/AS01E primary schedule without booster

R3R = RTS,S/AS01E primary schedule with booster

Primary case definition used in analysis of clinical malaria case.

N = number of subjects included in each group

n/T = person year rate in each group with:

For first or only episode:

 $n = number \ of \ subjects \ reporting \ at \ least \ one \ event \ in \ each \ group$

T(year) = sum of follow-up period expressed in years censored at the first occurrence of event in each group

For all cases of malaria:

 $n = number \ of \ episodes \ included \ in \ each \ group$

T(year) = person years at risk

Note: For point estimates shown in bold, the p-value was < 0.05.

Over the 18 months post Dose 3 FU period, VE against all episodes of clinical malaria stratified by age at the time of first dose was 23.77% (95% CI: 10.70 to 34.92) in infants aged 6 weeks and 30.88% (95% CI: 19.58 to 40.58) in infants aged 7-12 weeks (see Table 48). There was no evidence for an interaction between age (6 weeks vs. 7-12 weeks) and VE (interaction p=0.3825). Also, in the final multivariate exploratory model, there was no evidence for an interaction between age and VE.

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Table 48. Vaccine efficacy against all episodes of clinical malaria (primary case definition) of a primary vaccination schedule by age classification: infants aged 6 weeks

vs. 7-12 weeks at first dose (FU: M2.5-M20) (ATP cohort for efficacy)

								VE					
	R3R+F	R3C			C3C					95% CI			
Sub-group	N	n	T (year)	n/T	N	n	T (year)	n/T	(%)	LL	UL	p-value	
6W	1910	1837	2586.79	0.71	968	1150	1284.01	0.9	23.77	10.70	34.92	0.0008	
7-12W	2080	2023	2800.32	0.72	1039	1317	1388.85	0.95	30.88	19.58	40.58	<.0001	
OVERALL	3990	3860	5387.11	0.72	2007	2467	2672.86	0.92	26.45	20.14	32.25	<.0001	

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

N = number of subjects included in each group (without missing values)

n = number of episodes included in each group

T(year) = person years at risk

n/T = person year rate in each group

VE (%) = Vaccine efficacy unadjusted for covariates, except for study site (Negative binomial random-effects model)

LL, UL = 95% Lower and Upper confidence limits

P-value from Negative binomial random-effects model

From one to 12 months after the booster dose (Months 21 to 32, post-booster analysis), VE against all episodes of clinical malaria stratified by age at the time of the first dose was 31.81% (95% CI: 16.73 to 44.16) in infants aged 6 weeks and 29.96% (95% CI: 14.83 to 42.41) in infants aged 7-12 weeks (see Table 49), and there was no evidence for an interaction between age and VE (interaction p=0.8495).

Table 49. Vaccine efficacy against all episodes of clinical malaria (primary case definition) of a primary vaccination schedule with booster by age classification: infants aged 6 weeks vs. 7-12 weeks at first dose (FU: M21-M32) (ATP cohort for efficacy)

										VE					
	R3R				C3C					95% CI					
Sub-group	N	n	T (year)	n/T	N	n	T (year)	n/T	(%)	LL	UL	p-value			
6 weeks	823	730	783.91	0.93	868	1042	823.70	1.27	31.81	16.73	44.16	0.0002			
7-12 weeks	920	790	878.34	0.9	894	970	847.30	1.14	29.96	14.83	42.41	0.0004			
OVERALL	1743	1520	1662.25	0.91	1762	2012	1670.99	1.2	30.33	22.98	36.97	<.0001			

R3R = RTS,S/AS01E primary schedule with booster; C3C = Control

 $N = number \ of \ subjects \ included \ in \ each \ group \ (without \ missing \ values)$

n = number of episodes included in each group

T(year) = person years at risk

n/T = person year rate in each group

VE (%) = Vaccine efficacy unadjusted for covariates, except for study site (Negative binomial random-effects model)

LL, UL = 95% Lower and Upper confidence limits

P-value from Negative binomial random-effects model

No significant VE against severe malaria was observed in infants over the entire study period, irrespective of the administration of a booster dose. However, infants with a booster dose had a point estimate for VE against severe malaria (20.7%), which was consistent with the significant level of VE observed against clinical malaria (26.7%). The incremental VE against severe malaria provided by the booster dose was 26.4% during the post-booster FU period up to SE.

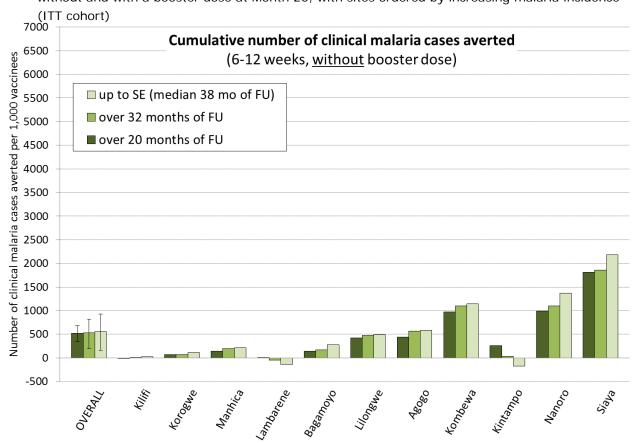
Vaccination with RTS,S/AS01 $_{\rm E}$ significantly reduced malaria hospital admissions in infants who received a booster dose (VE=27.1% [95% CI: 7.1 to 42.9] over the entire FU period), but not in those not having received a booster dose. Over the entire FU period, no VE against incident severe anaemia was shown in infants whether or not they had received a booster dose. No fatal malaria cases meeting the primary case definition were accrued in infants up to SE, probably due to the high level of care in the trial.

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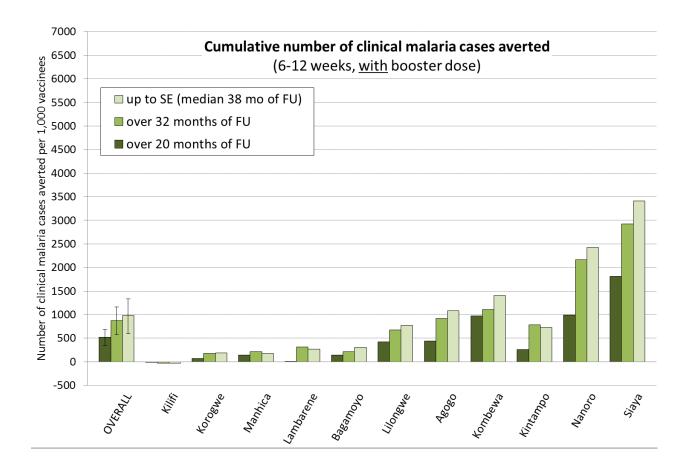
VE against all endpoints evaluated was lower among infants aged 6-12 weeks at enrolment compared to the 5-17 months age group. There was a lower immune response to the vaccine in infants, which could be due to the infant's immature immune system itself, the co-administration of RTS,S/AS01_E with routine EPI vaccines, an inhibitory effect of maternally derived anti-CS antibodies and/or a suppressive effect from maternal malaria in utero [The RTS,S Clinical Trials Partnership, 2012].

The vaccine was shown to be able to provide benefit, especially in study sites located in high transmission areas (Figure 12).

Figure 12. Cumulative number of clinical malaria cases averted overall and by site per 1,000 infants 6-12 weeks of age and vaccinated according to a RTS,S/AS01E primary vaccination schedule without and with a booster dose at Month 20, with sites ordered by increasing malaria incidence



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Across all sites, 8 and 12 severe malaria and 14 and 18 malaria hospitalisation were averted over the entire study period per 1000 infants vaccinated with a primary schedule without and with a booster dose, respectively.

For the assessment of vaccine impact on fatal malaria, the more sensitive secondary case definition 4 (taking into account the fatalities in the community) was used. Over the entire study period in the ITT cohort of infants, there were 26 cases of fatal malaria meeting this case definition, i.e., 8 in the R3R group, 12 in the R3C group and 6 in the C3C group. Failure to detect an impact of the RTS,S/AS01E vaccine on fatal malaria was ascribed by the applicant to the total low fatality rate. An investigator-initiated case control study was conducted at the Siaya site to quantify the reduction in mortality among children enrolled in study Malaria-055 vs. children not enrolled in Malaria-055, but living in the same area. Children enrolled in study Malaria-055 experienced a marked reduction in all-cause mortality of 70 % as compared to children not enrolled in study Malaria-055 [Hamel, 2014].

The modelling approach applied to estimate the PHI of RTS,S/AS01_E suggests that a substantial number of malaria deaths as well as clinical and severe malaria cases may be prevented by RTS,S/AS01_E, especially in moderate and high transmission settings across sub-Saharan Africa. The potential effect of the booster dose appears to be modest, although administration of a booster dose is expected to delay the limited rebound observed for severe malaria and malaria deaths in the absence of a booster dose. Overall, the PHI of RTS,S/AS01E is projected to remain positive without and with booster for all evaluated endpoints.

In infants, there was no evidence of increased incidence of clinical or severe malaria in RTS,S/AS01 $_{\rm E}$ vaccinees during the entire FU period. However, during the post-primary vaccination FU period (M2.5-M20), there was a higher incidence of severe malaria observed in RTS,S/AS01 $_{\rm E}$ recipients as compared to controls between 12 and 18 months FU post Dose 3 (rates: 0.03 per person/year in RTS,S/AS01 $_{\rm E}$

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versus 0.02 per person/year in controls). During subsequent FU periods (M21-M32 and M21-SE), the number of severe cases was similar in infants who did not receive a RTS,S/AS01 $_{\rm E}$ booster and the control group, and there was a trend towards a benefit in infants who received a RTS,S/AS01 $_{\rm E}$ booster.

3.5.2.4. Summary of vaccine efficacy in infants aged 6-12 weeks up to study end with and without a 4th dose

Table 50. Summary table of vaccine efficacy against malaria endpoints over all evaluated FU periods in infants aged 6-12 weeks having received RTS,S/AS01E according to the primary vaccination schedule without and with a booster dose at Month 20 (ATP cohort of efficacy)

Vaccine efficacy after co	mpletion	of primar	y vaco	cinatio	n scl	nedule (b	efore the	booster)							
		Primary	(M14)	analy	/sis°			M2	0 ana	lysis					
Endpoint	n/T R3	n/T C3C	VE	LL	UL	p-value	n/T R3	n/T C3C	VE	LL	UL	p-value			
First or only case*	0.37	0.48	31.2	24.5	37.4	<0.0001	0.34	0.43	29.2	23.0	34.9	<0.0001			
All cases of malaria*	0.64	0.91	32.9	26.4	38.9	< 0.0001	0.71	0.92	26.6	20.3	32.4	<0.0001			
	n/N R3	n/N C3C	VE	LL	UL	p-value	n/N R3	n/N C3C	VE	LL	UL	p-value			
Severe malaria*	58/3993	46/2007	36.6	4.6	57.7	0.0211	100/3996	59/2007	14.9	-19.5	38.9	0.3486			
Incident severe anaemia*	17/3993	14/2007	39.0	-33.7	71.7	0.1829	27/3996	14/2007	3.1	-99.9	51.0	1.0000			
Malaria hospitalisation*	102/3993	75/2007	31.6	6.6	49.8	0.0121	165/3996	100/2007	17.1	-7.3	35.7	0.1427			
Fatal malaria*	0/3993	0/2007	-	-	-	-	0/3996	0/2007	1	-	ı	-			
Vaccine efficacy of a prin	nary vacc	ination s	chedu	le wit	hout	a booster	dose								
	/accine efficacy of a primary vaccination schedule without a booster dose Final (M32) analysis SE analysis														
Endpoint	n/T R3C	n/T C3C	VE	LL	UL	p-value	n/T R3C	n/T C3C	VE	LL	UL	p-value			
First and only case*	ND	ND	-	-	-	-	ND	ND	-	-	ı	-			
All cases of malaria*	0.88	1.03	20.4	13.5	26.8	<0.0001	0.95	1.08	18.2	11.4	24.5	<0.0001			
	n/N R3C	n/N C3C	VE	LL	UL	p-value		n/N C3C	VE	LL	UL	р			
Severe malaria*	79/2005	89/2007	11.1	-21.7	35.2	0.4782	89/2005	102/2007	12.7	-17.2	35.0	0.3737			
Incident severe anaemia*	21/2005	23/2007	8.6	-72.2	51.9	0.8797	27/2005	31/2007	12.8	-50.9	49.9	0.6919			
Malaria hospitalisation*	130/2005	145/2007	10.3	-14.5	29.7	0.3818	143/2005	165/2007	13.2	-9.2	31.1	0.2130			
Fatal malaria*	0/2005	0/2007	-	-	-	-	0/2005	0/2007	-	-	ı	-			
Vaccine efficacy of a prin	nary vacc	ination s	chedu	le wit	h a bo	oster do	se								
		Final (analy	sis				anal	ysis					
Endpoint		n/T C3C	VE	LL	UL	p-value	n/T R3R		VE	LL	UL	p-value			
First and only case*	ND	ND	-	-	-	-	ND	ND	-	-	-	-			
All cases of malaria*	8.0	1.03	28.4	22.1	34.2	<0.0001	0.86	1.08	26.7	20.5	32.4	<0.0001			
	n/N R3C	n/N C3C	VE	LL	UL	p-value	n/N R3C	n/N C3C	VE	LL	UL	p-value			
Severe malaria*	73/1985	89/2007	17.1	-14.3	40.0	0.2300	80/1985	102/2007	20.7	-7.3	41.6	0.1289			
Incident severe anaemia*	19/1985	23/2007	16.5	-60.3	57.0	0.6424	21/1985	31/2007	31.5	-23.1	62.6	0.2090			
Malaria hospitalisation*	108/1985	145/2007	24.7	2.7	41.9	0.0229	119/1985	165/2007	27.1	7.1	42.9	0.0067			
Fatal malaria*	0/1985	0/2007	-	-	-	-	0/1985	0/2007	-	-	-	-			
R3C = RTS,S/AS01E primary vaccir	nation schedu	le without bo	oster						1						

R3C = RTS,S/AS01E primary vaccination schedule without booster

n/N=number of subjects reporting at least one event in each group/ number of subjects in analysis for respective endpoint.

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 $R3R = RTS, S/AS01E \ primary \ vaccination \ schedule \ with \ booster$

R3 = RTS,S/AS01E primary schedule vaccination before the booster (R3C + R3R pooled)

C3C = Contro

[°] VE assessed in all infants enrolled in the 6-12 weeks age category with 95% CI at the time of the M20 analysis is provided to allow comparison with analyses at other timepoints and with other endpoints at M14 only assessed in the M20 analysis. Note that VE against primary objective as provided in initial Application package was analysed with 97.5%CI as provided in Malaria-055 Annex report 3.

^{*} Primary case definition used in analysis of clinical malaria case, severe malaria case, incident severe anaemia case, malaria hospitalisation case (case definition 1) and fatal malaria case.

n/T = person year rate in each group with n = number of episodes included in each group and T(year) = person years at risk

Note: For point estimates shown in bold, the p-value was < 0.05. VE (%) = Vaccine Efficacy unadjusted for covariates, except for study site LL, UL = 95% Lower and Upper CI limits SE = Study end

Additional efficacy analysis provided during the evaluation for infants aged 6-12 weeks

Vaccine impact analysis was performed in the ITT cohort and in the ATP cohort for efficacy for the same endpoints and case definitions. Of note, the ATP cohort for efficacy includes fewer subjects than the ITT cohort because subjects not compliant to the per-protocol procedures were excluded from the analysis. Also the follow-up periods are shorter (case count starts 14 days after Dose 3 and Dose 4 in the ATP cohort for efficacy, whereas it starts from Day 1 after Dose 1 and Dose 4 in the ITT cohort). In the analysis up to SE, the methodology used to calculate the number of cases averted was different as compared to the one used to present vaccine impact up to Month 20, i.e., upon request from a WHO advisory body on malaria vaccines in phase III and phase IV studies, the follow-up (FU) period was divided into 3-monthly periods.

The average number of cases of clinical malaria, malaria hospitalisation, severe malaria, malaria death and severe anaemia averted per 1,000 vaccinated infants in the ATP cohort for efficacy and ITT cohort up to SE are presented in Table 51.

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Table 51. Number of cases averted per 1,000 infants 6-12 weeks of age and vaccinated according to a RTS,S/AS01E primary vaccination schedule without and with a 4th dose

			per 1000 vaccinees in the age category
	FU period post Dose 1	Schedule without a 4th dose (R3C)	Schedule with a 4th dose (R3R)
		n (LL ; UL)	n (LL ; UL)
ATP cohort for efficacy (new a			
Clinical malaria	M0-M20*		5 ; 643)
(secondary case definition 1)	M0-M32	473 (131 ; 786)	855 (559 ; 1160)
	M0-SE	520 (96 ; 905)	982 (596 ; 1359)
Malaria hospitalisation	M0-M20*	8 (-8	; 24)
(case definition 1)	M0-M32	7 (-14 ; 30)	16 (-4 ; 38)
	M0-SE	13 (-10 ; 38)	17 (-7 ; 42)
Severe malaria	M0-M20*	7 (-6	; 19)
(secondary case definition 1)	M0-M32	7 (-10 ; 27)	14 (-3 ; 31)
	M0-SE	7 (-12 ; 29)	13 (-6 ; 31)
Fatal malaria (ICD10 code)	M0-M20*	-1 (-	3 ; 2)
(secondary case definition 4)	M0-M32	-2 (-5 ; 1)	-1 (-4 ; 2)
	M0-SE	-3 (-7 ; 0)	-2 (-6 ; 2)
Incident severe anaemia	M0-M20*	0 (-9	; 10)
(secondary case definition 3)	M0-M32	0 (-13 ; 14)	5 (-8 ; 18)
	M0-SE	-1 (-15 ; 15)	3 (-11 ; 18)
ITT cohort (previously reporte	d analysis in respo	nse to Day 120 Question 109)	
Clinical malaria	M0-M20*	518 (34	1;687)
(secondary case definition 1)	M0-M32	526 (200 ; 819)	873 (573 ; 1158)
	M0-SE	558 (158 ; 926)	983 (592 ; 1337)
Malaria hospitalisation	M0-M20*	8 (-9	; 25)
(case definition 1)	M0-M32	5 (-17 ; 27)	14 (-10 ; 35)
	M0-SE	14 (-13 ; 39)	18 (-8 ; 42)
Severe malaria	M0-M20*	5 (-8	; 18)
(secondary case definition 1)	M0-M32	5 (-13 ; 24)	9 (-8 ; 28)
	M0-SE	8 (-13 ; 28)	12 (-6 ; 32)
Fatal malaria (ICD10 code)	M0-M20*	-1 (-	3;2)
(secondary case definition 4)	M0-M32	-1 (-5 ; 2)	-2 (-5 ; 2)
	M0-SE	-3 (-7 ; 1)	-2 (-6 ; 2)
Incident severe anaemia	M0-M20*		;11)
(secondary case definition 3)	M0-M32	0 (-13 ; 15)	3 (-9 ; 16)
	M0-SE	-1 (-16 ; 15)	3 (-11 ; 17)

Clinical malaria secondary case definition = illness in a child brought to a study facility with a measured temperature of \geq 37.5° C or reported fever within the last 24 hours and P. falciparum asexual parasitaemia at a density of > 0 parasites/ μ L. This definition was used for this analysis as, during routine clinical practice, these children would normally receive a full course of anti-malarial treatment.

Malaria hospitalisation case definition 1 = a medical hospitalisation with confirmed P. falciparum asexual parasitaemia at a density of > 5000 parasites/ μ L. Severe malaria secondary case definition 1 = P. falciparum asexual parasitaemia at a density of > 5000 parasites/ μ L with one or more markers of disease severity, including cases in which a coexisting illness was present or could not be ruled out. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of \leq 2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia, acidosis, elevated lactate level, or haemoglobin level of < 5 g/dL. Co-existing illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.

Fatal malaria (ICD10 code) = a fatal case associated with International Classification Disease (ICD10) code B50, B53, B54.

Incident severe anaemia secondary case definition 3 = a documented haemoglobin < 5.0 g/dL identified at clinical presentation to morbidity surveillance system.

n = number of cases averted per 1000 vaccinees.

LL, UL = 95% Lower and Upper CI limits

SE = Study end

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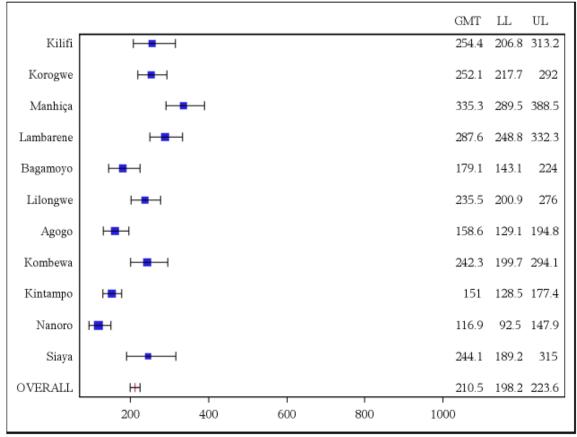
^{*} For M0-M20, the schedule without a 4th dose (R3C) and the schedule with a 4th dose (R3R) were pooled (R3R+R3C) to calculate the number of cases averted

Immunogenicity for the first 200 per study site aged 6-12 weeks at enrolment

In the 2200 subjects in this analysis the screening anti-CS seropositivity rate was comparable and relatively high (34.4% and 35.2%) in the two groups but the GMTs were very low (0.4 EU/mL in both groups). The seropositivity rates one month post-dose 3 were 99.8% vs. 5.7% with GMTs 210.5 vs. 0.3, indicating that maternal antibody decreased in the control group within the first few months of life.

The anti-CS antibody response by study site did not show variability in terms of seropositivity rates but the GMTs ranged from 117 in Nanoro (72% seropositive pre-vaccination; highest value observed at screening) to 335 in Manhiça (5% seropositive pre-vaccination; lowest value observed at screening).

Figure 13. Malaria-055: Anti-CS antibody GMCs one month post Dose 3 in RTS,S/AS01E recipients 6-12 weeks of age by increasing malaria incidence at each study centre (ATP cohort for immunogenicity)



Body weight category had no impact on seropositivity rates or GMTs. However, RTS,S/AS01E recipients who were 6 weeks old at first vaccination had a lower M3 anti-CS response compared to those who were 7-12 weeks old (p=0.0031). RTS,S/AS01E recipients who were seropositive for anti-CS at baseline had a lower M3 response compared to those who were seronegative at baseline (p<0.0001).

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Table 52. Determinants of anti-CS response, results from linear regression analysis ([6-12] weeks) (FU: M2.5-M20) (ATP population for efficacy)

Parameter	Parameter Estimate	Std. Error	VIF	p-value
N	1222			
Root MSE	0.44			
Adjusted R-squared	0.11			
Intercept	2.451	0.026	0.000	<.0001
Male vs. female	0.028	0.025	1.014	0.2781
Anti CS Positive at Baseline vs. negative	-0.306	0.029	1.116	<.0001
Age 6w vs. 7-12w	-0.076	0.026	1.017	0.0031
Incidence in controls	-0.024	0.014	1.139	0.1013
Vitamin A usage	-0.023	0.078	1.038	0.7706
Low HAZ vs. normal HAZ	0.045	0.031	1.167	0.1509
Low WAZ vs. normal WAZ	-0.053	0.051	1.166	0.2927

Std. Error = Standard Error

VIF = Variance Inflation Factor

P-value from linear regression

The exploratory analysis of factors influencing the incidence of clinical malaria showed that higher anti-CS titres at M3 were associated with a lower incidence of clinical malaria (p=0.0003) but RTS,S/AS01E recipients who were seropositive at baseline had a higher clinical malaria incidence (p=0.0001).

Table 53. Effect of anti-CS response on the incidence of clinical malaria (primary case definition) in RTS,S/AS01E recipients ([6-12] weeks) (FU: M2.5-M20) (ATP population for efficacy)

Parameter	Incident rate ratio	95% CI LL	95% CI UL	p-value
Intercept	0.001	0.001	0.002	<.0001
Male vs. female	1.098	0.920	1.310	0.3014
Anti CS Positive at Baseline	1.455	1.204	1.758	0.0001
Anti-CS titre at M3	0.691	0.566	0.844	0.0003
Age 6w vs. 7-12w	0.903	0.756	1.079	0.2620
Incidence in controls	3.078	2.775	3.413	<.0001
Vitamin A usage	0.719	0.457	1.132	0.1542
Low HAZ vs. normal HAZ	0.995	0.796	1.245	0.9684
Low WAZ vs. normal WAZ	1.181	0.828	1.685	0.3581
Dispersion	2.466	2.077	3.046	_

LL = Lower Limit

UL = Upper Limit

P-value from negative binomial regression

In the analysis of the anti-CS antibody response one month after the booster dose (Month 21) stratified by age at the time of the first dose (6 weeks vs. 7-12 weeks), all, except one infant in each age sub-category were seropositive for anti-CS antibodies. Anti-CS antibody GMCs were increased compared to the pre-booster anti-CS antibody levels (Month 20) in both the 6 weeks and 7-12 weeks age sub-categories. However, these were lower in the 6 weeks of age sub-category than in the 7-12 weeks of age sub-category, with non-overlapping 95% CIs, i.e., 144.8 EU/ml (95% CI: 123.7 to 169.5) and 193.4 EU/ml (95% CI: 170.5 to 219.3), respectively (see Table 54).

Hence, a booster dose restored the immune response in terms of anti-CS antibody GMCs in both age sub-categories, although not to the same level as observed after the primary series.

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Table 54. Seropositivity rates and GMTs for anti-CS antibodies at Month 20 and Month 21 (post-booster dose) by age classification: infants aged 6 weeks vs. 7-12 weeks at

first dose (ATP population for immunogenicity)

				≥ 0.5	EU/ML			GMT			
							95% C			95% CI	
Antibody	Sub-group	Group	Timing	N	n	%	LL	UL	value	LL	UL
Anti-CS	6w	R3R	PIII(M20)	235	214	91.1	86.7	94.4	4.9	4.1	6.0
			PIV(M21)	225	224	99.6	97.5	100	144.8	123.7	169.5
	[7-12]w	R3R	PIII(M20)	295	277	93.9	90.5	96.3	6.8	5.7	8.0
			PIV(M21)	278	277	99.6	98.0	100	193.4	170.5	219.3

6w = 6 weeks at the time of dose 1; [7-12]w = 7 to 12 weeks at the time of dose 1

R3R = RTS,S/AS01E primary schedule with booster

GMT = geometric mean antibody titre calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with titre equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum

PIII(M20) = 18 months post Dose 3; PIV(M21) = 1 month post booster

3.5.2.5. Immunogenicity relevant to Hepatitis B

In the study Malaria-055, anti-HBs antibody responses were measured pre-vaccination (Month 0) and one month post Dose 3 (Month 3) in the first 200 subjects enrolled in each study site (11 study sites) and for each age category.

In the 5-17 months age category, 85% in both the R3R + R3C and C3C groups had received a full hepatitis B vaccination course (3 doses) prior to study participation. The anti-HBs GMC at M3 was significantly higher for RTS,S/AS01E.

 Table 55.
 Seroprotection rates and GMTs for anti-HBs antibodies (ATP population for

immunogenicity) in the 5-17months age category

>= 10 MIU/ML								>= 100 MIU/ML					GMC			
	95% CI			6 CI			95% CI		95% CI							
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL	Min	Max
Anti-HBs	R3R+R3C	Screening	1017	906	89.1	87.0	90.9	656	64.5	61.5	67.4	166.3	148.0	186.8	<10.0	131926.5
		PIII(M3)	1029	1028	99.9	99.5	100	1027	99.8	99.3	100	81567.7	75442.7	88189.9	<10.0	2204505
	C3C	Screening	515	461	89.5	86.5	92.0	324	62.9	58.6	67.1	168.6	142.8	199.2	<10.0	57468.9
		PIII(M3)	526	458	87.1	83.9	89.8	314	59.7	55.4	63.9	127.5	108.8	149.4	<10.0	276996.6

R3R+R3C = RTS,S primary schedule with or without booster

C3C = Control

GMC = geometric mean antibody concentration calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum

Screening = Screening visit

PIII(M3) = 1 month post Dose 3

Anti-HBs antibody responses were assessed pre-boost (Month 20) and one month post booster dose (Month 21). The evaluation of pre- and post-boost anti-HBs antibody responses was performed as an ad hoc analysis, after study completion. Anti-HBs antibodies were measured in subjects assigned to receive the booster dose of RTS,S/AS01E (R3R group) among the first 200 subjects enrolled in each age category in three study sites: Lambaréné, Korogwe and Lilongwe.

In the 5-17 months age category, 98.9% of RTS,S/AS01E recipients (R3R group) had seroprotective levels (≥10 mIU/ml) of anti-HBs antibodies both pre-booster (Month 20) and one month post booster dose of RTS,S/AS01E (Month 21). The same proportion of subjects (98.9%) had anti-HBs antibodies ≥100 mIU/ml at Month 20 and Month 21. The anti-HBs antibody GMCs were 5,068.5 mIU/ml (95% CI:

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3,711.3 to 6,922.0) pre-booster and 95,206.4 mIU/ml (95% CI: 72,395.4 to 125,204.9) one month post-booster dose.

Table 56. Seropositivity rates, seroprotection rates, percentages of subjects with anti-HBs antibodies ≥100 mIU/mI and GMCs for anti-HBs antibodies at Month 20 and Month 21 (5-17 months) (ATP cohort for immunogenicity).

		2	6.2	MIU/N	ΛL	l		MIU/N otect			≥ 100	MIU/I	ML		GMC			
				95%	CI			95%	CI			95%	6 CI		95%	% CI		
Timing	N	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL	value	LL	UL	Min	Max
PIII(M20)	95	94	98.9	94.3	100	94	98.9	94.3	100	94	98.9	94.3	100	5068.5	3711.3	6922.0	<6.2	100271.3
PIV(M21)	94	93	98.9	94.2	100	93	98.9	94.2	100	93	98.9	94.2	100	95206.4	72395.4	125204.9	<6.2	659800.0

R3R = RTS,S/AS01E primary schedule with booster

GMC = geometric mean antibody concentration calculated on all subjects

N = number of subjects with available results and from Korogwe, Lilongwe or Lambarene sites

n/% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = lower limit, UL = upper limit

MIN/MAX = minimum/maximum

PIII(M20) = 18 months post Dose 3; PIV(M21) = 1 month post boost

In the 6-12 weeks age category, pre-vaccination about 20% in each group were seroprotected against HBV but < 1% had a birth dose. At M3 99.9% in the RTS,S/AS01E group and 96.3% of controls were seroprotected and over 90% per group had \geq 100 mIU/mL. The GMT was significantly higher in the RTS,S/ASO1_E group.

Table 57. Seroprotection rates and GMTs for anti-HBs antibodies (ATP population for immunogenicity), in the 6-12 weeks age category

					>= 10 N	IIU/ML			>= 100 l	MIU/ML			GMC			
						959	% CI			959	6 CI		959	% CI		
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL	Min	Max
Anti-HBs	R3R+R3C	Screening	1120	225	20.1	17.8	22.6	81	7.2	5.8	8.9	8.6	8.0	9.3	<10.0	14486.0
		PIII(M3)	1213	1212	99.9	99.5	100	1210	99.8	99.3	99.9	13674.3	12811.5	14595.3	<10.0	359317.7
	C3C	Screening	561	115	20.5	17.2	24.1	40	7.1	5.1	9.6	8.5	7.7	9.4	<10.0	6338.0
		PIII(M3)	627	604	96.3	94.5	97.7	570	90.9	88.4	93.0	728.8	643.6	825.2	<10.0	27126.5

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

GMC = geometric mean antibody concentration calculated on all subjects

N = number of subjects with available results

n% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum

Screening = Screening visit

PIII(M3) = 1 month post Dose 3

In the 6-12 weeks age category, 99.3% of RTS,S/AS01E recipients (R3R group) had seroprotective levels (≥ 10 mIU/ml) of anti-HBs antibodies pre-booster (Month 20) and 100% of subjects were seroprotected one month post booster dose of RTS,S/AS01E (Month 21). At Month 20, 97.8% of subjects had anti-HBs antibodies ≥ 100 mIU/ml and one month post-booster dose 100% of subjects were ≥ 100 mIU/ml. The anti-HBs antibody GMCs were 1,532.5 mIU/ml (95% CI: 1,240.6 to 1,893.2) pre-booster and 116,458.1 mIU/ml (95% CI: 86,865.7 to 156,131.6) one month post-booster dose.

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Table 58. Seropositivity rates, seroprotection rates, percentages of subjects with anti-HBs antibodies ≥100 mIU/mI and GMCs for anti-HBs antibodies at Month 20 and Month

21 (6-12 weeks) (ATP cohort for immunogenicity)

		2	6.2	MIU/N	ΛL	_	10 N ropro		_	2	100	MIU/I	ИL		GMC			
				95%	CI			95%	CI			95%	6 CI		95%	% CI		
Timing	N	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL	value	LL	UL	Min	Max
PIII(M20)	134	134	100	97.3	100	133	99.3	95.9	100	131	97.8	93.6	99.5	1532.5	1240.6	1893.2	6.2	19526.0
PIV(M21)	48	48	100	92.6	100	48	100	92.6	100	48	100	92.6	100	116458.1	86865.7	156131.6	1888.2	902600.0

R3R = RTS,S/AS01E primary schedule with booster

GMC = geometric mean antibody concentration calculated on all subjects

N = number of subjects with available results and from Korogwe, Lilongwe or Lambarene sites

n% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = lower limit, UL = upper limit

MIN/MAX = minimum/maximum

PIII(M20) = 18 months post Dose 3; PIV(M21) = 1 month post boost

3.5.2.6. Immunogenicity relevant to anti-CS antibody response after 4th dose for both age groups

In infants as well as in children enrolled in study Malaria-055, the immune response to the 4th dose in terms of geometric mean concentrations (GMCs) of anti-CS antibodies was not as usually expected for a "classical" booster immune response. As shown in Figure 14 the anti-CS antibody GMCs after the 4th dose were lower than after the 3rd dose, while the classical booster response pattern was observed for the HBsAg specific antibody response after the 4th dose of RTS,S/AS01E (see Tables 56-58).

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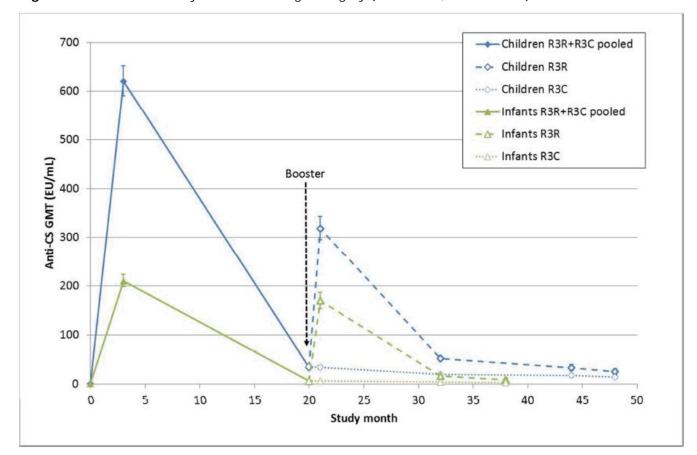


Figure 14. Anti-CS antibody GMCs in each age category (ATP cohort, Malaria- 055)

The Applicant clarified that it could be hypothesised there are similarities between the observed antibody response to the CS portion included in RTS,S antigen and typically T-cell independent humoral responses to non-conjugated bacterial polysaccharide vaccines, for which the potential to induce hyporesponsiveness to further antigen exposure has been demonstrated [O'Brien, 2007; Poolman, 2011]. The potential of the portion of the CS protein included in the RTS,S/AS01_E vaccine to predispose to humoral hyporesponsiveness by a similar mechanism as observed for non-conjugated polysaccharides seems unlikely because of the following reasons:

- Induction of robust T-cell responses by RTS,S/AS01E vaccination in the target population has been demonstrated. This is supported by the evidence for an association between CD4+ T-cell response and protection against Plasmodium falciparum infection as was observed in controlled human malaria infection trials (CHMI) performed in malaria-naïve adults.
- Induction of CS-specific memory B cells in response to RTS,S/AS01 E [Agnandji, 2011] as well as antibody avidity maturation is documented [Ajua, 2015]

The difference in antibody response to the 2 antigens present in the same vaccine might be explained by the fact that only a portion of the CS protein is included in the RTS,S antigen, and it is present in lower quantity than the HBs (S) antigen (5 μ g CS portion vs. 20 μ g HBs). Therefore, the HBs antigen may have an immunodominant role in RTS,S, i.e., more T-cell and B-cell epitopes are present in HBs than in the CS antigen, leading to more and earlier HBs-specific T-cell and B-cell responses than CS-specific T-cell and B cell responses.

This hypothesis of competition between immune response to HBs and CS was further explored by analysing the correlation between individual pre Dose 4 anti-HBs antibody concentrations (at Month

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20) and post Dose 4 anti-CS antibody concentrations (at Month 21) by age category (only available from 3 sites). No correlation was found between pre Dose 4 anti-HBs antibody concentrations and post Dose 4 anti-CS antibody concentrations in both age categories, suggesting that the level of anti-HBs antibodies at the time of the 4th dose administration does not influence the anti-CS antibody response induced by this 4th dose.

3.5.2.7. Concomitant use of other vaccines

Information on the effects of concomitant use comes from study 055 in the 6-12 weeks age group at enrolment (anti-polio antibody only) and from the dedicated interaction studies 050 and 063 - see section 2.5.1).

Study Malaria-055

Pre-vaccination in children aged 6-12 weeks >70% of infants were already seroprotected against OPV1 and 2 but only 37% were seroprotected against OPV3. At one month post-dose 3 the seroprotection rates were comparable between groups for each OPV type, although slightly numerically higher for controls, but the actual rates in both groups were lower for OPV3 vs. OPV1 and OPV2.

For each of the 3 polio serotypes, the RTS,S/AS01 $_{\rm E}$ group was non-inferior to the control group in terms of seroprotection rates post vaccination.

Table 59. Seroprotection rates and GMTs for anti-OPV1/OPV2/OPV3 antibodies (ATP population for OPV immunogenicity)

					≥81	ED50			GMT			
						959	6 CI		959	6 CI		
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
Anti-OPV1	R3R+R3C	Screening	928	719	77.5	74.7	80.1	47.4	41.7	53.8	<8.0	8192.0
		PIII(M3)	913	839	91.9	89.9	93.6	334.9	295.2	379.8	<8.0	8192.0
	C3C	Screening	469	354	75.5	71.3	79.3	43.3	36.2	51.9	<8.0	8192.0
		PIII(M3)	464	437	94.2	91.6	96.1	417.6	351.4	496.2	<8.0	8192.0
Anti-OPV2	R3R+R3C	Screening	928	726	78.2	75.4	80.8	38.6	34.6	43.2	<8.0	2896.0
		PIII(M3)	913	865	94.7	93.1	96.1	372.1	334.5	414.0	<8.0	8192.0
	C3C	Screening	468	357	76.3	72.2	80.1	40.3	34.2	47.5	<8.0	4096.0
		PIII(M3)	466	455	97.6	95.8	98.8	450.8	393.9	516.0	<8.0	8192.0
Anti-OPV3	R3R+R3C	Screening	931	346	37.2	34.1	40.4	9.4	8.6	10.3	<8.0	2048.0
		PIII(M3)	913	729	79.8	77.1	82.4	80.0	71.0	90.1	<8.0	8192.0
	C3C	Screening	474	171	36.1	31.7	40.6	9.1	8.0	10.3	<8.0	2048.0
		PIII(M3)	466	391	83.9	80.2	87.1	95.9	82.0	112.2	<8.0	8192.0

R3R+R3C = RTS,S/AS01E primary schedule with or without booster

C3C = Control

GMT = geometric mean antibody titer calculated on all subjects

N = number of subjects with available results

 $\ensuremath{\text{n}}\xspace/\%$ = number/percentage of subjects with titer equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum

Screening = Screening visit

PIIIM(3) = 1 month post dose 3

For subjects who had received a neonatal dose of OPV the pre-vaccination seroprotection rates were 80.1%, 80.9% and 38.4% for the three respective OPV types in both groups. One month post-dose 3, at least 92.3% were seroprotected against OPV 1 and at least 95.1% for OPV 2. For OPV3 the rates were 81.7% in the RTS,S/AS01E group and 85.9% for controls.

For subjects who had not received a neonatal dose of OPV the pre-vaccination seroprotection rates were at least 63.6%, 64.7% and 28.3% for respective OPV types. One month post-dose 3, at least

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90.9% and 93.7% were seroprotected for anti-OPV1 and 2 but rates for OPV3 were 75.1% in the RTS,S/AS01E group and 78.9% for controls.

Study Malaria -063

Phase III randomized, open, controlled study to evaluate the immune response to the hepatitis B antigen of the RTS,S/AS01E candidate vaccine, when administered as primary vaccination integrated into an EPI regimen to infants living in sub-Saharan Africa.

The three groups that received RTS,S/AS01E also tested three commercial scale vaccine lots and this resulted in randomisation into 11 subgroups. Currently immunogenicity data are reported to M3.

0		0. 1 5 .	Study population		Numbe	r of Subje	ects
Study	Objective(s)	Study Design Schedule	Age Country	Study groups	TVC	ATP	ATP
Malaria- 063	1°: Non-inferiority of anti-HBs immune response vs. Engerix-B	Phase III, open, randomized <i>0-1-2 months</i>	Healthy male and female infants 8 - 12 weeks <i>Burkina Faso, Ghana</i>	3 study groups with 3 lots of RTS,S/AS01 _E , 25μg/0.5ml + Co-Ad (Infanrix/Hib + OPV + Synflorix) + Rotarix staggered 3 study groups with 3 lots of RTS,S/AS01 _E , 25μg/0.5ml + Co-Ad (Infanrix /Hib + OPV + Rotarix) + Synflorix staggered 3 study groups with 3 lots of RTS,S/AS01 _E , 25μg/0.5ml + Co-Ad (Infanrix /Hib + OPV) + staggered (Synflorix + Rotarix) 1 study group with Engerix-B + Co-Ad (Infanrix /Hib + OPV + Synflorix) + Rotarix staggered 1 study group with Engerix-B + Co-Ad (Infanrix /Hib + OPV + Rotarix) + Synflorix Staggered	705	-	immuno 656 RTS,S/AS01 _E = 402 HepB = 254

- The mean age at the time of the first dose was 8.3 weeks with an approximately equal gender split.
- Term infants were not to have had prior vaccination against the primary series antigens and were to have mothers confirmed to be HIV and HBsAg negative during the pregnancy. However, 16-18% per group had seroprotective anti-HBS at screening.
- All groups received measles vaccine at 6 months post-dose 3 and yellow fever vaccine was given at the same time if this was in accordance with local treatment regulations.
- It is planned that all groups will receive Synflorix and Infanrix/Hib booster vaccinations at 18 months of age (Visit 11) and an Engerix-B booster at 48 months after the third dose in the primary series.

In the primary analysis for anti-HBs at M3 in the ATP cohort non-inferiority was demonstrated based on the UL of the 95% CI around the difference in seroprotection rates of -2.16%. There was a large difference in GMTs such that percentages reaching 100 mIU/ml were 100% vs. 83.4% compared with pre-vaccination rates of 4.3% and 5.6%. Thus, RTS,S/AS01E alone was concluded to satisfactorily protect against HBV when administered at the EPI schedule to infants without a birth dose.

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Table 60. Non-inferiority assessment of anti-HBs antibody seroprotection rates in RTS,S/AS01E relative to Engerix-B recipients, Month 3 (ATP cohort for immunogenicity)

						Difference in seropro (Group 2 minus G			
								95 9	% CI
Group 1	N	%	Group 2	N	%	Difference	%	LL	UL
pool_RTSS	397	100	Pool_HepB	253	96.0	Pool_HepB - pool_RTSS	-3.95	-7.12	-2.16

pool_RTSS = All study groups with RTS,5/AS01E vaccine (REP[R0]_1 + REP[R0]_2 + REP[R0]_3 + RER0[P]_1 +

 $RERo[P]_2 + RERo[P]_3 + RE[RoP]_1 + RE[RoP]_2 + RE[RoP]_3)$

Pool_HepB = All study groups with Engerix-B vaccine (HEP[Ro] + HERo[P])

N = number of subjects with available results

% = percentage of subjects with anti-HBs antibody titre \ge 10 mIU/ml

95% CI = 95% Standardized asymptotic confidence interval; LL = lower limit, UL = upper limit

Table 61. Anti-HBs seroprotection rates and GMTs in RTS,S/AS01E vs Engerix-B recipients, Month 3 (ATP cohort for immunogenicity)

					≥ 6.2	mIU/ml			≥ 10	mIU/ml			GMT			
						95%	6 CI			959	6 CI		959	6 CI		
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL	Min	Max
anti-HBs antibody	pool_RTSS	SCREENING	398	83	20.9	17.0	25.2	63	15.8	12.4	19.8	5.0	4.5	5.7	<6.2	3364.0
		PIII(M3)	397	397	100	99.1	100	397	100	99.1	100	6412.7	5732.9	7173.0	158.9	85526.0
	Pool_HepB	SCREENING	251	57	22.7	17.7	28.4	45	17.9	13.4	23.2	5.4	4.7	6.4	<6.2	2670.0
		PIII(M3)	253	246	97.2	94.4	98.9	243	96.0	92.9	98.1	377.4	310.6	458.7	<6.2	13120.0

pool_RTSS = All study groups with RTS,S/AS01E vaccine (REP[Ro]_1 + REP[Ro]_2 + REP[Ro]_3 + RERo[P]_1 + RERo[P]_2 + RERo[P]_3 + RE[RoP]_1 + RERO[P]_2 + RERO[P]_3 + RERO[P]_3 + RERO[P]_3 + RERO[P]_4 + RERO[P]_5 + RERO[P]_6 +

 $RE[RoP]_2 + RE[RoP]_3$

Pool_HepB = All study groups with Engerix-B vaccine (HEP[Ro] + HERo[P])

GMT = geometric mean antibody titre calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with titre equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum SCREENING = Pre-vaccination PIII(M3) = Post Dose 3, Month 3

One month post-dose 3, 97.7% of subjects were seropositive for anti-RF1 (≥ 33 EU/ml) in the RTS,S/AS01E pool vs. 35.3% of controls with GMTs of 307.8 EU/ml and 27.0 EU/ml, respectively.

The effects of co-administration were analysed as follows:

Anti-HBs GMTs were lower in the RTS,S/AS01E and control groups when Synflorix was co-administered but percentages reaching 10 or 100 mIU/mL within each group were not affected. The anti-RF1 responses in the RTS,S/AS01E groups showed the same pattern.

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Table 62. Anti-HBs seroprotection rates and GMTs per co-administration vaccination regimen, Month 3 (cut-off=100) (ATP cohort for immunogenicity)

					≥ 6.2	mIU/ml			≥ 100	mIU/m			GMT			
						959	6 CI			959	% CI		959	% CI		
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL	Min	Max
anti-HBs antibody	REP[Ro]	SCREENING	140	25	17.9	11.9	25.2	5	3.6	1.2	8.1	4.8	4.0	5.8	<6.2	3364.0
		PIII(M3)	140	140	100	97.4	100	140	100	97.4	100	5467.6	4493.8	6652.5	201.8	58466.0
	RERo[P]	SCREENING	122	24	19.7	13.0	27.8	6	4.9	1.8	10.4	4.9	4.0	6.0	<6.2	412.3
		PIII(M3)	123	123	100	97.0	100	123	100	97.0	100	6989.9	5747.5	8501.0	362.0	82160.0
	RE[RoP]	SCREENING	136	34	25.0	18.0	33.1	6	4.4	1.6	9.4	5.4	4.4	6.7	<6.2	981.8
		PIII(M3)	134	134	100	97.3	100	134	100	97.3	100	6998.7	5779.1	8475.7	158.9	85526.0
	HEP[Ro]	SCREENING	134	30	22.4	15.6	30.4	5	3.7	1.2	8.5	5.3	4.3	6.5	<6.2	2670.0
		PIII(M3)	135	131	97.0	92.6	99.2	111	82.2	74.7	88.3	334.4	253.4	441.4	<6.2	7478.0
	HERo[P]	SCREENING	117	27	23.1	15.8	31.8	9	7.7	3.6	14.1	5.6	4.4	7.2	<6.2	1553.0
		PIII(M3)	118	115	97.5	92.7	99.5	100	84.7	77.0	90.7	433.4	329.5	570.1	<6.2	13120.0

REP[Ro] = RTS,S/AS01E + EPICoAd (Infanrix/Hib + Polio Sabin + Synflorix) + Rotarix staggered

RERo[P] = RTS,S/AS01E + EPICoAd (Infanrix/Hib + Polio Sabin + Rotarix) + Synflorix staggered

RE[RoP] = RTS,S/AS01E + EPICoAd (Infanrix/Hib + Polio Sabin) + staggered (Synflorix + Rotarix)

HEP[Ro] = Engerix-B + EPICoAd (Infanrix/Hib + Polio Sabin + Synflorix) + Rotarix staggered

HERo[P] = Engerix-B + EPICoAd (Infanrix/Hib + Polio Sabin + Rotarix) + Synflorix staggered

GMT = geometric mean antibody titre calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with titre equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

 $\mathsf{MIN/MAX} = \mathsf{Minimum/Maximum}$

SCREENING = Pre-vaccination

PIII(M3) = Post Dose 3, Month 3

Within each of the 5 groups the anti-HBs GMTs were consistently lower for Ghana vs. Burkina Faso and some 95% CI did not overlap between the two. The anti-RF1 responses in the RTS,S/AS01E groups showed the same pattern.

The anti-CS GMT was lowest when RTS,S/AS01E was given with Synflorix but the 95% CI all overlapped. Within each of the 5 co-administration groups the anti-CS responses were not notably different between Ghana and Burkina Faso.

Table 63. Anti-CS seropositivity rates and GMTs per co-administration regimen, Month 3 (ATP cohort for immunogenicity)

					≥ 0.5	EU/m	l		GMT			
						959	6 CI		959	% CI		
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
anti-CS antibody	REP[Ro]	SCREENING	141	91	64.5	56.0	72.4	0.7	0.6	0.9	<0.5	5.8
		PIII(M3)	141	141	100	97.4	100	142.2	116.4	173.7	0.9	1855.4
	RERo[P]	SCREENING	124	87	70.2	61.3	78.0	8.0	0.7	0.9	<0.5	15.2
		PIII(M3)	123	123	100	97.0	100	188.5	156.5	227.0	4.2	1443.7
	RE[RoP]	SCREENING	137	80	58.4	49.7	66.7	0.6	0.6	0.8	<0.5	14.9
		PIII(M3)	136	135	99.3	96.0	100	205.5	167.3	252.5	<0.5	1836.1
	HEP[Ro]	SCREENING	136	84	61.8	53.0	70.0	0.6	0.6	0.7	<0.5	6.5
		PIII(M3)	135	16	11.9	6.9	18.5	0.3	0.3	0.3	<0.5	263.5
	HERo[P]	SCREENING	118	75	63.6	54.2	72.2	0.7	0.6	8.0	<0.5	4.2
		PIII(M3)	118	12	10.2	5.4	17.1	0.3	0.3	0.4	<0.5	145.1

The anti-PT, anti-FHA and anti-PRN antibody responses in terms of GMCs were numerically slightly lower in the pooled RTS,S/AS01E group vs. pooled control group but were concluded to be non-inferior vs. controls based on the UL of the 95% CI around GMC ratios of 1.20, 1.21 and 1.22, respectively. Anti-FHA seropositivity was very high before the first dose. All subjects were seropositive against all three antigens after the third dose regardless of co-administered vaccines. Responder rates to these antigens were at least 94% in baseline seropositive and 100% in baseline seronegative subjects with no discernible difference between RTS,S/AS01E and control groups.

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Table 64. Anti-PT, anti-FHA and anti-PRN seropositivity rates and GMCs in subjects vaccinated with acellular pertussis vaccine in co-administration with RTS,S/AS01E vs Engerix-B, Month 3 (ATP cohort for immunogenicity)

					≥5	EU/ml			GMC			
						95	% CI		95	% CI		
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
anti-PT antibody	pool_RTSS	SCREENING	401	116	28.9	24.5	33.6	3.8	3.6	4.1	<5.0	168.0
		PIII(M3)	387	387	100	99.1	100	105.9	99.2	113.1	12.0	1296.0
	Pool_HepB	SCREENING	253	90	35.6	29.7	41.8	4.3	3.9	4.8	<5.0	292.0
		PIII(M3)	247	247	100	98.5	100	114.2	104.8	124.5	18.0	514.0
anti-FHA antibody	pool_RTSS	SCREENING	399	359	90.0	86.6	92.7	13.9	12.7	15.2	<5.0	188.0
•		PIII(M3)	386	386	100	99.0	100	271.1	252.8	290.8	36.0	1933.0
	Pool_HepB	SCREENING	253	237	93.7	89.9	96.3	15.7	14.1	17.5	<5.0	214.0
		PIII(M3)	247	247	100	98.5	100	292.9	268.9	319.1	48.0	2681.0
anti-PRN antibody	pool_RTSS	SCREENING	401	70	17.5	13.9	21.5	3.2	3.0	3.4	<5.0	85.0
•		PIII(M3)	387	387	100	99.1	100	164.1	153.6	175.3	18.0	1051.0
	Pool_HepB	SCREENING	253	48	19.0	14.3	24.4	3.2	3.0	3.5	<5.0	34.0
		PIII(M3)	247	247	100	98.5	100	179.7	164.4	196.5	20.0	1383.0

pool_RTSS = All study groups with RTS,S/AS01E vaccine (REP[Ro]_1 + REP[Ro]_2 + REP[Ro]_3 + RERo[P]_1 + RERo[P]_2 + RERo[P]_3 + RE[RoP]_1 + RERO[P]_2 + RERO[P]_3 + RERO[P]_4 + RERO[P]_5 + RERO[P]_6 + RERO[P]_7 + RERO[P]_8 +

RE[RoP]_2 + RE[RoP]_3)

Pool_HepB = All study groups with Engerix-B vaccine (HEP[Ro] + HERo[P])

GMC = geometric mean antibody concentration calculated on all subjects

N = number of subjects with available results

n% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum SCREENING = Pre-vaccination

PIII(M3) = Post Dose 3, Month 3

When further analysed according to co-administration with/without Synflorix or Rotarix there was no effect apparent for anti-PT or anti-FHA. The anti-PRN GMTs were numerically lower when these antigens were co-administered with Synflorix, regardless of whether or not RTS,S/AS01E was given.

The comparison of responses to the pneumococcal serotypes between pooled RTS,S/AS01E and pooled controls in the two staggered Rotarix groups demonstrated non-inferiority for 9/10 types based on the UL of the 95% CI around ELISA GMC ratios in the range 1.27 to 1.65, the exception being 18C for which the UL of the 95% CI was 2.38. The GMCs were consistently lower in the RTS,S/AS01E group vs. controls except for serotype 14 although the 95% CI overlapped except for 18C (and 9V just overlapped). The percentages with \geq 0.2 µg/mL and \geq 1 µg/mL antibody for each serotype were comparable between groups (except lower for RTS,S/AS01E at the higher cut-off for 18C and 19F) and lowest in both groups for anti-6B.

Breakdown of these pooled data by study site did not show a consistent pattern of higher or lower GMCs across the 10 serotypes within the RTS,S/AS01E or control groups. Where differences between sites were observed they tended to be small.

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Table 65. Anti-pneumococcal serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F antibody seropositivity rates and GMCs (ELISA) following Synflorix vaccination in co-administration with RTS,S/AS01E or Engerix-B, Month 3 (ATP cohort for immunogenicity)

					≥ 0.05	μg/n	nl		≥ 0.2	μg/m	ıl	G	MC			J.
						95%	6 CI			95%	6 CI		95%	6 CI		
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL	Min	Max
anti-1 antibody	REP[Ro]	PIII(M3)	141	141	100	97.4	100	141	100	97.4	100	3.1	2.8	3.6	0.5	19.6
	HEP[Ro]	PIII(M3)	135	135	100	97.3	100	135	100	97.3	100	3.6	3.1	4.2	0.4	25.0
anti-4 antibody	REP[Ro]	PIII(M3)	141	141	100	97.4	100	140	99.3	96.1	100	3.5	3.0	4.0	0.2	17.7
	HEP[Ro]	PIII(M3)	134	134	100	97.3	100	134	100	97.3	100	4.2	3.5	_	0.2	28.8
anti-5 antibody	REP[Ro]	PIII(M3)	141	141	100	97.4	100	141	100	97.4	100	5.1	4.5	_	8.0	28.7
	HEP[Ro]	PIII(M3)	135	135	100	97.3	100	135	100	97.3	100	6.5	5.6		0.5	36.5
anti-6B antibody	REP[Ro]	PIII(M3)	141	_	96.5		98.8	_	87.2		92.3	1.1	8.0	1.3	<0.1	16.6
	HEP[Ro]	PIII(M3)	135	129	95.6	90.6	98.4	118	87.4	80.6	92.5	1.2	1.0	1.6	<0.1	15.4
anti-7F antibody	REP[Ro]	PIII(M3)	141	141	100	97.4		141	100	97.4	100	4.4	3.9		0.9	25.1
	HEP[Ro]	PIII(M3)	135	135	100	97.3	100	135	100	97.3	100	4.9	4.3	_	0.5	39.4
anti-9V antibody	REP[Ro]	PIII(M3)	141	140	99.3	96.1	100	137	97.2	92.9	99.2	2.8	2.4	3.3	<0.1	21.1
	HEP[Ro]	PIII(M3)	135	135	_	97.3	100	134	99.3	95.9	100	3.7	3.3		0.1	23.7
anti-14 antibody	REP[Ro]	PIII(M3)	141	141	100	97.4	100	141	100	97.4	100	5.8	5.0		0.2	45.3
	HEP[Ro]	PIII(M3)	134	134	100	97.3	100	132	98.5		99.8	5.7	4.7	7.0	0.1	78.6
anti-18C antibody	REP[Ro]	PIII(M3)	141	141	100	97.4	100	139	98.6	95.0	99.8	3.4	2.8	4.1	0.1	41.3
	HEP[Ro]	PIII(M3)	134	134	100	97.3	100	134	100	97.3		6.2	5.1		0.3	135.6
anti-19F antibody	REP[Ro]	PIII(M3)	141	141	100	97.4	100	139	98.6	95.0	99.8	4.2	3.4	5.2	0.1	55.5
	HEP[Ro]	PIII(M3)	134	134	100	97.3	100	129	96.3		98.8		4.1	6.4	0.1	66.6
anti-23F antibody	REP[Ro]	PIII(M3)	140	134	95.7	90.9	98.4	129	92.1	86.4	96.0	1.3	1.1	1.6	<0.1	16.8
	HEP[Ro]	PIII(M3)	134	128	95.5	90.5	98.3	120	89.6	83.1	94.2	1.5	1.1	1.9	<0.1	16.7

$$\label{eq:RepRo} \begin{split} \text{REP[Ro]}: & \text{RTS,S/AS01}_{\text{E}} + \text{PCV} + \text{DTPa/Hib} + \text{OPV, Rota staggered} \\ \text{HEP[Ro]}: & \text{Hepatitis B vaccine} + \text{PCV} + \text{DTPa/Hib} + \text{OPV, Rota staggered} \end{split}$$

The comparison of OPA between pooled RTS,S/AS01E and pooled controls in the two staggered Rotarix groups showed lower percentages with titres ≥ 1:8 in the former group for serotypes 1, 4, 9V, 18C and 19F but the actual differences were within 4 percentage points except for 19F, which was within 5 percentage points. The OPA GMTs were lower for RTS,S/AS01E for 7/10 serotypes (exceptions were 6B, 14 and 23F) but all 95% CI overlapped. Similar results applied in the TVC.

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Table 66. Anti-pneumococcal serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F antibody seropositivity rates and GMTs (OPA) following Synflorix vaccination in coadministration with RTS,S/AS01E or Engerix-B, Month 3 (ATP cohort for immunogenicity

					≥8	1/DIL			GMT			
						959	% CI		959	% CI		
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
OPA-1	REP[Ro]	PIII(M3)	132	89	67.4	58.7	75.3	48.9	34.6	68.9	<8.0	3015.0
	HEP[Ro]	PIII(M3)	124	88	71.0	62.1	78.8	65.0	45.0	93.7	<8.0	3366.0
OPA-4	REP[Ro]	PIII(M3)	130	127	97.7	93.4	99.5	768.3	617.6	955.8	<8.0	10157.0
	HEP[Ro]	PIII(M3)	123	123	100	97.0	100	810.9	676.5	972.0	50.0	11686.0
OPA-5	REP[Ro]	PIII(M3)	133	126	94.7	89.5	97.9	77.6	61.9	97.3	<8.0	4898.0
	HEP[Ro]	PIII(M3)	124	116	93.5	87.7	97.2	93.8	73.6	119.6	<8.0	1131.0
OPA-6B	REP[Ro]	PIII(M3)	128	107	83.6	76.0	89.5	444.4	295.0	669.5	<8.0	13106.0
	HEP[Ro]	PIII(M3)	121	98	81.0	72.9	87.6	389.3	250.1	606.1	<8.0	8943.0
OPA-7F	REP[Ro]	PIII(M3)	132	132	100	97.2	100	3774.0	3232.7	4405.8	162.0	27999.0
	HEP[Ro]	PIII(M3)	124	124	100	97.1	100	3947.4	3338.3	4667.7	59.0	98164.0
OPA-9V	REP[Ro]	PIII(M3)	132	128	97.0	92.4	99.2	1257.7	977.3	1618.7	<8.0	11574.0
	HEP[Ro]	PIII(M3)	122	121	99.2	95.5	100	1469.3	1180.4	1828.8	<8.0	42178.0
OPA-14	REP[Ro]	PIII(M3)	132	131	99.2	95.9	100	1426.3	1136.0	1790.9	<8.0	26900.0
	HEP[Ro]	PIII(M3)	123	118	95.9	90.8	98.7	1269.0	965.1	1668.6	<8.0	10167.0
OPA-18C	REP[Ro]	PIII(M3)	124	110	88.7	81.8	93.7	192.6	139.2	266.4	<8.0	4325.0
	HEP[Ro]	PIII(M3)	118	109	92.4	86.0	96.5	249.7	185.0	337.0	<8.0	5289.0
OPA-19F	REP[Ro]	PIII(M3)	129	105	81.4	73.6	87.7	159.3	109.9	231.0	<8.0	3768.0
	HEP[Ro]	PIII(M3)	123	106	86.2	78.8	91.7	228.8	160.4	326.3	<8.0	8494.0
OPA-23F	REP[Ro]	PIII(M3)	132	109	82.6	75.0	88.6	760.9	476.3	1215.5	<8.0	27030.0
	HEP[Ro]	PIII(M3)	121	99	81.8	73.8	88.2	735.6	456.3	1185.9	<8.0	17182.0

REP[Ro] : RTS,S/AS01E + PCV + DTPa/Hib + OPV, Rota staggered

HEP[Ro]: Hepatitis B vaccine + PCV + DTPa/Hib + OPV, Rota staggered

At M3 all subjects were seropositive for anti-PD (≥ 100 EU/ml) but the GMTs were 2435.3 EU/ml in the RTS,S/AS01E group and 2956.7 EU/ml for controls with 95% CI that only just overlapped.

On comparing the two staggered Synflorix groups the anti-rotavirus IgA concentrations were slightly lower in the RTS,S/AS01E group vs. controls at one month after the second dose of Rotarix but the UL of the 95% CI around the GMC ratio (1.61) was within the pre-defined acceptance limit.

Table 67. Anti-RV seropositivity rates and GMCs following Rotarix vaccination in coadministration with RTS,S/AS01E or Engerix-B, Month 3 (Total vaccinated cohort)

					≥ 20 U/ml				GMC			
						95%	6 CI		95%	6 CI		
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
anti-rotavirus IgA antibody	RERo[P]	PII(M3)	121	45	37.2	28.6	46.4	25.1	19.5	32.2	<20.0	1499.0
	HERo[P]	PII(M3)	121	45	37.2	28.6	46.4	28.6	21.5	37.9	<20.0	3386.0

RERo[P] = RTS,S/AS01E + EPICoAd (Infanrix/Hib + Polio Sabin + Rotarix) + Synflorix staggered

 $\mathsf{HERo}[\mathsf{P}] = \mathsf{Engerix} - \mathsf{B} + \mathsf{EPICoAd} \; (\mathsf{Infanrix} / \mathsf{Hib} + \mathsf{Polio} \; \mathsf{Sabin} + \; \mathsf{Rotarix}) \; + \; \mathsf{Synflorix} \; \mathsf{staggered}$

 $\label{eq:GMC} \text{GMC} = \text{geometric mean antibody concentration calculated on all subjects}$

N = number of subjects with available results

 $\ensuremath{\text{n}}\xspace/\%$ = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum

PII(M3) = Post Dose 2, Month 3

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3.5.2.8. Lot-to lot- consistency

Study Malaria-063

Lot-to-lot consistency for anti-HBs elicited by RTS,S/AS01E was demonstrated based on the predefined limits around the GMT ratio (0.5-2). The anti-HBs responses for pooled lot data were comparable but the individual randomised groups showed GMTs that were consistently lowest in the first sub-group (Synflorix co-administered) and consistently higher in the groups that received staggered Synflorix.

Table 68. Lot-to-lot consistency of anti-HBs antibodies per RTS,S/AS01E lot, GMT ratios, Month 3 (ATP cohort for immunogenicity)

				GMT ratio					
					95% CI				
Group	N	GMT	Group	N	GMT	Ratio order	Value	LL	UL
description			description						
pool_L1	132	6214.3	pool_L2	134	6826.1	pool_L1 /pool_L2	0.91	0.69	1.20
pool_L1	132	6214.3	pool_L3	131	6209.2	pool_L1 /pool_L3	1.00	0.76	1.32
pool_L2	134	6826.1	pool_L3	131	6209.2	pool_L2 /pool_L3	1.10	0.84	1.45

pool_L1 = All study groups with RTS,S/AS01E Lot 1 (REP[Ro]_1 + RERo[P]_1 + RE[RoP]_1)

 $pool_L2 = AII \ study \ groups \ with \ RTS,S/ASO1E \ Lot \ 2 \ (REP[Ro]_2 \ + \ RERo[P]_2 \ + \ RE[RoP]_2)$

 $pool_L3 = All \ study \ groups \ with \ RTS,S/ASO1E \ Lot \ 3 \ (REP[Ro]_3 \ + \ RERo[P]_3 \ + \ RE[RoP]_3)$

GMT = geometric mean antibody titre

N = Number of subjects with post-vaccination results available

95% CI = 95% confidence interval for the GMT ratio (Anova model - pooled variance with more than 2 groups); LL = lower limit, UL = upper limit

The anti-CS responses for pooled lot data were comparable but the individual randomised groups showed GMTs that were consistently lowest in the first sub-group (Synflorix co-administered).

Study Malaria-061

Title: A phase III, double-blind, randomized, multi-center study to evaluate the consistency of immunogenicity of three commercial scale lots of GlaxoSmithKline Biologicals' RTS,S/AS01E candidate malaria vaccine and to demonstrate non inferiority of three commercial scale lots compared to a pilot scale lot when administered intramuscularly on a 0, 1, 2-month schedule to children aged 5 to 17 months in sub-Saharan Africa. This double-blind (with respect to vaccine lots) study compared three commercial and one pilot scale lot.

Study		Study Design	Study population		Number	of Subjects	6
Study	Objective(s)	Schedule	Age Country	Study groups	TVC	ATP efficacy	ATP immuno†
Malaria- 061	1°: Lot-to-lot consistency 3 commercial scale lots vs. pilot scale lot of RTS,S/AS01 _E	Phase III, double-blind, randomized (1:1:1:1), multicentre study with four groups 0-1-2 months	Healthy male and female children 5 - 17 months <i>Nigeria</i>	RTS,S/AS01 $_{\rm E}$, lot 1, 25 μ g/0.5ml RTS,S/AS01 $_{\rm E}$, lot 2, 25 μ g/0.5ml RTS,S/AS01 $_{\rm E}$, lot 3, 25 μ g/0.5ml Control RTS,S/AS01 $_{\rm E}$, 25 μ g/0.5ml	320	-	72 72 73 72

The primary objectives were:

- 1. To demonstrate lot-to-lot consistency for anti-CS antibody elicited by 3 commercial scale lots one month post-dose 3 95% CI around the GMT ratios were all to be within [0.5, 2]
- 2. If the first primary objective was met then the co-primary objective was to demonstrate non-inferiority of the commercial scale lots vs. the pilot scale lot. One month post-dose 3 the UL of the 95% CI around the GMT ratio for pilot vs. pooled commercial scale lots was to be < 2

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An additional sensitivity analysis was included in the SAP prior to unblinding of study data to address the impact of having the third dose of the primary series of HBsAg vaccine within 30 days of the first RTS,S/AS01E dose (i.e. it was confined to those with at least 30 days between these vaccinations).

The study population had a mean age at enrolment between 9.8 and 10.2 months. Male subjects accounted for 45.7%, 49.4% and 58.8% in the commercial scale groups and 62.5% in the pilot scale group.

All subjects were anti-CS seropositive at one month post-dose 3 with GMTs from 241-320 EU/mL.

Table 69. Seropositivity rates and geometric mean titers (GMT) for anti-CS antibody titers (ATP cohort for immunogenicity)

							_					
				≥	0.5	EU_n	nl		GMT			
						95% CI			95%	6 CI		
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
Anti-CS	lot_1	PRE	72	2	2.8	0.3	9.7	0.3	0.2	0.3	<0.5	1.3
		PIII(M3)	72	72	100	95.0	100	319.6	268.9	379.8	25.9	1830.5
	lot_2	PRE	72	0	0.0	0.0	5.0	0.3	0.3	0.3	<0.5	<0.5
		PIII(M3)	72	72	100	95.0	100	241.4	207.6	280.7	63.0	1981.6
	lot_3	PRE	73	2	2.7	0.3	9.5	0.3	0.2	0.3	<0.5	4.2
		PIII(M3)	73	73	100	95.1	100	302.3	259.4	352.3	60.5	1021.5
	pooled lot	PRE	217	4	1.8	0.5	4.7	0.3	0.2	0.3	<0.5	4.2
		PIII(M3)	217	217	100	98.3	100	285.8	260.7	313.3	25.9	1981.6
	control	PRE	72	1	1.4	0.0	7.5	0.3	0.2	0.3	<0.5	1.1
		PIII(M3)	72	72	100	95.0	100	271.7	228.5	323.1	41.2	1729.3

 $lot_1 = RTS, S/AS01E$ commercial scale lot -Lot 1

lot_2 = RTS,S/AS01E commercial scale lot -Lot 2

lot_3 = RTS,S/AS01E commercial scale lot -Lot 3

pooled lot = RTS,S/AS01E commercial scale Lot 1 + RTS,S/AS01E commercial scale -Lot 2 + RTS,S/AS01E commercial scale Lot 3 control = RTS,S/AS01E 20L

GMT = geometric mean antibody titer calculated on all subjects

N = number of subjects with available results

 $\ensuremath{\text{n}}\xspace/\%$ = number/percentage of subjects with titer equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum

 $\mathsf{PRE} = \mathsf{Pre}\text{-}\mathsf{vaccination}$

PIII(M3) = Post Dose 3, Month 3

The three RTS,S/AS01E commercial scale lots were concluded to be consistent based on 95% CI around the GMT ratios between 0.64 and 1.65.

Table 70. Consistency assessment in terms of GMT ratios between the three commercial scale lots for anti-CS, Post Dose III, Month 3 (ATP cohort for immunogenicity)

			GMT ratio						
			95%	6 CI					
Group	N	GMT	Group	N	GMT	Ratio order	Value	LL	UL
description			description						
lot_1	72	319.6	lot_2	72	241.4	lot_1 /lot_2	1.32	1.06	1.65
lot_1	72	319.6	lot_3	73	302.3	lot_1 /lot_3	1.06	0.85	1.32
lot_2	72	241.4	lot_3	73	302.3	lot_2 /lot_3	0.80	0.64	1.00

lot_1 = RTS,S/AS01E commercial scale Lot 1

 $lot_2 = RTS, S/AS01E$ commercial scale Lot 2

lot_3 = RTS,S/AS01E commercial scale Lot 3

GMT = geometric mean antibody titer

 $N = Number\ of\ subjects\ with\ post-vaccination\ results\ available$

The pooled commercial scale lots were non-inferior to the pilot scale based on an UL of the 95% CI around the GMT ratio of 1.15.

Table 71. Non-inferiority assessment in terms of GMT ratios between the pooled commercial scale lots and the Pilot scale lot for anti-CS, Post Dose III, Month 3 (ATP cohort for immunogenicity)

			eenengemen,									
				GMT ratio								
				(control / pooled lot)								
CO	ntrol	pooled lot			95% CI							
N	GMT	N	GMT	Value	LL	UL						
72	271.7	217	285.8	0.95	0.79	1.15						

pooled lot = RTS,S/AS01E commercial scale Lot 1 + RTS,S/AS01E commercial scale-Lot 2 + RTS,S/AS01E commercial scale-Lot 3

control = RTS,S/AS01E pilot scale

GMT = geometric mean antibody titer

N = Number of subjects with post-vaccination results available

95% CI = 95% confidence interval for the GMT ratio (Anova model - pooled variance); LL = lower limit, UL = upper limit

For anti-HBs more than 90% were seroprotected at baseline and at least two-thirds had \geq 100 mIU/mL. All subjects were seroprotected and all had \geq 100 mIU/mL at one month post-dose 3. The GMTs were 46067.3-67384.7 mIU/mL for the commercial lots and 74105.0 mIU/mL for the pilot scale lot. All 95% CI overlapped between the four groups.

Table 72. Number and percentage of subjects with an anti-HBs antibody concentration equal to or above the cut-offs of 10 and 100 mIU/ml and GMCs (ATP cohort for immunogenicity)

					≥ 10 i	mIU/m	ı		≥ 100	mIU/n	ıl		GMC	
						95%	% CI			95%	6 CI		959	% CI
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL
Anti-HBs	lot_1	PRE	72	69	95.8	88.3	99.1	52	72.2	60.4	82.1	352.8	217.1	573.4
		PIII(M3)	72	72	100	95.0	100	72	100	95.0	100	54250.2	43293.6	67979.7
	lot_2	PRE	72	68	94.4	86.4	98.5	48	66.7	54.6	77.3	202.3	131.1	312.3
		PIII(M3)	72	72	100	95.0	100	72	100	95.0	100	46067.3	33919.2	62566.2
	lot_3	PRE	73	67	91.8	83.0	96.9	50	68.5	56.6	78.9	293.7	170.5	506.1
		PIII(M3)	73	73	100	95.1	100	73	100	95.1	100	67384.7	52271.4	86867.7
	pooled	PRE	217	204	94.0	90.0	96.8	150	69.1	62.5	75.2	275.8	208.4	365.1
		PIII(M3)	217	217	100	98.3	100	217	100	98.3	100	55273.5	47508.3	64308.0
	control	PRE	72	69	95.8	88.3	99.1	54	75.0	63.4	84.5	313.7	201.7	487.6
		PIII(M3)	72	72	100	95.0	100	72	100	95.0	100	74105.0	58613.6	93690.7

lot_1 = RTS,S/AS01E commercial scale Lot 1

lot_2 = RTS,S/AS01E commercial scale Lot 2

lot_3 = RTS,S/AS01E commercial scale Lot 3

 $pooled = \mathsf{RTS}, \mathsf{S/ASO1E} \ commercial \ scale \ \mathsf{Lot} \ 1 + \mathsf{RTS}, \mathsf{S/ASO1E} \ commercial \ scale \ \mathsf{Lot} \ 2 + \mathsf{RTS}, \mathsf{S/ASO1E} \ commercial \ scale \ \mathsf{Lot} \ 3$

control = RTS,S/AS01E pilot scale

 $\label{eq:GMC} \text{GMC} = \text{geometric mean antibody concentration calculated on all subjects}$

N = number of subjects with available results

n/% = number/percentage of subjects with concentration equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

 $\mathsf{PRE} = \mathsf{Pre}\text{-}\mathsf{vaccination}$

PIII(M3) = Post Dose 3, Month 3

There were 60/298 ATP subjects across all groups who had received their third dose of HBsAg within 30 days of the first RTS,S/AS01E dose but there was no consistent effect on the final GMTs.

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3.5.2.9. Other data of importance

Malaria-058 in HIV-infected subjects

Data up to the final visit at month 14 (12 months post-dose 3) was initially provided. Any scheduled EPI vaccines appropriate for the age group (since this covered infants and toddlers) were to be administered at least one week apart from study vaccines.

		Ctudy Docian	Study population		Number of	Subjects	
Study	Objective(s)	Study Design Schedule	Age Country	Study groups	HVC:	ATP efficacy	ATP immuno†
Malari 058	Safety and immunogenicity	Phase III, double-blind, 0-1-2 months	HIV-infected aged 6 weeks - 17 months Kenya	RTS,S/AS01 _E , 25µg/0.5ml Rabies vaccine	99 101	87 93	82 74

Eligible subjects were to be HIV-infected (documented positive by DNA PCR; see laboratory assays), whether or not taking HIV ART, and born following at least 36 weeks gestation. Excluded were those with Grade III or IV AIDS (WHO paediatric AIDS clinical staging). The collection, categorisation and analysis of data related to vaccine efficacy were as for study 055.

Randomisation was planned to enrol equivalent numbers aged 6 weeks to 4 months and 5 to 17 months and with CD4% levels <10%, 10-14%, 15-19% and \geq 20%. There were 200 subjects enrolled (99 RTS,S/AS01E, 101 controls) of which 82 (17 aged 6 weeks to 4 months) and 74 (13 aged 6 weeks to 4 months) were in the ATP and 87 and 93 were assessed for efficacy. Most were WHO HIV/AIDS Stage 1 and >80% had HIV RNA loads > 400 copies/mL.

One month post-dose 3 all subjects in the RTS,S/AS01E group vs. 12.7% in the control group were seropositive for anti-CS with GMTs 329.2 EU/ml and 0.3 EU/ml. At 12 months post-dose 3, 98.6% and 9.0% in respective groups were seropositive. The GMT in the RTS,S/AS01E group had fallen to 18.4 EU/ml and the GMT in the control group was unchanged (0.3 EU/ml).

Table 73. Seropositivity rates and GMTs for anti-CS antibodies (ATP population for immunogenicity)

					≥ 0.	5 EU/m	nl		GMT			
						95%	% CI		959	% CI		
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
anti-CS antibody	RTSS	SCREENING	81	16	19.8	11.7	30.1	0.3	0.3	0.4	<0.5	7.8
		PIII(M3)	79	79	100	95.4	100	329.2	260.6	415.8	16.7	1732.7
		PIII(M14)	73	72	98.6	92.6	100	18.4	13.3	25.5	<0.5	571.8
	Control	SCREENING	73	13	17.8	9.8	28.5	0.3	0.3	0.4	<0.5	7.2
		PIII(M3)	71	9	12.7	6.0	22.7	0.3	0.3	0.3	<0.5	2.0
		PIII(M14)	67	6	9.0	3.4	18.5	0.3	0.3	0.3	<0.5	1.6

RTSS = RTS,S/AS01E vaccine

Control = Rabies vaccine

GMT= geometric mean antibody titre calculated on all subjects

N = number of subjects with available results

 $\ensuremath{\text{n}}\xspace/\%$ = number/percentage of subjects with titre equal to or above specified value

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

MIN/MAX = Minimum/Maximum

 ${\sf SCREENING} = {\sf Screening} \ {\sf visit}$

PIII(M3) = 1 month post Dose 3

PIII(M14) = 12 months post Dose 3

The incidence of all clinical malaria episodes meeting the primary case definition was 0.551 vs. 0.838 episode per subject per year in the RTS,S/AS01E vs. control group in the TVC.

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Over 12 months of follow-up post-dose 3 in the ATP population for efficacy:

- VE against first or only episodes of clinical malaria meeting the PDef was 30.9% (95% CI: -18.7% to 59.8%; p-value = 0.1809)
- VE against all episodes of clinical malaria was 37.2% (95% CI: -26.5% to 68.8%; p-value = 0.1919)

In the same period in the TVC one case of severe malaria met the PDef in the RTS,S/AS01E group vs. 8 controls. There were one and 10 cases in respective groups that met the SDef 1. At 12 months post-dose 3 7/99 RTS,S/AS01E group and 3/101 (3.0%) controls had parasitaemia (p=0.21).

At baseline, use of ART was reported in 64.6% and 63.4% per group but at month 14 all subjects were on ART. Co-trimoxazole (CTX) was used by 88-89% at baseline and by 97.7% and 93.3% at month 14.

The median viral load at baseline was 149,000 copies/ml in the RTS,S/AS01E group and 157,000 copies/ml in the control group. Viral loads decreased in both groups on study, reaching 3125 and 584 copies/ml in respective groups at 1 month post-dose 3, 3790 and 400 copies/ml at 6 months post-dose 3 and 947 vs. 400 copies/ml at 12 months post-dose 3.

The mean CD4+ % at baseline was $27.55 \pm 8.48\%$ in the RTS,S/AS01E group and $26.52 \pm 8.48\%$ in the Rabies vaccine group. Mean values were 29.70% vs. 29.92% at 1 month post-dose 3, 32.70% vs. 31.07% at 6 months post-dose 3 and 32.80% vs. 31.61% at 12 months post-dose 3 (TVC). The mean CD4+ absolute cell counts did not change appreciably in either group from baseline to 12 months post-dose 3 (when they were 1995.36 cells/µl and 2003.70 cells/µl (TVC).

At baseline, 81.8% RTS,S/AS01E and 81.2% control subjects had Stage 1 HIV/AIDS and the remainder had Stage 2 HIV/AIDS. At 12 months post-dose 3, 80.4% and 73.4% in respective groups had Stage 1 HIV/AIDS, 10.9% and 19.1% had Stage 2 and 3.3% vs. 1.1% had Stage 3. There were 2.1% of subjects in the control group with Stage 4.

The mean HAZ at baseline was -1.67 in the RTS,S/AS01E group and -1.98 in the control group, with no improvement during follow up (-1.74 vs. -2.26 at 12 months post-dose 3 (TVC). There was only a slight improvement in the mean WAZ during this time (from -1.38 and -1.67 at baseline to -1.10 and -1.47).

Malaria-026

This was a large Phase 2 study in children aged 1-4 years in Mozambique. Cohort 1 (N=1,605) was followed using PCD to evaluate vaccine efficacy against clinical malaria and Cohort 2 (N=417) was followed to evaluate vaccine efficacy against incident infection. All children received 3 doses of RTS,S/AS02A or a control vaccine at 0, 1 and 2 months.

During the first 6 months double-blind phase VE against first clinical malaria episodes was 29.9% (95% CI: 11.0 to 44.8) and against all malaria episodes was 27.4% (95% CI: 6.2 to 43.8). VE against severe malaria was 57.7% (95% CI: 16.2 to 80.6) and VE against all hospital admissions was 32.3% (95% CI: 1.3 to 53.9). In the extension Malaria-039 over 21 up to 45 months post-dose 1, VE against all clinical malaria episodes was maintained at 25.6% (95% CI: 11.9 to 37.1, p<0.001) while VE against severe malaria over 42 months was 38.3% (95% CI: 3.4 to 61.3) and VE against all hospital admissions was 22.2% (95% CI: -3.8 to 41.7). However, when the analysis of all episodes was broken down by time period there was no efficacy in the period 30.5 to 42.5 months.

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 Table 74. Efficacy against all episodes of clinical malaria in cohort 1 of Malaria-026/

039 (ATP efficacy)

Time post-dose 3	RTS,S/AS02	Control		VE adjusted for covariates								
	Subjects (N)	No. of events	PYAR	Rate	Subjects (N)	No. of events	PYAR	Rate	(%)	95% CI		P value
Months 0.5 to 6	745	153	340.96	0.45	745	190	330.10	0.58	27.4	6.2	43.8	0.014
Months 6 to 18.5	723	157	663.5	0.24	719	193	642.3	0.30	28.8	6.2	45.9	0.016
Months 18.5 to 30.5	650	252	591.27	0.43	645	291	577.30	0.50	22.7	1.4	39.4	0.038
Months 30.5 to 42.5	638	99	600.85	0.16	629	100	597.28	0.17	8.8	-32.7	37.3	0.630
Months 0.5 to 42.5	745	658	2194.3	0.30	745	774	2142.8	0.36	25.6	11.9	37.1	<0.001

All Clinical Episodes = those occurring over total time at risk; the presence of P. falciparum asexual parasitaemia > 2,500 per µL and the presence of fever

Control = Prevenar and Hiberix (< 24 months)/Engerix-B (>24 months)

Covariates: age, geographical area, bednet use, distance from nearest health centre

Efficacy of RTS, S/AS02 against different P. falciparum strains

The RTS,S recombinant protein is expressed in yeast using the CS sequence from the *P. falciparum* strain NF54, 3D7 clone. There are different variants of the CS protein in the parasite population, the most variable domains being the T-cell epitopes near the C-terminus of the protein, called Th2R and Th3R. The repeat domain, target of the neutralising antibodies, is well conserved amongst strains and the applicant expected that at least the humoral response induced by RTS,S/AS01 and RTS,S/AS02 would cross-react with different *P. falciparum* strains.

In ancillary studies researchers investigated if protection against infection or clinical malaria provided by RTS,S/AS was sequence-dependent with regard to the Th2R and Th3R epitopes of CS protein.

- In Malaria-005, Malaria-044 and Malaria-026, analysis of the sequences of the Th2R and Th3R regions showed no relevant difference in the prevalence of vaccine-type or other allele sequences between vaccine and control groups.
- In Malaria-005 and Malaria-026, there was virtually no infection with vaccine—type *P. falciparum*.
- In Malaria-044, the proportion of *P. falciparum* isolates with non-vaccine type residues was significantly different in vaccinees vs controls for one amino-acid in Th2R and one amino-acid in Th3R. These differences were in opposite direction (one more prevalent in vaccine group and the other more prevalent in control group). Such a difference was also observed for one amino-acid in pre-vaccination samples and it was concluded that there was no biologically relevant overall effect.

These genotyping results were considered to support the hypothesis that RTS,S/AS provides allele-independent protection against *P. falciparum* infections. No evidence was found for vaccine-induced selection of escape mutants or for strain-specific protection. An ongoing ancillary study Malaria-066 is evaluating the genetic polymorphism of the CS protein of *P. falciparum* found in infected subjects from the RTS,S/AS01E or control groups in study Malaria-055 with results expected in early 2016.

Public Health Impact - Modelling

The applicant also provided estimates of the vaccine's efficiency over 15 years using 4 different models by GSK, Swiss Tropical and public health Institute, Imperial College (London) and the Institute for Disease Modeling (Seattle). All models were developed independently and covered estimates for clinical disease, severe disease (except for Imperial) and deaths (regardless of co-morbidities) by malaria. All models were applied to the older age group with a vaccination scheme of 6, 7.5 and 9

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^{≥ 37.5°}C at the time of presentation and occurring in a child who is unwell and brought for treatment to a healthcare facility

months of age with or without a 4th dose given 18 months post 3rd dose. GSK, Imperial College and Swiss TPH have applied their model to the younger age group as well. All models were calibrated using the data derived by study Malaria-055.

Table 75. Total number of malaria cases occurring over 15 years by transmission intensity as estimated by the 4 different PHI modelling approaches

Parasite prevalence		GSK			IDM	// Imperial Swiss TPH		Swiss TPH			
	Clinical malaria	Severe malaria	Malaria deaths	Clinical malaria	Severe malaria	Malaria deaths	Clinical malaria	Malaria deaths	Clinical malaria	Severe malaria	Malaria deaths
3%	15,543 (14,560; 16,593)	537 (332; 788)	149 (92; 219)	90,826 (87,602; 95,099)	4,186 (2,032; 7,975)	691 (335; 1,316)	120,483 (85,394, 155,572)	951 (812; 1,089)	274,744 (243,510 ; 308,010)	3,699 (2,022 ; 5,269)	705 (505 ; 1,021)
10%	120,544 (117,253; 123,530)	3,145 (2,571; 3,739)	872 (713; 1,037)	299,982 (281,142; 317,376)	10,253 (5,295; 18,500)	1,692 (874; 3,052)	378,787 (279,117; 478,456)	1,717 (1,503; 1,931)	1,066,008 (944,818; 1,195,080)	14,332 (7,845 ; 20,455)	2,734 (1,963 ; 3,961)
30%	371,703 (364,220; 378,963)	8,505 (7,471; 9,459)	2,360 (2,073; 2,624)	755,729 (635,608; 862,482)	14,208 (6,823; 26,910)	2,344 (1,126; 4,440)	767,599 (578,466; 956,733)	2,513 (2,218; 2,809)	1,937,575 (1,665,277; 2,273,172)	19,420 (11,713 ; 21,381)	3,445 (2,937 ; 4,132)
50%	562,462 (552,075; 573, 142)	12,277 (11,004; 13,397)	3,406 (3,053; 3,717)	971,621 (761,434; 1,198,648)	15,589 (7,902; 29,506)	2,572 (1,304; 4,868)	985, 647 (765,991; 1,205,302)	2,699 (2,442; 2,957)	2,089,482 (1,815,883; 2,479,398)	19,648 (13,539 ; 22,234)	3,750 (3,357; 4,230)

95% confidence intervals (CIs) are provided between brackets

A population of 100,000 persons followed over 15 years leads to a total population of 1,500,000 subjects (100,000 each year). In the GSK model, the subset of the total population over 15 years below the age of 15 years was estimated to be 383,878 for 3% parasite prevalence, 379,717 for 10% parasite prevalence, 370,744 for 30% parasite prevalence and 364,267 for 50% parasite prevalence, taking into account the assumed mortality rate across the different parasite prevalence settings.

Data on public health impact (PHI) from models were provided by parasite prevalence (3%, 10%, 30% and 50%). Data are presented as cumulative number of malaria cases (clinical, severe and deaths) averted over 15 years in infants vaccinated with RTS,S/ASO1E according to a vaccination schedule at 6, 10, 14 weeks of age without or with a 4th dose 18 months post Dose 3. These numbers vary between modelling groups because of the difference in case definitions and baseline assumptions used by the different modelling groups. These data are shown in Table 76.

Table 76. Cumulative number of malaria cases (clinical, severe and deaths) averted over 15 years per 100,000 RTS,S/AS01E vaccinees by transmission intensity and by 3 different PHI modelling groups in infants for a primary vaccination schedule at 6, 10, 14 weeks without or with a 4th dose 18 months post Dose 3

	6, 10, 14	weeks schedule without a	a 4 th dose	6, 10,	14 weeks schedule with a 4 ^t	^h dose
Parasite	GSK	Imperial	SwissTPH	GSK	Imperial	SwissTPH
prevalence		·				
Clinical malaria	cases averted					
3%	2,951 (1,920 ; 3,981)	12*103 (-23*103; 29*103)	5,307 (1,342 ; 11,673)	3,004 (2,072 ; 3,942)	16*10³ (-17*10³ ; 34*10³)	7,385 (4,225 ; 13,542)
10%	17,192 (12,957 ; 20,582)	30*10 ³ (14*10 ³ ; 63*10 ³)	20,593 (5,208 ; 45,292)	18,577 (15,144 ; 22,123)	44*10³ (25*10³ ; 81*10³)	28,655 (16,394 ; 52,545)
30%	36,104 (26,861 ; 45,220)	64*103 (37*103; 103*103)	48,803 (43,719 ; 53,985)	43,031 (34,729 ; 51,436)	93*10³ (56*10³ ; 135*10³)	65,629 (60,986 ; 71,185)
50%	43,972 (25,312 ; 63,564)	82*10 ³ (47*10 ³ ; 122*10 ³)	62,614 (54,480 ; 66,455)	55,955 (40,608 ; 74,345)	120*10³ (73*10³ ; 168*10³)	85,791 (74,454 ; 89,832)
Severe malaria	cases averted					
3%	77 (-100 ; 282)	-	152 (60 ; 306)	80 (-61 ; 235)	-	249 (134 ; 389)
10%	353 (-55 ; 871)	-	591 (235 ; 1,189)	409 (71 ; 833)	-	967 (521 ; 1,511)
30%	764 (20 ; 1,424)	-	1,017 (761 ; 1,330)	819 (264 ; 1,528)	-	1,369 (1,230 ; 1,768)
50%	894 (-297 ; 1,865)	-	951 (388 ; 1,305)	1,075 (43 ; 1,952)	-	1,302 (744 ; 1,753)
Malaria deaths	averted					
3%	21 (-28 ; 78)	102 (-23 ; 226)	36 (1 ; 67)	22 (-17 ; 65)	145 (12 ; 279)	43 (14 ; 76)
10%	98 (-15 ; 242)	194 (98 ; 305)	140 (6 ;261)	113 (20 ; 231)	277 (142 ; 431)	169 (54 ;296)
30%	211 (6 ; 395)	280 (146 ; 442)	277 (176 ; 339)	227 (73 ; 424)	398 (224 ; 613)	351 (246 ; 413)
50%	248 (-82 ; 517)	235 (23 ; 550)	347 (277 ; 430)	298 (12 ; 542)	355 (78 ; 719)	443 (340 ; 533)

PHI data on severe malaria are not available with the model from Imperial College model

The Applicant concluded that despite differences between modelling approaches developed by independent expert groups, the PHI estimates were generally well aligned and as a consequence increases the confidence in the model estimates.

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A consistent observation, irrespective of the model, was that when RTS,S/AS01E was administered according to the EPI schedule, a substantial number of clinical and severe malaria cases and malaria deaths are expected to be prevented, especially in moderate and high transmission settings across sub-Saharan Africa.

The vaccine impact is estimated to be higher when a 4th dose is administered 18 months after completion of the primary vaccination course.

High transmission areas show a higher number of cases averted (confirmed by the clinical Ph III study) but over the 15 years modelling time a higher rate of rebounds is also expected in these settings but is not expected in the low transmission areas. Obviously the effect of the 4th dose is best seen in the earlier years but over the 15 years the 4th dose does not have such a tremendous effect on the number of cases averted as could have been expected based on the data from the study only. A 4th dose also affects the time of a possible rebound by delaying the acquisition of natural infection. In the younger age group a rebound for clinical infection might be observed earlier, while in the older age group the 4th dose delays rebound for clinical disease by 7 years and by 4 years for severe disease.

Although the different models arrive at different numbers according to the methods and groups concerned the relative percentage of cases averted is similar between GSK and Swiss TPH for all prevalence rates and case definitions. Both estimate an additional 4% of cases averted if the 4th dose is given across all transmission rates.

Ongoing studies

- Malaria-063 Will continue to follow participants with two further analyses at month 26 and month 51 to evaluate long-term safety and immunogenicity. Final results are expected in 3Q2018.
- Malaria-066 Evaluates genetic polymorphism of the CS protein of *P. falciparum* found in all subjects who develop malaria in Malaria-055. Results are planned to be available early 2016.
- EPI Malaria-001 BOD AME Investigates malaria transmission intensity (MTI) caused by *P. falciparum* in catchment areas of Malaria-055 to obtain longitudinal estimates of parasite prevalence and serological conversion rates at study sites.

Planned studies

- Malaria-076 will extend Malaria-055 for another 3 years to describe the incidence of severe
 malaria at three study centres (Korogwe, Tanzania; Nanoro, Burkina Faso; Kombewa, Kenya).
 The same data collection systems and case definitions will be used as in the primary study.
 Secondary endpoints will include the description of clinical malaria, parasite prevalence and
 SAEs of special interest.
- Malaria-073 will evaluate the non-inferiority of immune response and the safety of RTS,S/AS01E, when administered as primary vaccination with or without co-administration of measles, yellow fever and rubella vaccines at 6, 7.5 and 9 months of age to children living in sub-Saharan Africa.

3.5.3. Discussion on clinical efficacy

1. Formulation, dose and schedule

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When combining antigen with adjuvant the number of permutations that can be studied clinically will be limited, especially when age range and schedules are additional factors that can have an important effect on the magnitude of the immune response.

The selection of RTS,S/AS01E at 0,1,2 months for Malaria-055 was broadly supported. In particular:

- The CHMI studies in malaria-naïve adults supported inclusion of an adjuvant based on enhancement of the anti-CS response by AS01B or AS02A. In adults and children AS01 elicited a better anti-CS response than AS02. In each case the anti-HBs responses were very satisfactory.
- CHMI studies also supported the switch to lyophilised RTS,S.
- In children aged \geq 1 year 3 x 25 µg RTS,S was as immunogenic as 3 x 50 µg when each was given with ASO2A 0.25 mL at 0, 1 and 3 months based on anti-CS and anti-HBs responses.
- The data from CHMI studies and in children supported 3-dose regimens over 2-dose regimens.
- Data for RTS,S/AS01E in children aged 6-12 weeks and 5-17 months supported 0,1,2 over 0,1,7.

In one CHMI study with ASO2A the use of a fractionated and delayed third dose gave unusually high efficacy, albeit in small numbers. A further CHMI study (Malaria-071) is ongoing in 51 malaria-naïve adults to re-assess protection after a delayed and fractional third dose (0.1 mL at month 7).

2. Age at time of the first dose

Administering the vaccine from 6 weeks of age using the 3-dose 0,1,2-month schedule would allow its inclusion into the existing EPI programme visits.

- i) Anti-CS responses and VE against malaria were lower when initiating vaccination at age 6-12 weeks compared to 5-17 months. Anti-CS at M3 was lower for those aged 6 weeks vs. 7-12 weeks at the time of the first dose and lower for those with detectable maternal anti-CS at baseline.
- ii) Antigen doses $> 25 \mu g$ have not been evaluated in infants (aged < 1 year). Higher antigen doses could potentially improve the anti-CS immune response in this age group.
- iii) Co-administration data indicate some negative effects of RTS,S-AS01E on immune responses (at least on GMTs) to co-administered antigens at the EPI schedule. Higher antigen doses could potentially exert a greater negative impact on responses to co-administered EPI vaccines.
- iv) The ASO1 adjuvant is not a constituent of any licensed vaccine in any age group. Although the MPL component is in ASO4 this adjuvant is not in vaccines given below 9 years of age. The lack of any post-marketing safety data for ASO1 is a potential safety concern that must be taken into account when reviewing the protective efficacy of the vaccine in both age groups.

3. Immune responses

Anti-CS

Antigen and assay

Within the RTS,S antigen the RTS portion comprises a fusion protein derived from selected parts (a target of neutralising antibody [R] and T-cell epitopes [T]) of the circumsporozoite surface protein of *P. falciparum* strain NF54. These are fused to the amino terminal end of the HBV S protein to form RTS,S. The RTS and S proteins are co-expressed in yeast and spontaneously assemble into mixed particles.

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In the WRAIR ELISA anti-CS was measured by ELISA (assay cut-off 1 μ g/ml) using plate adsorbed R32LR antigen with a standard reference antibody as a control.

The R32LR coating antigen is the well conserved repetitive domain of the *P. falciparum* CS protein and consists of four amino acids (Asn-Ala-Asn-Pro = NANP) repeated several times. A number of different recombinant and synthetic (NANP)n peptides have been used to develop assays for anti-CS. In the applicant's ELISA R32LR antigen (not stated to be identical to that used by WRAIR) was used for coating and the WRAIR standard reference antibody was run. The assay cut-off was $0.5 \, \text{EU/ml}$ and the applicant has classed all sera with $\geq 0.5 \, \text{EU/mL}$ as seropositive.

It is essential to appreciate the limitations of this anti-CS assay. Nevertheless, there is no feasible functional antibody assay and measuring IgG that binds to a well-conserved part of the CS protein at least provides some broad idea of the interaction between the vaccine and the human immune system. In addition, since the assay picked up pre-vaccination anti-CS that varied across regions and age groups, showed little change over study durations in controls, showed rapid decay of maternal antibody in infant controls and gave a fairly typical antibody decay curve in vaccinees, it seems clear that the assay is measuring a relevant part of the immune response to RTS,S.

Pre-vaccination anti-CS

The pre-vaccination anti-CS seropositivity rates have varied between age groups and geographical areas. However, even when baseline seropositivity rates (i.e. anti-CS detectable using the assay) have been higher the GMTs have been very low in children. For example:

- In children aged 5-17 months in Malaria-047 (W. Africa) baseline seropositivity rates were 10-20% compared to <5% in Malaria-049 in E. Africa but GMTs were <1 EU/mL.
- In children aged 6-12 weeks in Malaria-050 baseline seropositivity rates were 25-30% but GMTs were <1 EU/mL.
- In Malaria-055, with a wide spread of sites across Africa, the overall pre-vaccination seropositivity rates per group were ~10% in children aged 5-17 months. The overall rate in infants was ~35% with a range from 5% up to 72% although all baseline GMT values were ≤ 1 EU/mL.
- In children enrolled at 6-12 weeks the anti-CS seropositivity rate decreased from 35% to 6% at M3 in controls in Malaria-055, indicating decay of maternal antibody.

Post-vaccination anti-CS

In studies with control groups there has been no appreciable increase in anti-CS antibody during the time span of the studies. Anti-CS responses to RTS,S/AS01E have been very clearly superior to controls but the GMTs at one month post-dose 3 have varied between studies, age groups and geographical sites.

GMTs one month after the third dose of RTS,S/AS01B tended to be higher in malaria-na $\ddot{}$ ve adults with almost no baseline seropositivity (143.5 μ g/ml in Malaria-027 and 160.3 EU/ml in Malaria-048) than in adults living in malaria-endemic areas with at least two-thirds seropositive at baseline (21.8 μ g/ml in Malaria-005 and 41.4 EU/ml in Malaria-044).

Within Malaria-055 the M3 GMT for all children aged 5-17 months was 621 EU/mL but varied from 48.4 to 787 EU/mL across sites and with a higher anti-CS GMT in the 5-11 months age sub-group, even though efficacy was lower than in the 12-17 months subgroup. For all infants aged 6-12 weeks the M3 GMT was 210.5 but values varied from 117 to 335 across sites. The analysis of covariates showed that

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those seropositive at baseline had a lower response than those who were seronegative, indicating a negative effect of maternal antibody. Body weight category had no impact but anti-CS responses were lower for those aged 6 weeks vs. 7-12 weeks at the time of the first dose.

In both age groups the GMCs observed after a 4th dose were lower than those observed after the 3rd dose of the primary series. In the absence of a suitable control group to interpret the findings (i.e., previously unvaccinated children of the same age and resident in the same endemic region) it is not possible to conclude on the observation but it does suggest that the vaccine does not truly boost the anti-CS response. This is unexplained and has potential consequences should further follow-up suggest that 5th or more doses are needed to prevent a rebound phenomenon. Meanwhile, in light of the vaccine construct and the extremely high anti-HBsAg titres that are observed before and after a 4th dose, it cannot be ruled out that the anti-HBsAg antibody may be somehow inhibiting the immune response to the CS antigenic region of the construct.

The CHMP initially expressed concerns whether the vaccine actually predisposes to some degree of hyporesponsiveness to sequential doses. The Applicant considers that the lower response to CS than expected is not due to a similar mechanism of hyporesponsiveness as observed for non-conjugated polysaccharides vaccines. Regarding the impact of the high anti-HBs antibody response on the anti-CS antibody response, the Applicant concluded from the correlation analysis that no correlation was found between pre Dose 4 anti-HBs antibody concentrations and post Dose 4 anti-CS antibody concentrations in both age categories, however, some competition between the CS and HBs epitopes included in the RTS,S antigen with immunodominance of HBs over the CS portion cannot be ruled out. Therefore, the lower anti-CS antibody response following Dose 4 compared to that following Dose 3 remains unexplained. However, even if hyporesponsiveness to CS would exist, this would not impact the development of natural immunity that is mostly targeting blood stage antigens, and not the CS antigen. In conclusion, no clinical impact in terms of increased susceptibility to malaria-related events is expected. In addition, the benefit of the 4th dose in terms of incremental vaccine efficacy was observed in study Malaria-055.

Anti-HBs

Antigen and assay

In RTS,S the HBsAg-related antigen is the amino terminal end of the HBV S protein and is the same as that used in all the applicant's licensed vaccines for HBV. Various assays for anti-HBs have been used over the duration of the programme with different assay cut-offs. In addition, in 2006 an in-house validated ELISA was used with an assay cut-off 3.3 mIU/ml. This assay was used to test baseline and M3 sera in Malaria-055 and Malaria-061 and it was among those assays investigated from 2012 onwards. The final conclusion was that the assay over-estimated anti-HBs in the low range (< 100 mIU/ml). At least 99.8% of subjects in the RTS,S/AS01E groups across studies had post-vaccination anti-HBs >100 mIU/ml and on this basis the conclusions drawn about responses to RTS,S were not affected.

Most importantly, sera obtained in Malaria-063, which was pivotal for describing the ability of RTS,S/AS01E to elicit anti-HBs and replace other HBsAg-containing vaccines, were assayed using a chemiluminescence enzyme immunoassay (CLIA) with a cut-off of 6.2 mIU/mI.

Anti-HBs protection

All the studies have supported the ability of RTS,S/AS01E to elicit very high levels of anti-HBs in infants from 6 weeks of age, with or without a birth dose, and in the 5-17 month age groups (in which it is acting as a booster).

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Malaria-063 was the major study conducted in infants aged 8-12 weeks who had not received a birth dose of HBsAg to support use of RTS,S/AS01E to replace vaccination with HBsAg-containing products. This study clearly showed that RTS,S/AS01E elicited higher GMCs and higher proportions with ≥100 mIU/mL at M3. The actual GMC observed for the pooled RTS,S/AS01E groups in Malaria-063 was ~6400, which is about half the GMC observed in Malaria-055 in this same age group despite the fact that the pre-vaccination anti-HBs seroprotection rates and GMCs were comparable between studies.

Anti-HBs persistence and response to a 4th dose

Malaria-055 provided anti-HBs at M3 (one month post-dose 3) in both age groups, including infants who did or did not have a birth dose, and data up to month 20 plus after a 4th dose at Month 21. In both age groups the pre-4th dose titres were still very high.

In the 6-12 weeks and the 5-17 months group almost all had pre-4th dose anti-HBsAg >100 IU/mL, which is astoundingly high at 18 months after the last dose of the primary series. There was a major response to the 4th dose in both age groups. However, as is the case for the pre/post 4th dose anti-CS, the numbers and the individuals sampled were not the same as those sampled post-dose 3 so any comparison have to be made with caution. The post-4th dose GMCs for anti-HBsAg are higher than observed after the 3rd dose (116,458 for 6-12 weeks and 95,206 in the 5-17 months groups). This suggests that the anti-HBsAg response is boosted but the anti-CS response is not.

In children aged 6-12 weeks the M3 GMCs were >13,000 mIU/mL for RTS,S/AS01E vs. 730 mIU/mL for Tritanrix HepB/Hib. Therefore no important negative effect of maternal antibody applied to either treatment group.

Malaria-063 used a different assay but it was also conducted in infants who had no birth dose. The prevaccination seroprotection rates were 16-18%, which is in line with the other studies quoted above, and the M3 GMCs were >6,400 for RTS,S/AS01E vs. 377 for Engerix-B. Again, if there is a negative effect of maternal antibody it seems unlikely it will be important given the magnitude of the post-vaccination anti-HBs levels.

Cell-mediated responses to CS

In CHMI studies the CMI data were consistent with the hypothesis that the functional antibody response and elicitation of sensitised CD4+ T-cells expressing IFN-γ play an important role in protection. CS-specific CD4+ T-cell responses were directed against several epitopes but were predominantly focussed on the Th2R immunodominant polymorphic C-terminal region of the CS protein. CS-specific CD8+ cytotoxic lymphocytes were not detected. Immune responses waned after a second dose but were boosted by a third dose given 5 months later. Later studies with RTS,S/AS01 included the following:

- Malaria-044 in semi-immune adults showed similar CS-specific CD4+ T-cell responses in RTS,S/AS01B and RTS,S/AS02A groups with no response observed in the control group.
- Malaria-048 in malaria-naïve adults showed that CD4+ CS-specific T-cell responses in recipients of RTS,S/AS01B were of greater magnitude of response vs. RTS,S/AS02A but CD8+ CS-specific T-cell responses could not be detected in any group.
- Malaria-047 indicated that RTS,S/AS01E elicited sensitised CD4+ cells (but not CD8+ cells) with higher frequencies after 3 vs. 2 doses and with 0,1,2 vs. 0,1,7 dosing in children aged 5-17 months.

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 Malaria-049 in the same age group supported these findings and showed persistence of sensitised CD4+ cells at M14. In addition, CS-specific CD8+ 8cells were detected with a higher frequency in the RTS,S/AS01E group at M14.

Co-administration data

In Malaria-063 at the EPI schedule co-administration with DTaP/Hib plus Rotarix or, to a greater extent, plus Synflorix had a negative effect on the anti-CS GMT, although the 95% CI all overlapped.

As a result, the applicant proposes the following statement in section 4.5 of the SmPC:

Mosquirix can be given concomitantly with any of the following monovalent or combination vaccines including diphtheria (D), tetanus (T), whole cell pertussis (Pw), acellular pertussis (Pa), hepatitis B (HepB), Haemophilus influenzae type b (Hib), oral polio (OPV), measles, yellow fever, rotavirus and pneumococcal conjugate vaccines (PCV). The co-administration of Mosquirix with PCV increases the risk of fever within 7 days post-vaccination (see section 4.8).

Concomitant administration of rotavirus and pneumococcal conjugate vaccines with Mosquirix may reduce the antibody response to the circumsporozoite (CS) antigen of Mosquirix. The impact of this observation on the level of protection induced by Mosquirix is currently unknown.

The proposal for the SmPC seems acceptable but the observation is pertinent to generally lower immune responses as age decreases, the negative effect of maternal antibody on anti-CS and the lower efficacy when RTS,S/AS01E is given from the age of 6 weeks-12 weeks vs. 5-17 months.

Assays for antibody to co-administered antigens have used the applicant's usual methodologies and have been the subject of a detailed review in the last few years. Overall the results of the investigations have not invalidated any study conclusions and on this basis the results are accepted.

The non-inferiority of the immune response was demonstrated for D, T, Pw, Pa, Hib, polio and pneumococcal antigens (except for pneumococcal serotype 18C); although there was a trend for lower antibody geometric mean concentrations (GMC) for these antigens when compared to the control group. These observations were considered as not clinically significant.

In Malaria-055 the co-administered antigens were DTPw, HBsAg and Hib plus OPV. Co-administration with RTS,S/AS01E had a small negative effect on responses to OPV 1, 2 and 3 but this seems unlikely to be clinically important.

Anti-D, anti-T and anti-PRP responses were not measured in Malaria-055 or Malaria-063 and antibody elicited by Pw as not measured in Malaria-055. Thus, the only data to support the claims for co-administration of RTS,S/AS01E with these antigens come from Malaria-050 in which it was given with the first two or all three doses of Tritanrix-HepB/Hib. Co-administration resulted in a general trend to lower GMCs/GMTs, especially when all three doses coincided, but proportions reaching the usual threshold values applied were unaffected.

In Malaria-063 RTS,S/AS01E did not per se have an effect on responses to PT, FHA and PRN. RTS,S/AS01E did appear to have a negative effect on GM antibody measured by ELISA and OPA to most of the conjugated pneumococcal polysaccharides in Synflorix. The clinical significance of these decreases in antibody is unknown. The serological findings also need to be viewed in light of the safety data relevant to co-administration with Synflorix.

For co-administration with yellow fever and measles vaccine the available data come only from Malaria-050.

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Malaria-057 is ongoing and there were no data available from this study during the procedure. Due to the lower anti-CS observed when vaccination commenced from 6-12 weeks compared to older subjects and the negative effects of co-administration with EPI vaccines, Malaria-057 was initiated to investigate possibilities to optimize the anti-CS response in infants. This study evaluates the safety and immunogenicity of seven schedules with co-primary endpoints of SAEs up to month 10 and anti-CS antibody concentrations at one month post-dose 3 (M3) of RTS,S/AS01E.

There is also a planned study Malaria-073 to evaluate the non-inferiority of immune response and the safety of RTS,S/AS01E, when administered as primary vaccination with or without co-administration of measles, yellow fever and rubella vaccines at 6, 7.5 and 9 months of age to children living in sub-Saharan Africa.

4. Vaccine efficacy against malaria

Accumulation of evidence supporting VE

The human challenge studies in malaria-naïve adults provided an initial demonstration of proof of concept for adjuvanted RTS,S in non-immune subjects, raising hopes that such a vaccine could be protective in early life and so impact on paediatric mortality rates and the burden of malaria disease. These studies also supported selection of ASO2A over ASO3 or ASO4, use of a 3-dose schedule over a few months and the switch to a lyophilised preparation of RTS,S. One study suggested protection might be short-lived.

Since one study with ASO2A using a fractionated third dose gave unusually high efficacy a further CHMI study (Malaria-071) is ongoing in 51 malaria-naïve adults to re-assess protection after a delayed and fractional third dose (0.1 mL at month 7). Malaria-026/039 documented modest (20-30%) efficacy after RTS,S/ASO2A was given at 0,1,2 months to children aged 1-4 years. Efficacy was borderline significant in the period 18-30 months post-dose 3 and was not detected thereafter. Subsequently Malaria-038 and -040 showed short-term efficacy (~65% efficacy against first or only episode of *P. falciparum* malaria infection) over 3.5 to 6.5 months post-dose 3 in infants who received RTS,S/ASO2D at 10, 14, 18 weeks of age staggered by 2-week intervals from EPI vaccines or received co-administered EPI vaccines at 8, 12 and 16 weeks of age.

Malaria-050 was the first study to report on the efficacy of RTS,S/AS01E when given to infants with EPI vaccines at the 0,1,2 schedule. Although not formally designed to assess efficacy it suggested that over ~17 months post-dose 3 VE against first (PDef) malaria was ~50% and was even higher (>60%) in the first 6 months of follow-up. This study also showed a preliminary relationship between anti-CS antibody and reduction in risk.

The Phase 2b study Malaria-049 was confined to children aged 5-17 months (mean 11 months). VE assessed at a mean of 8 months post-dose 3 was estimated at 50-60% regardless of the case criteria and mode of analysis. However, over 12 months post-dose 3 VE was estimated at ~40%. Further follow-up by investigators in Kilifi (noting that the study was not powered to estimate efficacy only at this site) in Malaria-059 indicated that the point estimate of VE halved between years 1 and 2 (with lower 95% CI below zero) and was negative after year 3, i.e. cases/PYAR were higher in the RTS,S/AS01E group in years 4, 5 and 6. No data were collected on the severity of malaria but it seems that there were no fatal cases in either treatment group and no excess of SAEs relating to clinical malaria in the RTS,S/AS01E group.

The applicant acknowledges that a rebound effect cannot be ruled out. The pivotal efficacy study Malaria-055 was designed and conducted in accordance with the recommendations of a WHO consultation group and it was the subject of CHMP scientific advice. The overall study design is

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acceptable in that it sought to establish VE using an unvaccinated control group and in different settings of seasonality and transmission intensity and used PCD.

The study aimed to demonstrate VE against a background of routine control measures, most importantly ITNs. However, ITN coverage was incomplete and it seems that it was far from optimal due to frequent use of nets with holes. In addition, there was little use of indoor residual spraying.

Efficacy in children vaccinated when aged 5-17 months

In the first 6000 children enrolled at age 5-17 months (not including children enrolled at Korogwe due to delayed study initiation) follow-up for ~12 months starting from 2 weeks post-dose 3 gave VE point estimates in the range 50-60%. Estimates of VE were very consistent with those reported in this age group in Phase 2 and also consistent across the various analyses (i.e. using different case definitions, counting first/only or all malaria episodes and in ATP and ITT populations). Additionally, efficacy against severe malaria was apparent (VE 47%).

During follow-up over 20 months from the first dose in the full cohort of children aged 5-17 months VE against clinical malaria was 46% and VE for first/only PDef episodes was 49% while VE against severe malaria was 36% and against prevalent parasitaemia 31%.

In the analysis up to M20 there was a significant site interaction. Although the lower limit of the 95% CI for each site was above zero (mostly >25%) the point estimates ranged from 40-77%. In addition, the actual numbers of cases and attack rates in the vaccine and control groups were very different between sites (lowest in Kilifi [Kenya] and highest in Siaya [also in Kenya] and Nanoro [Burkina Faso]). However, VE did not vary by transmission intensity.

In the final model of determinants of malaria incidence, the only factor with a a significant interaction with vaccine assignment is moderate anemia.

Even over the first 12 months of case ascertainment proportionality of hazard was not demonstrated. Very importantly, VE from 2 weeks post-dose 3 (M2.5 on study) dropped from 68% up to M8 to 41% in the period M8 to M14 and 26% in the period M14-M20. The lower limit of the 95% CI remained above zero. At M20 half of the RTS,S/AS01 $_{\rm E}$ group received a booster and all groups were further followed .

The additional data up to ~M48 (range 41-55 months; 21-35 months post-boost) in this age group were provided during the evaluation. Current analyses suggest that VE against clinical malaria is minimal or lost in the non-boosted group in the last period of the follow-up. A VE of ~40% is shown in this age group against clinical disease (primary case definition) and of ~30% against severe disease (primary definition) over the complete study time (median 46 months post 3rd dose) if a fourth dose is given. The VE tends to be lower in high transmission areas. The long-term public health impact model for this age group performed by the Applicant, estimates a significant number of cases averted (clinical disease, severe disease and deaths due to malaria) for mid-high transmission areas over 15 years post introduction of the vaccination. However, it remains unclear whether lack of a fourth dose predisposes vaccinated children to more severe malaria after Month 20.

Although vaccine efficacy was demonstrated before and after a 4th dose, several concerns remain regarding the overall and long term benefit of the vaccine. The data indicate the need for at least a 4th dose, however in the absence of clinical data, a 5th or 6th vaccine dose cannot be recommended at present.

In this regard, the Applicant has already undertaken a number of research activities, such as the ongoing study Malaria-057 (evaluating different schedules of the RTS,S/AS01_E vaccine with variation in

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age at first dose, in dose spacing and in co-administration of EPI vaccines in infants) and the ongoing study Malaria-071 (a challenge study evaluating efficacy against infection of a delayed 3rd fractional dose in malaria-naïve adults), and several exploratory studies where RTS,S/AS vaccine is used in combination with other candidate malaria vaccine antigens in view of the development of a next generation malaria vaccine. The preliminary efficacy results obtained with the delayed 3rd fractional dose of the RTS,S/AS01_E vaccine after the primary vaccination phase of study Malaria-071 are encouraging, and the Applicant will therefore further investigate the potential utility of this regimen in a new clinical study in the paediatric population in malaria endemic areas in Sub-Saharan Africa. This proposed study will be a randomised, multicentre, controlled, partially observer blind, Phase IIb study in children aged 5 to 17 months at first dose living in high malaria endemic transmission areas. All study subjects will receive the RTS,S/AS01_E vaccine, either in a full or fractional (Fx) dose, i.e., 1/5th RTS,S/AS01_E. The initial study is planned to include the older age group only and this is endorsed to avoid possibly unnecessary exposure of the very young infants and also avoids a possible interference with the other vaccination schedules.

Efficacy in children vaccinated when aged 6-12 weeks

The ATP subjects in this age group received three doses of Tritanrix HepB/Hib with the three doses of RTS/S/AS01E or MenC. No pneumococcal conjugate (PnC) vaccine was given, because not part of the routine practice at the time of study initiation. Impact on the efficacy in routine use due to the effects of PnC on anti-CS responses can therefore not be excluded.

RTS,S/AS01E was less effective than in the older age cohort. In the ATP population VE over 12 months post-dose 3 was consistently in the range 30-40% regardless of the mode of analysis and including VE against severe malaria. In the ITT population VE was demonstrated for all clinical malaria but not for severe malaria.

During follow-up over 20 months from the first dose in the full cohort of children aged 6-12 weeks VE against clinical malaria was 27% and VE for first/only PDef episodes was 29%. Although VE varied across study sites there was no significant interaction detected and it did not vary by transmission intensity. There was no demonstrable VE against severe malaria, prevalent parasitaemia or moderate anaemia. In the final exploratory model the significant factors affecting malaria incidence, other than treatment, were outpatient distance, anti-CS site average and gender. The model also pointed towards an interaction between treatment and age, with different vaccine efficacy in those children under 6 weeks and those above. The anti-CS GMT at M3 did not significantly interact with treatment, meaning that the vaccine efficacy does not vary by anti-CS antibody GMC at the level of the site.

As in the older children proportionality of hazard was not demonstrated up to M14 or M20.

VE from 2 weeks post-dose 3 (M2.5 on study) dropped from 47% up to M8 to 23% in the period M8 to M14 and 11% (lower 95% CI only just above zero) in the period M14-M20. A VE of ~27% was shown in this age group against clinical disease (primary case definition) and of ~20% against severe disease (primary definition) over the complete study time (median of 36 months post 3rd dose) if a fourth dose was given. With a 4-dose schedule, VE tended to be higher in low transmission areas. No rebound was seen during the study time.

With regard to the 4th dose, it is agreed that unfortunately there are no data to support any other timing. Indeed, the immune responses to the 4th dose might suggest that giving it earlier would likely be even less effective than giving it at month 20. This means that vaccine efficacy is close to being lost completely near the time of the 4th dose and in the vaccinated group that did not receive a 4th dose it was clear that there was no efficacy after month 20.

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Finally with regard to efficacy, all of the data indicate that vaccine efficacy will be restored for only a brief time after the 4th dose, after which the data already available in the older cohort need to be taken into account. The applicant has acknowledged that a rebound effect cannot be ruled out.

5. HIV positive subjects

The HIV-positive subset in Malaria-055 was very small and the majority were in the older age cohort. Nevertheless, within each age cohort anti-CS GMTs at M3 were significantly lower than in the total study population and with a lower GMT for the 6-12 weeks vs. 5-17 months subsets.

In Malaria-058 the age range spanned 6 weeks to 17 months and EPI vaccines were staggered. At M3 (one month post-dose 3) the anti-CS GMT was 329.2 EU/ml vs. 0.3 EU/ml for controls but at M14 the GMT in the RTS,S/AS01E group had fallen to 18.4 EU/ml. Although an impact of vaccination on rates of malaria was apparent the differences vs. controls in this small study of 200 subjects were not significant.

Although the median viral load at baseline was not higher in the RTS,S/AS01E group the M3, M8 and M14 data showed higher median loads in vaccinees. There is not an obvious explanation for this finding, which may be spurious. In the exploratory model no effect of the vaccine (positive or negative) was observed on HIV progression in terms of viral load or CD4% at 1 or 12 months post-dose 3 and differences in viral loads at subsequent time points were explained by differences in baseline after controlling for other variables6. Other efficacy data

Vaccine efficacy against P. falciparum prevalent parasitaemia

The prevalence of *P. falciparum* parasitaemia was generally lower in the RTS,S/AS group vs. controls and reached significance (p-value <0.05) in Malaria-026/-039, Malaria-049 and in children 5-17 months of age at first dose enrolled in Malaria-055.

Efficacy against different P. falciparum strains

The most variable domains of the CS protein are the T-cell epitopes Th2R and Th3R. Malaria-005, -044 and -026 sequencing of the Th2R and Th3R regions did not show relevant differences between vaccine and control groups in the prevalence of vaccine-type or other allele sequences. Malaria-005 and -026, showed virtually no infection with vaccine-type *P. falciparum*.

Malaria-044 did show that proportions of *P. falciparum* with non-vaccine type residues were significantly different in vaccinees vs. controls for one amino-acid in Th2R and one amino-acid in Th3R but one was more prevalent in the vaccine group and the other more prevalent in control group. Such a difference was also observed for one amino-acid in pre-vaccination samples and it was concluded that there was no biologically relevant overall effect.

Malaria-066 is an ancillary study of study Malaria-055 planned to evaluate the genetic polymorphism of the CS protein of P. falciparum found in infected infants and children from the RTS,S/AS01_E or control groups. The Applicant committed to provide the final study report.

3.5.4. Conclusions on the clinical efficacy

VE is higher for those aged 5-17 months at the time of the first dose compared to infants aged 6-12 weeks. There is marked waning of vaccine efficacy, especially in the younger cohort, after the third dose. It remains unclear when the 4th dose is best given, how long protection may continue, whether further doses are needed to prevent possible rebound phenomena and whether the protection afforded by sequential doses will continue to drop below that observed after the primary series, even though the vaccinees are increasing in age.

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Thus far the data do show that after the 4^{th} dose is given in the respective R3R groups VE increases but to a level that is slightly lower than after the initial 3 doses and then decreases over the next 2 years of follow-up in a similar fashion in both age groups. In the R3C groups where no 4th dose was given VE decreases further as seen during the last months of the primary vaccination phase and the point estimates of VE against clinical malaria reach $0\% \sim 34$ months after the last dose of the primary vaccination.

The models for the public health impact confirm the data from the study Malaria-055. Lower VE but higher impact on clinical, severe disease and deaths due to malaria can be expected for mid to high-transmission areas. It nevertheless will be of paramount importance to maintain or even extend the malaria prevention and therapy standards already in place as all data (clinical study as well as modelled analyses) have taken this into account.

Concomitant use of EPI vaccines demonstrates some lower immune responses to certain antigens that are difficult to interpret. The data in HIV+ infants and children suggest that VE and the immunogenicity are lower than in the healthy peers.

No concerns with regard to hepatitis B indication were identified.

3.6. Clinical safety

A pooled analysis of safety data was performed on data collected in the target population, i.e. children 6 weeks to 17 months (6w-17m) of age living in malaria-endemic regions of Sub-Saharan Africa, who have been administered at least one dose of RTS,S/ASO1_E (final formulation) or control vaccine on a 0, 1, 2-month vaccination schedule. The six clinical trials included in the pooled analysis were Malaria-047, Malaria-049, Malaria-050, Malaria-055, Malaria-061 and Malaria-063 (primary vaccination course only, i.e. no booster dose).

Patient exposure

A total of 5123 infants and 6985 Children 5-17 months of age received at least one dose of their vaccination with Mosquirix.

Table 77. Number of subjects and doses in the pivotal studies

Study	Age	N	RTS,S/AS01E	RTS,S/AS01E	Control
			#subjects	#doses	#subjects
Malaria-047	5-17m	540	270	711	45
Malaria-049	5-17m	894	447	1320	447
Malaria-061	5-17m	320	320	954	N/A
Malaria-055‡	5-17m	8922	5948#	17306	2974
	6-12w	6537	4358	12739	2179
Malaria-063	6-12w	705	425	1241	280
Malaria-050	6-10w	511	340	1001	171
Malaria-058	6w-17m	200 (40 Infants)	99	288	101

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Study	Age	N	RTS,S/AS01E	RTS,S/AS01E	Control
			#subjects	#doses	#subjects
Total	5-17m	10676	6985#	20291	3466
	6-12w	7753	5123	14981	2285
	6w-17m	18629	12207#	35560	5852

[‡] Malaria-055: including both first 3 doses and 4th dose dose vaccination.

Safety monitoring was done in all studies from start of the trial until at least 8 months after the first dose for the SAEs with a final visit or phone call for each subject.

Safety measures included the occurrence, duration, severity and relationship to vaccination of:

- Immediate solicited and unsolicited reactions (within 30 minutes after vaccination)
- Solicited AEs up to 7 days after vaccination:
 - o Local: erythema, pain, swelling at injection site
 - Systemic: fever, drowsiness, loss of appetite, irritability/fussiness
- Unsolicited AEs up to 30 days after vaccination
- All SAEs up to end of the study
- Clinical laboratory data

Some adverse events were rated as adverse events of special interest (known as identified or potential risks for paediatric vaccines):

- Seizures
- Rashes and Mucocutaneous lesions
- pIMDs (potential immune mediated disorders)

Adverse events

Solicited local symptoms in children aged 6 weeks to 17 months reported in Malaria-055

First three doses

In children 5-17m of age living in malaria-endemic regions, pain, redness and swelling at the injection site were reported following 12.4%, 3.1% and 9.6% of doses of RTS,S/AS01E, respectively and following 5.8%, 2.7% and 7.6% of doses of rabies vaccine, respectively. The incidence of pain, swelling and redness did not increase with the administration of subsequent doses of RTS,S/AS01E.

Grade 3 solicited local reactogenicity was infrequent (grade 3 pain, swelling and redness following 0.1%, 0.2% and 0.7% of doses of RTS,S/AS01E, respectively).

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[#] During monitoring, it was found that one subject belonging to the 5-17m age category, was enrolled twice under two different PID numbers. The same subject was presented for enrolment in a study site and a satellite site. The subject was excluded from the ATP analyses. Due to the removal of one PID from the database, the total number of subjects enrolled into the trial changed from 15,460 subjects (8,923 in 5-17m) as reported in previous analyses to 15,459 subjects (8,922 in 5-17m) in the final analyses reported in 2014.

In infants 6-12w of age living in malaria-endemic regions, pain, redness and swelling at the injection site were reported following 17.3%, 5.8% and 6.0% of doses of RTS,S/AS01E, respectively and following 13.6%, 6.3% and 5.7% of doses of MenC vaccine, respectively. The incidence of pain, swelling and redness did not increase with subsequent doses of RTS,S/AS01E.

Grade 3 solicited local reactogenicity was infrequent (grade 3 pain and swelling following 0.3% and 0.1% of doses of RTS,S/AS01E, respectively; grade 3 redness not reported for RTS,S/AS01E).

Fourth dose

In children 5-17m of age living in malaria-endemic regions, pain, redness and swelling at the injection site after the fourth dose were reported by 17.0%, 2.3% and 6.6% of children who previously received three first doses of RTS,S/AS01E and a fourth dose of RTS,S/AS01E at Month 20 (R3R group), respectively, by 7.0%, 2.0% and 5.5% of children who previously received three first doses of RTS,S/AS01E and a dose of comparator vaccine at Month 20 (R3C group), respectively, and by 6.5%, 1.3% and 4.7% of children receiving three primary doses of comparator vaccine and a dose of comparator vaccine at Month 20 (C3C group), respectively.

Grade 3 solicited local reactogenicity was infrequent (no grade 3 pain was reported; grade 3 redness and swelling were reported by 0.5% and 1.4% of children in the R3R group, respectively; grade 3 swelling was reported by 0.2% of children in the R3C group).

In infants 6-12w of age living in malaria-endemic regions, pain, redness and swelling at the injection site were reported by 9.7%, 1.5% and 7.4% of infants in the R3R group, respectively, by 4.6%, 1.9% and 4.5% of infants in the R3C group, respectively, and by 4.0%, 1.4% and 6.9% of infants in the C3C group, respectively.

Grade 3 solicited local reactogenicity was infrequent (no grade 3 pain was reported; grade 3 redness and swelling were reported by 0.2% and 0.8% of infants in the R3R group, respectively and grade 3 swelling was reported by 0.3% of infants in the C3C group).

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Table 78. Malaria-055: Incidence of solicited local symptoms reported during the 7-day (Day 0-6) post fourth dose period [5-17m (TVC)

					R3R					R30	;				C30	;	
						95 %	6 CI				95 %	6 CI				95 %	6 CI
Symptom	Product	Туре	N	n	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL
Pain	Total	All	641	109	17.0	14.2	20.1	639	45	7.0	5.2	9.3	633	41	6.5	4.7	8.7
		Grade 3	641	0	0.0	0.0	0.6	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
	Menjugate	All						639	45	7.0	5.2	9.3	633	41	6.5	4.7	8.7
		Grade 3						639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
	RTS,S/AS01 _E	All	641	109	17.0	14.2	20.1										
		Grade 3	641	0	0.0	0.0	0.6										
Redness	Total	All	641	15	2.3	1.3	3.8	639	13	2.0	1.1	3.5	633	8	1.3	0.5	2.5
		Grade 3	641	3	0.5	0.1	1.4	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
	Menjugate	All						639	13	2.0	1.1	3.5	633	8	1.3	0.5	2.5
		Grade 3						639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
	RTS,S/AS01 _E	All	641	15	2.3	1.3	3.8										
		Grade 3	641	3	0.5	0.1	1.4										
Swelling	Total	All	641	42	6.6	4.8	8.8	639	35	5.5	3.8	7.5	633	30	4.7	3.2	6.7
		Grade 3	641	9	1.4	0.6	2.6	639	1	0.2	0.0	0.9	633	0	0.0	0.0	0.6
	Menjugate	All						639	35	5.5	3.8	7.5	633	30	4.7	3.2	6.7
		Grade 3						639	1	0.2	0.0	0.9	633	0	0.0	0.0	0.6
	RTS,S/AS01 _E	All	641	42	6.6	4.8	8.8										
		Grade 3	641	9	1.4	0.6	2.6										

R3R = RTS, S/AS01E 4-dose schedule

R3C = RTS,S/AS01E 3-dose schedule

C3C = Control

N = number of subjects with the administered dose

 $\ensuremath{\text{n}}\xspace/\%$ = number/percentage of subjects reporting the symptom at least once

95% CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit

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Table 79. Malaria-055: Incidence of solicited local symptoms reported during the 7-day (Day 0-6) post fourth dose period [6-12w] (TVC)

					R3F	?				R30	;				C30	;	
						95	% CI				95 %	% CI				95 %	6 CI
Symptom	Product	Туре	N	n	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL
Pain	Total	All	608	59	9.7	7.5	12.3	625	29	4.6	3.1	6.6	621	25	4.0	2.6	5.9
		Grade 3	608	0	0.0	0.0	0.6	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
	Menjugate	All						625	29	4.6	3.1	6.6	621	25	4.0	2.6	5.9
		Grade 3						625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
	RTS,S/AS01 _E	All	608	59	9.7	7.5	12.3										
		Grade 3	608	0	0.0	0.0	0.6										
Redness	Total	All	608	9	1.5	0.7	2.8	625	12	1.9	1.0	3.3	621	9	1.4	0.7	2.7
		Grade 3	608	1	0.2	0.0	0.9	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
	Menjugate	All						625	12	1.9	1.0	3.3	621	9	1.4	0.7	2.7
		Grade 3						625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
	RTS,S/AS01 _E	All	608	9	1.5	0.7	2.8										
		Grade 3	608	1	0.2	0.0	0.9										
Swelling	Total	All	608	45	7.4	5.4	9.8	625	28	4.5	3.0	6.4	621	43	6.9	5.1	9.2
		Grade 3	608	5	8.0	0.3	1.9	625	0	0.0	0.0	0.6	621	2	0.3	0.0	1.2
	Menjugate	All						625	28	4.5	3.0	6.4	621	43	6.9	5.1	9.2
		Grade 3						625	0	0.0	0.0	0.6	621	2	0.3	0.0	1.2
	RTS,S/AS01 _E	All	608	45	7.4	5.4	9.8										
		Grade 3	608	5	8.0	0.3	1.9										

R3R = RTS,S/AS01E 4-dose schedule

R3C = RTS,S/AS01E 3-dose schedule

C3C = Control

N = number of subjects with the administered dose

 $\ensuremath{\text{n}}\xspace/\% = \ensuremath{\text{number/percentage}}\xspace$ of subjects reporting the symptom at least once

95% CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit

Solicited general symptoms in children aged 6 weeks to 17 months reported from Malaria-055

Drowsiness, irritability, loss of appetite and fever (defined as axillary temperature \geq 37.5°C) were the general symptoms solicited in all clinical trials. Grade 3 fever was defined as axillary temperature >39.0°C.

First three doses

In children 5-17m of age living in malaria-endemic regions, the occurrence of drowsiness, irritability and loss of appetite was reported following 6.6%, 11.5% and 11.4% of doses of RTS,S/AS01E, respectively, and following 4.4%, 5.3% and 7.4% of doses of rabies vaccine, respectively. Cases of fever and fever considered related to vaccination were reported following 31.1% and 16.9% of doses of RTS,S/AS01E, respectively, and following 13.4% and 5.9% of doses of rabies vaccine, respectively. The incidence of solicited general reactogenicity did not increase with the administration of subsequent doses of RTS,S/AS01E, except for fever in the RTS,S/AS01E group, which occurred more frequently after Doses 2 and 3 as compared to Dose 1.

Grade 3 solicited general reactogenicity was infrequent (grade 3 drowsiness, irritability and loss of appetite following 0.1% of doses, and fever following 2.5% of doses of RTS,S/AS01E).

In infants 6-12w of age living in malaria-endemic regions, the occurrence of drowsiness, irritability and loss of appetite was reported following 9.9%, 22.2% and 7.9% of doses of RTS,S/AS01E, respectively,

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and following 7.6%, 17.7% and 6.5% of doses of MenC vaccine, respectively. Cases of fever and fever considered related to vaccination were reported following 30.6% and 20.8% of doses of RTS,S/AS01E, respectively, and following 21.1% and 12.6% of doses of MenC vaccine, respectively. The incidence of solicited general reactogenicity did not increase with the administration of subsequent doses of RTS,S/AS01E.

Grade 3 solicited general reactogenicity was infrequent (only grade 3 irritability and fever reported, following 0.5% and 0.6% of doses of RTS,S/AS01E, respectively).

Fourth dose

In children 5-17m of age living in malaria-endemic regions, the occurrence of drowsiness, irritability and loss of appetite after the fourth dose was reported by 8.6%, 9.8% and 10.3% of children in the R3R group, respectively, by 3.4%, 3.9% and 4.2% of children in the R3C group, respectively, and by 3.3%, 2.8% and 3.3% of children in the C3C group, respectively. Cases of fever and fever considered related to vaccination were reported by 36.3% and 23.6% of children in the R3R group, respectively, by 11.0% and 4.5% of children in the R3C group, respectively, and by 7.1% and 2.5% of children in the C3C group, respectively.

Grade 3 solicited general reactogenicity was infrequent (grade 3 drowsiness, irritability and loss of appetite reported by 0.2% of children in the R3R group, and grade 3 fever by 5.3%, 0.9% and 0.8% of children in the R3R, R3C and C3C groups, respectively).

In infants 6-12w of age living in malaria-endemic regions, the occurrence of drowsiness, irritability and loss of appetite after the fourth dose was reported by 5.4%, 7.6% and 7.4% of infants in the R3R group, respectively, by 3.0%, 3.7% and 4.3% of infants in the R3C group, respectively, and by 2.4%, 3.7% and 2.9% of infants in the C3C group, respectively. Cases of fever and fever considered related to vaccination were reported by 25.0% and 13.2% of infants in the R3R group, respectively, by 8.3% and 2.4% of infants in the R3C group, respectively, and by 9.3% and 2.9% of infants in the C3C group, respectively.

No grade 3 drowsiness, irritability and loss of appetite were reported in any of the three groups. Grade 3 fever was reported by 1.5%, 1.1% and 1.6% of infants in the R3R, R3C and C3C groups, respectively.

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Table 80. Malaria-055: Incidence of solicited general symptoms reported during the 7day (Days 0-6) post fourth dose period [5-17m] (TVC)

		R3R				R3C	;		C3C							
					95 %	% CI				95	% CI				95 %	% CI
Symptom	Туре	N	n	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL
Drowsiness	All	641	55	8.6	6.5	11.0	639	22	3.4	2.2	5.2	633	21	3.3	2.1	5.0
	Grade 3	641	1	0.2	0.0	0.9	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
	Related	641	34	5.3	3.7	7.3	639	10	1.6	8.0	2.9	633	13	2.1	1.1	3.5
	Grade 3*Rel	641	0	0.0	0.0	0.6	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
Irritability	All	641	63	9.8	7.6	12.4	639	25	3.9	2.5	5.7	633	18	2.8	1.7	4.5
	Grade 3	641	1	0.2	0.0	0.9	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
	Related	641	40	6.2	4.5	8.4	639	12	1.9	1.0	3.3	633	8	1.3	0.5	2.5
	Grade 3*Rel	641	1	0.2	0.0	0.9	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
Loss of appetite	All	641	66	10.3	8.1	12.9	639	27	4.2	2.8	6.1	633	21	3.3	2.1	5.0
	Grade 3	641	1	0.2	0.0	0.9	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
	Related	641	39	6.1	4.4	8.2	639	14	2.2	1.2	3.6	633	13	2.1	1.1	3.5
	Grade 3*Rel	641	1	0.2	0.0	0.9	639	0	0.0	0.0	0.6	633	0	0.0	0.0	0.6
Fever	All	641	233	36.3	32.6	40.2	639	70	11.0	8.6	13.6	633	45	7.1	5.2	9.4
	Grade 3	641	34	5.3	3.7	7.3	639	6	0.9	0.3	2.0	633	5	0.8	0.3	1.8
	Related	641	151	23.6	20.3	27.0	639	29	4.5	3.1	6.5	633	16	2.5	1.5	4.1
	Grade 3*Rel	641	24	3.7	2.4	5.5	639	1	0.2	0.0	0.9	633	0	0.0	0.0	0.6

R3R = RTS,S/AS01E 4-dose scheduleschedule

R3C = RTS, S/AS01E 3-dose schedule

C3C = Control

N = number of subjects with the administered dose

n/% = number/percentage of subjects reporting the symptom at least once

95% CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit

Table 81. Malaria-055: Incidence of solicited general symptoms reported during the 7day (Day 0-6) post fourth dose period [6-12w] (TVC)

				R3R	}				R30	2				C30	С	
					95 %	% CI				95	% CI				95 '	% CI
Symptom	Туре	N	n	%	LL	UL	N	n	%	Ц	UL	N	n	%	LL	UL
Drowsiness	All	608	33	5.4	3.8	7.5	625	19	3.0	1.8	4.7	621	15	2.4	1.4	4.0
	Grade 3	608	0	0.0	0.0	0.6	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
	Related	608	19	3.1	1.9	4.8	625	6	1.0	0.4	2.1	621	5	8.0	0.3	1.9
	Grade 3*Rel	608	0	0.0	0.0	0.6	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
Irritability	All	608	46	7.6	5.6	10.0	625	23	3.7	2.3	5.5	621	23	3.7	2.4	5.5
	Grade 3	608	0	0.0	0.0	0.6	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
	Related	608	27	4.4	2.9	6.4	625	10	1.6	8.0	2.9	621	6	1.0	0.4	2.1
	Grade 3*Rel	608	0	0.0	0.0	0.6	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
Loss of appetite	All	608	45	7.4	5.4	9.8	625	27	4.3	2.9	6.2	621	18	2.9	1.7	4.5
	Grade 3	608	0	0.0	0.0	0.6	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
	Related	608	26	4.3	2.8	6.2	625	8	1.3	0.6	2.5	621	6	1.0	0.4	2.1
	Grade 3*Rel	608	0	0.0	0.0	0.6	625	0	0.0	0.0	0.6	621	0	0.0	0.0	0.6
Fever	All	608	152	25.0	21.6	28.6	625	52	8.3	6.3	10.8	621	58	9.3	7.2	11.9
	Grade 3	608	9	1.5	0.7	2.8	625	7	1.1	0.5	2.3	621	10	1.6	8.0	2.9
	Related	608	80	13.2	10.6	16.1	625	15	2.4	1.3	3.9	621	18	2.9	1.7	4.5
	Grade 3*Rel	608	5	8.0	0.3	1.9	625	1	0.2	0.0	0.9	621	3	0.5	0.1	1.4

R3R = RTS,S/AS01E 4-dose schedule R3C = RTS,S/AS01E 3-dose schedule

C3C = Control

N = number of subjects with the administered dose n/% = number/percentage of subjects reporting the symptom at least once 95% CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit

AEs of special interest (AESIs)

Febrile convulsions occurring within 7 days of vaccination

Febrile convulsions are single generalised tonic-clonic seizures of up to 15 minutes duration in infants and children 3 months through 6 years of age. Usually, the body temperature is above 38.0°C (Bonhoeffer 2004). Increased risk of febrile convulsions during the period of vaccine-induced fever has been reported after administration of other childhood vaccines, like measles vaccines. The specific causes of febrile convulsions and the mechanism by which fever generates febrile convulsions are still poorly understood.

Generalised convulsive seizures occurring within the 30 days post-vaccination were identified as an AE of specific interest in the RTS,S/AS01E programme and reported as SAE in trials in infants and children. For seizures occurring within 7 days of vaccination, data collection and presentation was done according to the Brighton Collaboration guidelines [Bonhoeffer 2004]

Febrile convulsions in the comparative pooled analysis of safety data

In the comparative pooled analysis of safety data in the target population with the final formulation, the incidence of generalised convulsive seizure within 7 days following the vaccination with the first 3 doses was analysed per age category. In children 5-17m, the incidence of generalised convulsive seizures (level 1-3) within 7 days of vaccination was 1.1 per 1,000 doses in the RTS,S/AS01E group (95% CI: 0.6-1.6) and 0.7 per 1,000 doses in the control group (rabies vaccine) (95% CI: 0.3-1.4) (Table 82). In infants 6-12w, the incidence of generalised convulsive seizures within 7 days of vaccination was 0.1 per 1,000 doses in the RTS,S/AS01E group (95% CI: 0.0-0.5) and 0.4 per 1,000 doses in the control group (MenC + DTPwHib/HepB) (95% CI: 0.1-1.1) (Table 83).

Table 82. Pooled analysis of safety data in the target population with final formulation: Incidence of seizures by diagnostic certainty level during the 7-day (Days 0-6) post-vaccination period (per 1,000 doses) [5-17m] (TVC)

Characteristics	Catagorias			/AS01 _E 8896		Control vaccine N = 10179				
Characteristics	Categories		m/4000	95%	CI		m/4000	95% CI		
		n	n/1000	LL	UL	n	n/1000	LL	UL	
Generalised convulsive seizure	Level 1 to 3	20	1.1	0.6	1.6	7	0.7	0.3	1.4	
Convulsive seizure	Level 1 to 5	24	1.3	0.8	1.9	9	0.9	0.4	1.7	
Diagnostic certainty level	Level 1	6	0.3	0.1	0.7	2	0.2	0.0	0.7	
	Level 2	14	0.7	0.4	1.2	5	0.5	0.2	1.1	
	Level 3	0	0.0	0.0	0.2	0	0.0	0.0	0.4	
	Level 4	3	0.2	0.0	0.5	1	0.1	0.0	0.5	
	Level 5	1	0.1	0.0	0.3	1	0.1	0.0	0.5	

N = number of doses; n = number of doses in a given category

n/1000 = n / Number of doses with available results x 1000

LL, UL for percentage = Exact 95% Lower and Upper confidence limits

Level 1: Witnessed sudden loss of consciousness AND generalised, tonic, clonic, tonic-clonic, or atonic motor manifestations

Level 2: History of unconsciousness AND generalised, tonic, clonic, tonic-clonic, or atonic motor manifestations

Level 3: History of unconsciousness AND other generalised motor manifestations

Level 4: Reported generalised convulsive seizure with insufficient evidence to meet the case definition

Level 5: Not a case of generalised convulsive seizure

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Table 83. Pooled analysis of safety data in the target population with final formulation: Incidence of seizures by diagnostic certainty level during the 7-day (Days 0-6) post-vaccination period (per 1,000 doses) [6-12w] (TVC)

Characteristics	Cotogorios		RTS,S/ N = 1			Control vaccine N = 7720				
Characteristics	Categories	n	n/1000	95%	CI		n/1000	95% CI		
		n	11/1000	LL	UL	n	11/1000	LL	UL	
Generalised convulsive seizure	Level 1 to 3	2	0.1	0.0	0.5	3	0.4	0.1	1.1	
Convulsive seizure	Level 1 to 5	2	0.1	0.0	0.5	3	0.4	0.1	1.1	
Diagnostic certainty level	Level 1	0	-	0	0.3	1	0.1	0.0	0.7	
	Level 2	2	0.1	0.0	0.5	2	0.3	0.0	0.9	
	Level 3	0	0.0	0.0	0.3	0	0.0	0.0	0.5	
	Level 4	0	0.0	0.0	0.3	0	0.0	0.0	0.5	
	Level 5	0	0.0	0.0	0.3	0	0.0	0.0	0.5	

N = number of doses; n = number of doses in a given category

n/1000 = n / Number of doses with available results x 1000

LL, UL for percentage = Exact 95% Lower and Upper confidence limits

Level 1: Witnessed sudden loss of consciousness AND generalised, tonic, clonic, tonic-clonic, or atonic motor manifestations

Level 2: History of unconsciousness AND generalised, tonic, clonic, tonic-clonic, or atonic motor manifestations

Level 3: History of unconsciousness AND other generalised motor manifestations

Level 4: Reported generalised convulsive seizure with insufficient evidence to meet the case definition

Level 5: Not a case of generalised convulsive seizure

Febrile convulsion in Malaria-055

First three doses

In trial Malaria-055, in children 5-17m, the incidence of generalised convulsive seizure within 7 days of vaccination (according to the Brighton Collaboration diagnostic certainty level of 1 to 3) was higher in the RTS,S/AS01E group than in the rabies group: 1.04 per 1,000 doses in the RTS,S/AS01E group (95% CI: 0.62-1.64) and 0.57 per 1,000 doses in the rabies group (95% CI: 0.19-1.34), with a risk ratio of 1.8 (95% CI: 0.6-4.9) (The RTS,S Clinical Trials Partnership2011). All convulsions occurred in children who had fever before or during the seizure.

Importantly, although the increase in febrile convulsions in children was observed within 7 days of vaccination, the overall rate of children experiencing a febrile convulsion reported as SAE was not increased in the RTS,S/AS01E group compared to the comparator group over 30 days post-vaccination (39 [1.0%] children and 17 [0.8%] children, respectively) and over the entire follow-up period (224 [3.8%] and 112 [3.8%] children, respectively).

In infants 6-12w, the incidence of generalised convulsive seizures within 7 days of vaccination was 0.16 per 1,000 doses in the RTS,S/AS01E group (95% CI: 0.02-0.57) and 0.47 per 1,000 doses in the MenC group (95% CI: 0.10-1.37), with a risk ratio of 0.3 (95% CI: 0.1-2.0) (The RTS,S Clinical Trials Partnership 2011). All seizures but one occurred in infants who had fever before or during the seizure.

Fourth dose

In children 5-17m, the incidence of generalised convulsive seizure within 7 days of booster vaccination was 2.5 per 1,000 doses in the R3R group (95% CI: 0.9 to 5.3), 1.2 per 1,000 doses in the R3C group

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(95% CI: 0.3 to 3.5) and 0.4 per 1,000 doses in the C3C group (95% CI: 0.0 to 2.3). All convulsions occurred in children with a history of fever, except for two children.

In infants 6-12w, the incidence of generalised convulsive seizure within 7 days from the fourth dose was 2.2 per 1,000 doses in the R3R group (95% CI: 0.6 to 5.6) and 0.5 per 1,000 doses in the C3C group (95% CI: 0.0 to 3.0). No cases were reported in the R3C group. All convulsions occurred in infants with a history of fever.

Meningitis

In the first analysis of trial Malaria-055 after 12 months follow-up a numerical imbalance of meningitis cases was noticed in both age categories. More cases of meningitis were observed among children aged 5-17 months who received RTS,S/AS901E than in controls (11 vs. 1).

At the time of the 3^{rd} analysis, 18 months after dose 3, Seventeen cases of meningitis of any cause were reported as SAE after primary vaccination in children 5-17m, 16 cases occurred in children receiving RTS,S/AS01E (N = 5,949 children) and one case was reported in the control group (N = 2,974 children) (RR = 8.0 [95% CI: 1.1-60.3]).

Table 84. Malaria-055: Analysis at study Month 20 for meningitis in both age groups after the three first doses per treatment group

		Number of c	ases (%)	
Age Group	Preferred Term	RTS,S/AS01 _E pooled groups	Control group	RR (%: 95% CI: LL-UL)
6-12w		N= 4358	N= 2179	
	Meningitis all aetiology (Total)	9	3	1.5 (0.4-5.5)
	Meningitis	3	2	
	Meningitis Pneumococcal	3	1	
	Meningitis Salmonella	3	0	
5-17m		N= 5949	N= 2974	
	Meningitis all aetiology (Total)	16	1	8.0 (1.1-60.3)
	Meningitis	9	1	
	Meningitis Meningococcal	4	0	
	Meningitis viral	1	0	
	Meningitis Haemophilus	1	0	
	Meningitis Pneumococcal	1	0	

No pathogen was identified for 9 vs. 1 case, a bacterial pathogen was found in 6 and one was reported as being of viral aetiology. Two additional cases occurred in children who did not attend the M20 visit, giving 18 vs. 1 cases.

In infants 6-12w, meningitis of any cause was reported as an SAE in 12 infants, nine cases occurred in infants receiving RTS,S/AS01E (N = 4.358) and three cases were reported in the comparator group (N = 2.179) (RR = 1.5 [95% CI: 0.4-4.5]). A pathogen was identified in seven cases of meningitis (MedDRA PT "Meningitis salmonella" [three cases] and "Meningitis pneumococcal" [four cases]).

Among the infants 6-12w who presented with meningitis, two of them receiving RTS,S/AS01E died (two cases of pneumococcal meningitis) and two receiving control vaccine died (one case of pneumococcal meningitis and one case with no pathogen retrieved). In children 5-17m, five of them

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receiving RTS,S/AS01E died (four cases with no pathogen retrieved and one case of pneumococcal meningitis) and one receiving control vaccine died (no pathogen retrieved).

All meningitis cases retrieved in the safety pooling come from Malaria-055. As yet there is no indication of an excess rate of meningitis in other studies.

In the 5-17 months age category, after the 4^{th} dose, 4 cases were reported, 1 in the R3R (children that received 3 doses of RTS,S/AS01_E and 4^{th} dose with RTS,S/AS01_E) and 3 in the R3C (3 doses with RTS,S/AS01_E and control vaccine at the time of the 4^{th} dose) group. None was reported in the control group.

In the 6-12 weeks age category, after the 4th dose, 2 cases were reported in the R3C group (3 doses with RTS,S/ASO1_F and control vaccine at the time of the 4th dose) and 3 cases in the control group.

The display of time to onset for all cases spanned < 28 days to more than one year, although it seems that about half occurred by day 120. The analysis by site showed a much higher rate in Lilongwe, Malawi (11 cases with 7 aged 5-17 months and 4 aged 6-12 weeks at entry; total 0.7%) than at other sites ($\leq 0.3\%$). According to the calendar date of the meningitis cases, no cluster over a limited time period, which could be interpreted as an outbreak, was observed in any of the trial sites participating in Malaria-055, including Lilongwe The applicant concluded that:

- The absence of temporal relationships to vaccination and of potential mechanisms do not argue in favour of a direct effect of RTS,S/AS01_E.
- After the 4th dose, only one case occurred after RTS,S/AS01
- The meningitis signal includes diverse aetiologies of meningitis and no biologically plausible explanation could be identified so far.
- The low incidence of meningitis in the control group (C3C) of the 5-17 months of age category is not explained.
- More than 40% of meningitis cases were reported from one study site, without explanation found like outbreak.
- An indirect effect of RTS,S/AS01_E on susceptibility to develop meningitis cannot be excluded.
- Based on the currently available data, the hypothesis of chance finding is more likely to explain the imbalance observed

Assessment All cases of CNS infections/inflammations, including the ones from Malaria-055, were reviewed by 2 external experts who concluded that there was no specific pattern of clinical presentation and that a causal relationship with RTS,S/AS01 was unlikely.

The applicant provided information on immunological hypothesis as well as a hypothesis on increased permeability of the blood brain barrier. All the hypotheses were very unlikely to explain the safety signal meningitis.

In conclusion, based on the currently available data, there is no evidence in favour of immunological hypotheses. Other hypothesis, like simple chance finding, should also be taken into account and it is more likely to explain the imbalance observed. Meningitis remains a potential risk that will be closely monitored and further assessed in the planned post-approval EPI-MAL-003 study included in the RMP.

Potential Immune mediated disorders (pIMDs)

pIMDs were to be reported as SAEs but there were no reports. Research of SAEs by MedDRA term identified some possible pIMDs. Overall 17 pIMDs were identified, including 3 encephalitis cases in

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RTS,S/AS01 recipients and 3 in controls. Single cases of SJS and psoriasis occurred in controls and one case of erythema multiforme occurred after RTS,S/AS.

Fatalities in children 6 weeks to 17 months of age vaccinated with RTS,S/AS01E - Fatalities in the comparative pooled analysis of safety data

In the comparative pooled analysis of safety data in the target population with final formulation, fatal SAEs were reported during the entire study period or until the DLP by 122 (1.2%) children 5-17m of age and 130 (1.7%) infants 6-12w of age. In the 5-17m age category, similar incidences of fatal SAEs were reported for children receiving RTS,S/AS01 $_{\rm E}$ or control vaccine (respectively 1.3% [95% CI: 1.0-1.6] and 1.1% [95% CI: 0.8-1.5]; RR = 1.18 [95% CI: 0.80-1.78], p = 0.4510). In the 6-12w age category, the incidence of fatal SAEs was slightly higher in infants receiving RTS,S/AS01E compared to infants receiving control vaccine but the difference was not significant (respectively 1.9% [95% CI: 1.5-2.3] and 1.4% [95% CI: 1.0-1.9]; RR = 1.39 [95% CI: 0.93-2.10], p = 0.1098).

Within the 30 days post-vaccination, 20 (0.2%) children 5-17m and 31 (0.4%) infants 6-12w old experienced a fatal SAE. In the 5-17m age category, similar incidences of fatal SAEs were reported for children receiving RTS,S/AS01E or control vaccine (respectively 0.2% [95% CI: 0.1-0.4] and 0.2% [95% CI: 0.1-0.4]; RR = 1.25 [95% CI: 0.45-3.96], p = 0.8460). In the 6-12w age category, the incidence of fatal SAEs was slightly higher in infants receiving RTS,S/AS01E compared to infants receiving control vaccine but the difference was not significant (respectively 0.5% [95% CI: 0.3-0.7] and 0.3% [95% CI: 0.1-0.5]; RR = 1.82 [95% CI: 0.76-5.00], p = 0.2150). No death was considered related to vaccination.

Serious adverse events

RTS, S/AS01E in children aged 5 to 17 months

- The incidence of SAEs over the entire study period was significantly lower for RTS,S/AS01E vs. controls (18.6% [95% CI: 17.7-19.6] vs. 22.6% [95% CI: 21.3-24.1]; RR = 0.82 [95% CI: 0.75-0.90], p <0.0001).
- However, this difference is at least partly driven by the fact that the most frequently reported SAE in both groups was malaria (6.6% vs. 9.3%; p<0.05). Other frequent SAEs were pneumonia (5.8% vs. 6.2%), gastroenteritis (4.4% vs. 5.5%; p<0.05), febrile convulsions (3.9% vs. 4.0%) and anaemia (3.2% vs. 4.9%; p<0.05).
- Within 30 days post-vaccination the incidence of SAEs was 5.1% for RTS,S/AS01E and 5.9% for controls. No PT showed a statistically significant higher rate for RTS,S/AS01E.
- SAEs considered related to vaccination were reported by 11 who received RTS,S/AS01E vs. one control. Febrile convulsions were reported and considered related to RTS,S/AS01E for 7 vs. 1.

RTS, S/AS01E in infants 6-12 weeks

- The incidence of SAEs was similar for RTS,S/AS01E and controls (20.4% [95% CI: 19.2-21.5] and 20.5% [95% CI: 19.0-22.1]; RR = 0.99 [95% CI: 0.89-1.10), p = 0.9146).
- The most frequently reported SAE in both groups was pneumonia (7.7% vs. 7.0%) followed by gastroenteritis (6.6% vs. 6.8%, respectively), malaria (5.5% vs. 5.8%), anaemia (2.7% vs. 3.3%) and febrile convulsions (2.5% in both groups). The only PTs with a significant difference between groups (p<0.05) were *P. falciparum* infection (more frequent in controls) and malnutrition (more frequent in the RTS,S/AS01E group).

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- Within the 30 days post-vaccination, the incidence of SAEs was 4.2% in both groups. Except for enteritis (4 controls, none RTS,S/AS01E; RR = 0.028) there was no significant difference for any PT.
- Over the entire study period, SAEs considered related to vaccination were reported for four infants who received RTS,S/AS01E (including one case of febrile convulsion) and for 3 controls.

Laboratory findings

In Malaria-038, -040, -046, -047, -049, -050 and -058 clinical safety laboratory evaluations were graded according to a pre-defined toxicity scale. In children at least 5 months of age no safety signal was identified on monitoring haematological and biochemical laboratory parameters in recipients of RTS,S/AS01E. In infants aged 6-12 weeks few haematology and biochemistry values were outside the normal range in any vaccine group.

Safety in special populations

Malaria-058 in HIV-infected children

From the data provided there is no clear negative impact of RTS,S/AS01E vaccination in HIV infected children could be observed. However, it should be noted that median viral loads were higher in subjects vaccinated with RTS,S/AS01E compared with subjects in the control group. Median viral loads were comparable in both groups at baseline: 149,000 copies/ml in the RTS, S/AS01E group versus 157,000 in the control group. Viral loads decreased in both groups but remained higher in the RTS,S/AS01E group reaching medians of 3125 and 583.5 copies/ml respectively at 1 month post-dose 3, 3790 and 400 copies/ml at 6 months post-dose 3 and 947 vs. 400 copies/ml at 12 months postdose 3. Furthermore the frequency of SAEs particularly within 30 days post vaccination was higher in the RTS,S/AS01E group compared with the control group (20.2% [95% CI 12.8;29.5] versus 11.9% [95% CI: 6.3; 19.8]). This was mainly driven by a higher frequency of pneumonia in the RTS,S/AS01E group (13.1% [95% CI 7.2; 21.4] versus 5.0% [95% CI 1.6; 11.2]). Up to month 14 frequencies of SAEs and pneumonia were more balanced in the two groups. SAEs were reported by 41.4% of subjects [95% CI 31.6; 51.8] versus 36.6% [95% CI 27.3; 46.8], pneumonia by 23.2% [95% CI 15.3; 32.8] versus 22.8% [95% CI 15.0; 32.2] (described in section "serious adverse events"). Also fever rates were higher in RTS,S/AS01E subjects compared with subjects in the control group (41% [95% CI 35 47] versus 18% [95% CI 14.5; 23] subjects reported vaccine related fever with grade 3 vaccine related fever in 4.2% versus 2.0% of subjects. Taking all this into account and considering the small sample size of 99 subjects in the RTS,S/AS01E vaccine group and 101 subjects in the Rabies control group the issue of safety and efficacy in HIV infected children needs to be further evaluated in a larger sample sizes. Safety in HIV infected children is addressed in the RMP.

In subjects not on ART the median CD4+ cells count and the minimum CD4+ cell count was notably lower in the RTS,S/AS01E vaccine group. The minimum CD4+ cell count in subjects not on ART was not ≤ 200 cells/µl, but notable. As the sample size of subjects not on ART is very low with 3 subjects in both groups and the only measurement was done only 1 month post dose 3 and not controlled at a later time point this can be a finding by chance.

Malaria-055 in HIV-infected children

In Malaria-055, children with known HIV/AIDS disease stage I and II (WHO AIDS staging) were eligible. It should be noted that HIV testing was not a study procedure; this analysis included

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therefore all children known to be HIV infected at enrolment or subsequently diagnosed on clinical suspicion.

At study end, 1.0% of the children and infants had a confirmed HIV positive status (51 in R3R, 54 in R3C and 48 in C3C groups) and a few additional children and infants (9 in total) had an SAE coded as retroviral infection that was not confirmed by PCR or HIV antibody test (suspected HIV positive status). Six children were known to be HIV infected at enrolment, the others were identified HIV-infected during study conduct. Therefore, most of the children included in this analysis were not under treatment at the time of RTS,S/AS01E vaccination. The adherence to treatment during the length of the study is unknown.

Although a trend for more pneumonia was reported as SAEs in Malaria-058 within the first 30 days, in Malaria-055, pneumonia within 30 days was experienced by 8.6% [95%CI: 4.0 to 15.6] of subjects in the RTS,S/AS01E group and 10.4% [95%CI: 3.5 to 22.7] of subjects in the control group. No imbalance in pneumonia SAEs was observed over the year following dose 3 and there was no indication of a trend in other invasive bacterial infections within 30 days post-vaccination or over the full study period.

The overall safety information from dose 1 to study end in the HIV-infected children showed they experienced similar incidence of SAEs and fatal SAEs in the 3 groups (R3R, R3C and C3C).

Malaria-055 subjects with low and very low weight for age

Within this subgroup the safety profile was generally comparable between RTS,S/AS01E and controls except that in very low weight for age infants at least one SAE was reported by 32.7% vs. 25.4% controls, nine of which were fatal SAEs.

Malaria-055 infants born prematurely (<37 weeks)

An evaluation of safety over 20 months post dose 1 was performed in 362 infants aged 6-12 weeks at first dose (244 in the RTS, S/ASO1_E group and 118 in the control group) who were born prematurely.

There were 244 RTS,S/AS01E and 118 controls born prematurely, mostly with a gestational age of 33-36 weeks. Up to month 20 at least one SAE was reported for 48 (19.7%) vs. 13 (11.0%) of these infants and most were cases of pneumonia. SAEs of malaria occurred in 4.1% vs. 1.7%. Fatal SAEs occurred in 8 (3.3%) RTS,S/AS01E recipients and one (0.8%) of the control subjects. None of the fatal SAEs were considered related to vaccination and there was no imbalance with a particular SAE.

Immunological events

Two cases of anaphylaxis were reported in Malaria-055 in the control group

Discontinuation due to AEs

Overall, in completed clinical trials (except for Malaria-055) 1,259 subjects did not complete the studies for reasons other than (S)AEs whereas 87 were withdrawn from further vaccination due to an AE or SAE, including 69 with a fatal SAE (31 any RTS,S/AS vaccine and 38 any control vaccine), 5 with a non-fatal SAE (4 vs. 1) and 13 subjects with a non-serious AE (9 vs. 4).

The 18 non-fatal SAEs leading to withdrawal from further RTS,S/AS01 and/or AS02 vaccination included:

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- In Malaria-027 one developed an urticarial reaction (grade 1) after the first dose, which
 resolved without sequelae. A second subject was withdrawn due to a cerebral infarct occurring
 99 days post Dose 3 of RTS,S/AS01_B. The event was categorised as an SAE and considered not
 related to study vaccination.
- In Malaria-046 one had a simple febrile seizure 18 days post-dose 2 and fully recovered. Another subject was found to have sickle cell anaemia five days post Dose 2.
- In Malaria-048 one developed grade 3 local redness post-dose 1 (320 mm on day 4; resolved by day 8) and one developed grade 3 gastrointestinal symptoms post-dose 1, which resolved by Day 14.
- In Malaria-020, one recipient of RTS,S/AS02A was withdrawn from further vaccination due to malaria and upper respiratory tract infection prior to Dose 3. One recipient of the control vaccine was withdrawn from further vaccination due to raised alanine aminotransferase (ALT) levels prior to Dose 2.
- In Malaria-025, two recipients of RTS,S/AS02A and two recipients of the control vaccine were
 withdrawn from further vaccination due to raised ALT levels post Dose 1. One recipient of the
 control vaccine had upper respiratory tract infection and pneumonia and was withdrawn on
 suspicion of immunodeficiency disease.
- In Malaria-026, one recipient of RTS,S/AS02A was withdrawn from further vaccination because of pneumonia post Dose 1 which led to the diagnosis of pulmonary tuberculosis associated with an underlying HIV infection. The child had three other admissions during the course of the surveillance, two for acute pneumonia due to bacterial superinfections and one for malaria and acute gastroenteritis. One recipient of RTS,S/AS02A was found to be malnourished, necessitating admission for nutritional support post Dose 2. The child recovered and did not require admission again during the course of the surveillance. One recipient of RTS,S/AS02A developed upper respiratory tract infection post Dose 1 and was withdrawn from further vaccination on suspicion of an immunodeficiency disease. One recipient of the control vaccine experienced a generalised urticarial rash within minutes of receiving Dose 2. The event resolved without treatment.
- In Malaria-058, one recipient of RTS,S/AS01E was withdrawn from further vaccination due to two SAEs post Dose 1: salmonella sepsis and pneumonia.

For Malaria -055, of the 8,922 children 5-17m who were enrolled, 475 children did not receive three primary doses of vaccine; six of them were withdrawn from further treatment because of a medical withdrawal (of which five received RTS,S/AS01_E), 10 of them because of death (of which seven received RTS,S/AS01_E), while five children were unwell (of which three received RTS,S/AS01_E). In total, 1,063 children 5-17m did not receive a 4th dose of vaccine; 12 of them were withdrawn from further treatment because of a medical withdrawal, 83 of them because of death, while 11 children were unwell. Of the 1,785 children not attending the visit at Month 32, 133 children died. Of the 2,027 children who were not enrolled in the extension, 133 children died and one child had an SAE. Of the 708 children not attending the visit at the end of the extension, 25 children died.

Of the 6,537 infants 6-12w who were enrolled, 303 infants did not receive three primary doses of vaccine; 11 of them were withdrawn from further treatment because of a medical withdrawal (of which seven received RTS,S/ASO1 $_E$), 22 of them because of death (of which 18 received RTS,S/ASO1 $_E$), while two infants were unwell (of which one received RTS,S/ASO1 $_E$). In total, 746 infants 6-12w did not

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receive a 4th dose of vaccine; five of them were withdrawn from further treatment because of a medical withdrawal, 81 of them because of death, while five infants were unwell. Of the 1,355 infants not attending the visit at Month 32, 141 infants were withdrawn from further vaccination because of death. Of the 1,497 infants who were not enrolled in the extension, 140 infants died and one infant had an SAE. Of the 403 infants not attending the visit at the end of the extension, seven infants died and two infants had an SAE.

Overall, the reasons for treatment discontinuation were similar between subjects receiving any RTS,S/AS vaccine formulation and subjects receiving a control vaccine, without any major imbalances.

3.6.1. Discussion on clinical safety

The most common solicited AEs in infants were pain and fever and this mostly in the same rate and grade in all vaccination groups with only few grade 3 reactions. Some exemptions apply:

- Pain and swelling were more frequent in groups receiving Tritanrix HepB, this is well known to be an effect of the whole-cell pertussis component
- Pain (also, not grade 3) was more frequent in toddlers of the RTS,S/AS01E groups.
- Fever was higher in the RTS,S/AS01E group in infants but low frequency of grade 3 were observed
- A higher risk of fever related convulsions during the first 2 days after the vaccination especially in the older age group.

A higher risk of febrile convulsions in the older age group was observed; however as clinical data with other paediatric vaccines suggest reduction of immune response, prophylactic use of antipyretics prevaccination is not recommended.

Meningitis cases are also more frequently observed in the Mosquirix groups and more often in the older age group, within 18 months follow-up period after the first 3 doses. The applicant discussed various underlying immunological and non-immunological reasons of which none alone explains the observation. It seems a multifactorial occurrence and will be further observed in a planned epidemiological study (EPI-Mal-003). Also, the unusual high amount of meningitis cases in one site could not be explained by an outbreak situation and the aetiology was not homogenous so that this finding can only be attributed to chance. No causal relationship was identified for meningitis cases observed in the pivotal clinical trial, however meningitis remains as a potential risk and an epidemiological study is planned post-marketing to assess this potential risk.

Regarding unsolicited AEs the infectious diseases of the gastrointestinal and the respiratory tract were predominant and in similar rate and grade in all vaccination groups. Plasmodium falciparum infections and cerebral malaria was more often seen in the control groups.

Serious adverse events coded to infections and infestations were more dominant and in particular pneumonia and gastroenteritis. The incidence of these serious adverse events was similar in all vaccination groups.

The rate and grade of SAEs was higher in the low-weight subjects in both age groups but similar between the vaccination groups. There does not seem to be any proneness to malaria due to the lower weight.

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HIV+ subjects

HIV+ subjects of study Malaria-055 (subgroup analysis) showed a similar rate and grade of SAEs in both vaccination groups. The age groups were pooled. Deaths were in most cases attributed to the HIV infection, Pneumonia or Gastroenteritis.

The rate of these SAEs is uniformly higher (>20%) than in the healthy cohort (~10%).

In this subgroup convulsions and febrile convulsions occurred in in similar frequencies (~10%) in all vaccine groups.

From the data provided from study Malaria-058, no clear negative impact of RTS,S/AS01E vaccination in HIV infected children could be observed. However, it should be noted that viral loads were higher in subjects vaccinated with RTS,S/AS01E and the frequency of unsolicited pneumonia in RTS,S/AS01E vaccinated subjects as well as the percentage of subjects reporting SAEs in the RTS,S/AS01E vaccine group compared to the Rabies control group particularly during the 30-day safety follow-up period (described in section "serious adverse events") was also higher.

As described above in subjects not on ART the median CD4+ cells count and the minimum CD4+ cell count was notably lower in the RTS,S/AS01E vaccine group. The minimum CD4+ cell count in subjects not on ART was not ≤ 200 cells/ μ l, but notable. As the sample size of subjects not on ART is very low with 3 subjects in both groups and the only measurement was done only 1 month post dose 3 and not controlled at a later time point this can be a finding by chance.

Preterm Infants

Over the time till the 4th dose 19.7% of the pre-term subjects in the RTS,S/AS01E group and 11% in the control group showed SAEs. 3.3% in the RTS,S/AS01E and 0.8% in the control group died of an SAE. None of the SAEs were considered related to any vaccination and all febrile convulsions seen in this subgroup occurred several months after the vaccinations. The most common SAEs were Gastroenteritis, Malaria and Pneumonia and the rate of these SAEs was not higher than in the termborn infants of the same age cohort.

There is no separate analysis of preterm subjects for the time after the 4th dose but, considering the results of the first three doses, no differences to the same age peers are expected.

Rebound of malaria

Rebound was suggested as a possibility after about 4 years follow-up of children vaccinated when aged 5-17 months in Malaria-049/059. In the pivotal study Malaria-055, the median follow-up was 36 months post dose 3 in the 6-12 week age category and of 46 months in the 5-17 months age category.

In children 5-17 months of age at first dose, the incidence of severe malaria decreased over time in all groups. In recipients of a primary vaccination course without 4th dose, a trend to increased incidence of severe malaria compared to control was observed from month 21 until the end of the trial. However, the confidence intervals around the incidence are wide and overlapping. This increased incidence was seen predominantly in sites with a moderate to high level of malaria parasite transmission. The overall impact in terms of cases averted tended to remain positive and there was no indication that the severe malaria cases observed after study month 20 resulted in a more severe outcome.

In infants 6 to 12 weeks of age at primary vaccination with RTS,S/AS01E, there is no evidence of increased incidence of clinical or severe malaria during the entire study period.

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Concomitant use

The frequency of fever in subjects vaccinated concomitantly with RTS,S/AS01E, DTPa/Hib, OPV and PCV (Synflorix) was almost twice as high as in RTS,S/AS01E schedules where Synflorix was not given concomitantly (RTS,S/AS01E + DTPa/Hib + OPV + Rotarix, Synflorix staggered (RERo[P] and RTS,S/AS01E + DTPa/Hib + OPV, Synflorix and Rotarix staggered). However, regarding the occurrence of fever it has to be noted that the frequency of Grade 3 fever was very low in the RTS,S/AS01E vaccine group where Synflorix was concomitantly given.

Detection of cytosolic yeast catalase

Cytosolic yeast catalase was detected in the RTS,S/AS01E vaccine lots manufactured at commercial scale but not in the vaccine lots manufactured at pilot scale. This safety finding is related to quality and therefore also in detail assessed in the quality assessment report.

The potential induction of cross-reactive immune response to human catalase was assessed in the children who were enrolled in study 061.

Amongst the 300 children tested for anti-human catalase antibodies with an in-house ELISA one child was positive one month post dose 3, with an antibody concentration of 3,622 EU/ml. The child was negative pre-vaccination and received the RTS,S final container lot DMALA017A, which was formulated from a commercial scale RTS,S PB lot with intermediate catalase consent. The child reported 2 AEs (upper respiratory tract infection and respiratory tract infection) and no SAE. At a follow-up contact 3 years, no developmental anomalies were observed.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

3.6.2. Conclusions on the clinical safety

The safety profile of this vaccine is acceptable and quite similar to others apart from a higher risk for febrile convulsions in the older age group within 7 days after a dose (mostly the third dose) of Mosquirix. There also is no safety signal from the supportive studies that might indicate a general problem with the antigens.

All identified potential safety issues (febrile convulsions, meningitis, auto-immune disorders, anaphylaxis, malaria rebound) are addressed in the RMP. Ongoing and planned studies will also provide new data for safety and especially following a possible rebound.

HIV + infected children

The higher incidence of adverse reactions observed in HIV+ infected children do not preclude the possibility of use of RTS,S/AS01E in this population considering the following:

- In phase III trials (Malaria-055 and Malaria-058), the benefit/risk balance of RTS,S/AS01_E in HIV-infected children (stage I and II) is considered to be positive
- There was no evidence of HIV progression in terms of CD4+ cell counts (CD4 % and absolute counts), HIV viral load and WHO HIV clinical classification
- Safety monitoring of HIV-infected children will be performed in phase IV studies...

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Concomitant use:

The frequency of fever in subjects vaccinated concomitantly with RTS,S/AS01E, DTPa/Hib, OPV and PCV (Synflorix) was almost twice as high as in RTS,S/AS01E schedules where Synflorix was not given concomitantly. This is mentioned in the SmPC. However, regarding the occurrence of fever it has to be noted that the frequency of Grade 3 fever was very low in the RTS,S/AS01E vaccine group where Synflorix was administered concomitantly.

Detection of cytosolic yeast catalase:

Amongst the 300 children tested for anti-human catalase antibodies with an in-house ELISA one child was positive one month post dose 3. The child reported 2 AEs (upper respiratory tract infection and respiratory tract infection) and no SAE. The child was followed-up for 3 years and showed no developmental anomalies so far. No further blood-draws were made.

3.7. Pharmacovigilance

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

3.8. Risk Management Plan

The PRAC considered that the risk management plan version 1.3 could be acceptable if the applicant implements the changes to the RMP as described in the PRAC endorsed PRAC Rapporteur assessment report.

The applicant implemented the changes in the RMP as requested by PRAC.

The CHMP endorsed the Risk Management Plan version 1.4 with the following content:

Safety concerns

Summary of safety concerns	
Important identified risks	Febrile convulsion
Important potential risks	Meningitis Hypersensitivity (including anaphylaxis) Potential Immune Mediated Diseases (pIMDs) Rebound effect Behavioural changes regarding usage of other malaria preventive measures
Missing information	Long term efficacy Impact/effectiveness P. falciparum strains replacement Plasmodium species replacement Fever upon co-administration with other EPI vaccines known to induce fever (DTPw-based combination vaccines and PCV) Immunogenicity of Mosquirix when co-administered with Measles and Yellow fever vaccines Cross immunisation against human catalase Vaccine efficacy and safety in subjects from regions other than sub-Saharan Africa Safety in HIV-infected children

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Pharmacovigilance plan

Study/activit y Type, title and category (1-3)	Objectives	Safety concerns addressed	Status	Date for submissio n of interim or final reports
Malaria-076 (200599) (interventional clinical trial, 3)	Primary objective: To describe the incidence of severe malaria in the long-term over a 3-year period (from January 2014 to December 2016) of follow-up pooled across transmission settings, in both age categories.	Rebound effect, long term efficacy	Ongoin g	4Q 2017
EPI-MAL-003 (115056) (observational cohort study, 3)	Co-primary objectives: To estimate the incidence of protocol-defined potential adverse events of special interest (AESI) and other adverse events leading to hospitalisation or death, in children vaccinated with RTS,S/ASO1 _E enrolled during the EPI-MAL-003 study. To estimate the incidence of aetiology confirmed meningitis in children vaccinated with RTS,S/ASO1 _E vaccine recipients, enrolled in the EPI-MAL003 study. Secondary objectives: Safety To estimate the incidence of aetiology confirmed and/or probable meningitis (final classification) in children vaccinated with RTS,S/ASO1 _E and children not vaccinated with RTS,S/ASO1 _E (enrolled during EPI-MAL-003 study). To estimate the incidence of probable meningitis (final classification) in children vaccinated with RTS,S/ASO1 _E (enrolled during EPI-MAL-003 study). To estimate the incidence of aetiology confirmed, probable and/or clinically suspected meningitis (final classification) in children vaccinated with RTS,S/ASO1 _E (enrolled during EPI-MAL-003 study). To estimate the incidence of aetiology confirmed, probable and/or clinically suspected meningitis (final classification) in children vaccinated with RTS,S/ASO1 _E (enrolled during EPI-MAL-003 study). To monitor trends over time of meningitis cases identified at site level (first line laboratory) in children vaccinated with RTS,S/ASO1 _E (aerrolled during EPI-MAL-002 or EPI-MAL-003 studies). To assess the potential association between vaccination and meningitis by comparing the incidence of aetiology confirmed meningitis in children vaccinated with RTS,S/ASO1 _E (enrolled during EPI-MAL-002 or EPI-MAL-003 studies). To assess the potential association between vaccination and meningitis by comparing the incidence of aetiology confirmed and/or probable meningitis in children vaccinated with RTS,S/ASO1 _E with the incidence of these events in children not vaccinated with RTS,S/ASO1 _E with the incidence of these events in children vaccinated with RTS,S/ASO1 _E with the incidence of feetiology confirm	Febrile convulsions, meningitis, pIMDs, anaphylaxis, fever when co- administered with other EPI vaccines known to induce fever (DTPw-based combination vaccines and PCV), vaccine effectiveness and impact and safety in HIV infected children	Draft protoco I	Q2 2023

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Study/activit y Type, title and category (1-3)	Objectives	Safety concerns addressed	Status	Date for submissio n of interim or final reports
	during EPI-MAL-002 or EPI-MAL-003 studies).			
	- To assess the potential association between vaccination and AESI, and other AE leading to hospitalisation or death by comparing the incidence of these events in children vaccinated with RTS,S/AS01 _E with the incidence of these events in children not vaccinated with RTS,S/AS01 _E (enrolled during EPI-MAL-002 or EPI-MAL-003 studies).			
	- To describe risk factors for AESI, other AE leading to hospitalisation or death, meningitis, and malaria in children vaccinated with RTS,S/AS01 $_{\rm E}$ and children not vaccinated with RTS,S/AS01 $_{\rm E}$ (enrolled during EPI-MAL-003 study).			
	- To describe the causes of hospitalisation (including AESI, other AE, meningitis and malaria) and death in children vaccinated with RTS,S/AS01 $_{\rm E}$ and children not vaccinated with RTS,S/AS01 $_{\rm E}$ (enrolled during EPI-MAL-003 study).			
	- To assess the risk of fever when RTS,S/AS01 _E is co- administered with other vaccines containing DTPw and PCV during the 7-day period following each administration.			
	- To assess the risk of febrile convulsions during the 7-day period and 1-month period following each dose of RTS,S/AS01 _E .			
	Effectiveness and impact: - To estimate the vaccine effectiveness (direct effect) and the impact (indirect, total and overall effects) of vaccination with RTS,S/AS01 _E on the incidence of any malaria and severe malaria (including <i>P. falciparum</i> malaria) diagnosed by rapid diagnostic test (RDT) and/or microscopy.			
	- To estimate the vaccine effectiveness (direct effect) and impact (indirect, total and overall effects) of vaccination with RTS,S/AS01 $_{\rm E}$ on:			
	 the prevalence of anaemia among hospitalised children. the incidence of all-cause hospitalisations and hospitalisations attributed to malaria (including <i>P. falciparum</i>). the mortality rate (all-cause mortality and 			
	deaths attributed to malaria [including <i>P. falciparum</i>]).			
EPI-MAL-005	Main objectives:	Behavioural changes	ongoing	2Q 2023
	- To obtain longitudinal estimates of <i>P. falciparum</i> parasite prevalence in order to characterise malaria transmission intensity in a standardised way at centres conducting the EPI-MAL-002 and EPI-MAL-003 studies before and after the introduction of the malaria vaccine RTS,S/AS01 _E in sub-Saharan Africa To obtain longitudinal estimates of the use of malaria control interventions in centres conducting the EPI-MAL-002 and EPI-MAL-003 studies before and after the introduction of the malaria vaccine RTS,S/AS01 _E in sub-Saharan Africa.	cnanges regarding usage of other malaria preventive measures and Plasmodium species replacement		
Ancillary study to EPI-MAL-	Main Objective:	<i>P. falciparum</i> strain	Planned	2Q 2023

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Study/activit y Type, title and category (1-3)	Objectives	Safety concerns addressed	Status	Date for submissio n of interim or final reports
005	To monitor the genetic diversity in CS sequences in parasite populations before and after the implementation of the vaccine in vaccinated and unvaccinated children	replacement		
Malaria-073 (200596) (interventional clinical trial, 3)	Key objectives - To demonstrate the non-inferiority of the antibody response to the CS antigen when RTS,S/AS01 _E is coadministered with YF vaccine and a combined measles and rubella vaccine versus RTS,S/AS01 _E administered alone. - To describe the antibody response to the human catalase after administration of a 3-dose course of RTS,S/AS01 _E	Immunogenicity of Mosquirix when co- administered with Measles, Rubella and Yellow fever vaccines, Cross immunisation against human catalase	Draft protoco I	1Q 2020
EPI-MAL-002 (115055) (observational cohort study, 3)	Key objectives - To estimate the incidence of AESI, of meningitis and of other AE leading to hospitalisation or death, in children, prior to implementation of RTS,S/AS01 _E	-	Final protoco I, planned to start	4Q 2019

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Important identified	d risks	
Febrile convulsions	Warning in section 4.4 to inform that vaccinees, especially those with a history of febrile convulsions, should be closely followed up as vaccine related fever may occur after vaccination. Listed in section 4.8	None
Important potential	risks	
Meningitis	Warning in section 4.4 to inform on the safety signal observed in the clinical trial.	None
Hypersensitivity (including anaphylaxis)	Contraindication in section 4.3 in case of previous hypersensitivity and warning in section 4.4 to have appropriate medical treatment and supervision.	None
pIMDs	None	None
Rebound effect	Warning in section 4.4 to inform that <i>Mosquirix</i> does not provide complete protection against malaria caused by <i>P. falciparum</i> and that the protection is waning during the period between the third and fourth doses and after the fourth dose. If symptoms compatible with malaria develop, appropriate diagnosis and treatment should be sought.	None
Behavioural changes regarding usage of other malaria preventive measures	Warning in section 4.4 to advise that the use of other malaria control measures recommended locally should not be interrupted	None
Missing information		
Long term efficacy	Warning in section 4.4 to inform that <i>Mosquirix</i> does not provide complete protection against malaria caused by <i>P. falciparum</i> and <i>andand</i> that the protection is waning during the period between the third and fourth doses and after the fourth dosedosedose. If symptoms compatible with malaria develop, appropriate diagnosis and treatment should be sought.	None
Impact/effectiveness	None	None
P. falciparum strains replacement	None	None

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Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Plasmodium species replacement	None	None
Fever upon co- administration with EPI vaccines	Statement in section 4.8 and 4.5 to inform of the higher risk of fever observed when <i>Mosquirix</i> is co-administered with PCV, DTPa/Hib and OPV.	None
Immunogenicity of Mosquirix when co- administration with Measles and Yellow fever vaccines	None	None
Cross immunisation against human catalase	None	None
Vaccine efficacy and safety in subjects from regions other than sub-Saharan Africa	Warning in 4.4 stating that data regarding the efficacy of Mosquirix are limited to children from sub-Saharan Africa	None
Safety in HIV infected children	Statement in 4.8 stating that data from clinical studies suggest that HIV-infected children are more likely to experience local and systemic reactogenicity compared to children of unknown HIV infection status.	None

3.9. Product information

3.9.1. User consultation

User testing of the Package Leaflet is not mandatory because the product is to be marketed outside the European Union.

3.10. Scientific advisory group consultation

The CHMP consulted the Scientific Advisory Group (SAG) Vaccines on the adequacy of the available safety, efficacy and vaccine co-administration evidence to support the recommendation for vaccination for RTS,S/AS01_E vaccine in both age groups (6-12 weeks and 5-17 months) evaluated in study malaria 055.

The SAG expressed the view that the RTS,S/AS01 $_{\rm E}$ vaccine can be considered sufficiently efficacious in both age groups to be recommended in the prevention of malaria, however it is unknown how the relatively low efficacy shown in clinical trial setting will translate into field settings with different transmission intensities.

There was a general agreement that the benefit observed in terms of prevention of clinical malaria could be of relevance mainly in regions with moderate and high malaria transmission rate, where a low efficacy vaccine may still have a substantial impact on the high numbers of cases. The experts were strongly of the view that post-approval studies should be undertaken to better understand the potential benefit of the vaccine, including against severe malaria.

The group was of the view that rebound is considered theoretical and likely depending on the transmission rates and on the efficacy of the vaccine over time. Nevertheless, the SAG considered important that the potential for rebound is followed up by appropriate post-approval activities.

The excess of febrile convulsions within 7 days post-vaccination in the age group 5-17 months was noted, however the SAG considered that the incidence observed is not much higher than observed for

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other vaccines (e.g. measles) and no imbalance in febrile convulsions between RTS,S/AS01_E and control was observed over a 30-day period post-vaccination or over longer follow-up.

The concerns around the observed excess rate of meningitis in vaccinated children were also discussed and it was concluded that a causative link is unlikely but at this stage cannot be excluded, hence there is the need for post-authorisation follow up.

The experts concluded that the available data do not allow formulation of recommendations on the timing when a fourth dose should be given, as only one single time point (18 months post Dose 3) was evaluated in the main clinical study.

Based on the available evidence, the SAG could not make any recommendation on the possibility that further doses should be administered after a fourth dose. It is not known how vaccine efficacy and immunogenicity will vary following multiple doses considering the lower anti-CS booster effect post-dose 4 (compared with post-primary). However, it cannot be excluded that it could be beneficial to administer additional doses in high transmission settings, so this possibility should be further investigated.

The SAG considered the data on HIV positive children to be broadly supportive for an indication for use in HIV-infected children from 6 weeks to 17 months of age.

4. Benefit-Risk Balance

Benefits

Beneficial effects

RTS,S/AS01E is the first malaria vaccine developed to target the pre-erythrocytic stage of *P. falciparum* for the age group 6 weeks to 17 months in which the burden of malaria is highest in sub-Saharan Africa. The primary endpoint in the main study 055 was vaccine efficacy against clinical malaria in infants 6-12 weeks and children 5-17 months of age. The study aimed to demonstrate VE against a background of routine control measures, most importantly ITNs (insecticide treated bednets). A 4-dose (0, 1, 2, 18-month) schedule studied and proposed, would allow its inclusion into the existing Expanded Program of Immunisation (EPI) visits.

Vaccine efficacy (VE) was shown against clinical malaria in both age groups. VE tends to be lower in regions with a high transmission rate and was higher in the older age group from 5-17 months. After the 4th dose, VE against clinical malaria increases in both age groups, however to slightly lower levels than after the 3 first doses. The decline over the next 12-15 months is similar to that seen during the 18 months post Dose 3. Overall, a VE against clinical disease (primary case definition 1) of ~40% is reached for the older age group and of ~27% for the younger age group over the complete observation time of 36-46 months post the third dose if a 4th dose is given.

Vaccine efficacy against severe disease (primary case definition 1) over the complete observation time is \sim 29% in the older and 20% in the younger age group if a 4th dose is given.

The number of clinical malaria cases averted over the entire study ranged from 205 to 6,565 (average 1,774) per 1,000 vaccinees for the older age group, while for the lower age group the number of clinical malaria cases averted over the entire study ranged from -30 to 3,406 (average 983) per 1,000 vaccinees when 4 doses of RTS,S/AS01E were administered.

In addition, four different mathematical models were developed to illustrate the potential long-term public health impact, which showed similar results for cases averted of clinical disease, severe disease and deaths due to malaria for a time-span of 15 years from implementation of the vaccination.

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The available data in HIV-infected subjects are limited; however they do support the extrapolation of benefit-risk considerations in uninfected children to HIV infected children.

When administered on a 0,1,2-months primary vaccination schedule, RTS,S/AS01E induced adequate anti-HBs immune response at least equal or better than that induced by a licensed hepatitis B vaccine.

Uncertainty in the knowledge about the beneficial effects

Anti-CS can be measured to describe immune responses to vaccination but there is no established correlate of protection. Some broad but not wholly consistent relationships between anti-CS and efficacy have been described, but the results cannot be used to predict VE.

VE was to be measured against a background of routine control measures, most importantly ITNs. ITN coverage was incomplete and often involved nets with holes. In optimised bite prevention settings additional benefit of RTS,S/AS01_E, could be lower.

The vaccine impact with seasonal malaria chemoprophylaxis has not been studied. Additional information will be available through planned surveillance studies.

In infants aged 6-12 weeks, vaccine efficacy was modest and short-lived. After a 4th dose some degree of vaccine efficacy against clinical malaria was restored but the available data point again to rapid waning.

In the group receiving the 4th dose no rebound effect was observed. The same is not true for the group that has not received a 4th dose and therefore an epidemiological study is planned to address this potential risk post-opinion.

In the older age group, additional data up to ~M49 (range 41-55 months; 21-35 months post-boost) suggest that VE is minimal or lost in the non-boosted group. A VE of ~40% is shown in this age group against clinical disease (primary case definition) and of ~35% against severe disease (primary definition) over the complete study time (46 months post 3rd dose) if a fourth dose is given. The VE tends to be lower in high transmission areas. The applicant's long-term public health impact model for this age group estimates a significant number of cases averted (clinical disease, severe disease and deaths due to malaria) for mid-high transmission areas over 15 years post introduction of the vaccination. However, it remains unclear whether lack of a fourth dose predisposes vaccinated children to more severe malaria after Month 20.

Although vaccine efficacy was demonstrated before and after a 4th dose, the long term benefit of the vaccine remains unclear.

In moderate to high transmission areas the VE modelled is higher as expected from the data of study Malaria-055 and a rebound might be possible. In low transmission settings rebound is not estimated. Natural immunity might be delayed by the vaccination but on the other hand older children are less prone to severe disease and deaths. The administration of a 4th dose (and its timing) does not show a high effect in the 15-year models.

Immune responses observed after a 4th dose were lower than those observed after the 3rd dose, which in the absence of a suitable control group to interpret the findings, suggest that the vaccine does not truly boost the anti-CS response. Further studies regarding alternative booster timepoints or fractional doses will address this issue.

Co-administration with a pneumococcal vaccine had a slightly negative impact on the anti-CS antibodies. Considering that pneumococcal vaccines were not administered in the pivotal study 055 in infants 6-12 weeks, the VE results could be even lower following co-administration. On the other hand

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the missing correlation of anti-CS antibodies and VE make a prediction of the real clinical effect highly speculative.

Also, in concomitant use with Synflorix the titre against serotype 18C was lower in the OPA and significantly lower in the ELISA. The clinical relevance is unknown.

Hyporesponsiveness on the immunogenicity level cannot be ruled out as the titres increased in both age groups after the 4th dose to levels not as high as after the third dose. VE was not higher after the 4th dose. This issue will be addressed in a planned study with earlier and annual doses. As long as the immunological effect of further doses is unknown they cannot be recommended even in view of a continuing risk of disease for the children.

Risks

Unfavourable effects

RTS,S/AS01E can be described as a relatively reactogenic vaccine, which is not surprising for a product that includes an adjuvant. The local reactogenicity however is not of a degree that would cause major concern. The systemic reactogenicity of RTS,S/AS01 $_{\rm E}$ is more problematical than its local effects. In both age cohorts in Malaria-055 the major issue was fever, which followed about one third of doses and in the older cohort increased in frequency between the first and the two subsequent doses.

The incidence of febrile convulsions within 7 days post-vaccination in the age group 5-17 months was higher in the RTS,S/AS01_E group, however no imbalance in febrile convulsions between RTS,S/AS01_E and control was observed over a 30-day period post-vaccination or over longer follow-up. As clinical data with other paediatric vaccines suggest reduction of immune response, prophylactic use of antipyretics pre-vaccination is not recommended.

An imbalance in meningitis cases was observed in the malaria vaccine group; however no clear relationship has been established but will be monitored in dedicated surveillance studies.

In HIV-infected subjects in Malaria-058 EPI vaccines were not given concomitantly. Local and systemic reactogenicity was greater for RTS, S/AS01 $_{\rm E}$ vs. the age-related controls and fever was very common. Solicited general reactogenicity increased with subsequent doses of RTS, S/AS01 $_{\rm E}$. A higher rate of SAEs within 30 days post-vaccination was observed. However, ultimately the risks do not preclude the possibility of use of RTS, S/AS01E in HIV-infected children.

Uncertainty in the knowledge about the unfavourable effects

The ASO1_E adjuvant is not a constituent of any licensed vaccine in any age group. Although the MPL component is already in ASO4, this adjuvant is not in vaccines given below 9 years of age. The lack of any post-marketing safety data for ASO1_E is a potential safety concern. Additional longer term safety will be gathered from safety studies as part of the pharmacovigilance plan.

The fever rates observed in Malaria-055 were without concomitant pneumococcal vaccines, which may be expected to be increased in light to the incidence of fever observed in a study that assessed co-administration of Mosquirix, PCV and DTPa-Hib (Malaria-063). In addition, data with pneumococcal vaccines relate to a single product and it is not known if rates would be similar with co-administration of RTS,S/AS01_E with other pneumococcal vaccines.

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Benefit-risk balance

Importance of favourable and unfavourable effects

In the absence of vaccination the risk of dying from falciparum malaria decreases with increasing age. RTS,S/AS01E does have at least some short-term efficacy and therefore has a potential to reduce morbidity and mortality in the age range most at risk of death.

So far no correlate of protection could be established beside many efforts. This leads to uncertainties regarding the observed (and expected) lower anti-CS titres in HIV+ children as well as the long-term protection.

The vaccine contains a novel adjuvant system that as expected is reactogenic. Febrile seizures are reported particularly in the 5-17 months age group.

Benefit-risk balance

Discussion on the benefit-risk balance

RTS,S/AS01E showed vaccine efficacy against clinical disease in infants and children aged from 6 weeks-17 months and vaccine efficacy is greater in older of age group. The vaccine provides higher benefit in low transmission rate regions. The protection is waning over time in both age groups. The timing of the 4th dose requires further consideration in both age groups; however at the present time the available clinical data do not support a timing different from 18 months post dose 3. The applicant committed to explore different schedules in planned studies.

The public health impact has been estimated by models from 4 different groups (including one conducted by the applicant) that show similar results for cases averted of clinical disease, severe disease and deaths due to malaria. Over the time of 15 years post implementation the VE modelled confirms the VE measured in the studies and also confirms the differences between the transmission areas. The additional effect of the 4th is also highest in the high-transmission areas.

It nevertheless will be of paramount importance to maintain or even extent the malaria prevention and therapy standards already in place as all data (studies as well as modelled) have taken this maintaining into account.

The safety profile observed is similar to other vaccines and a higher rate of febrile convulsions was seen in the older age group and is reflected in the product information. An imbalance in meningitis cases was observed in the malaria vaccine group; however no clear relationship has been established and will be monitored in dedicated surveillance studies.

5. Recommendations

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Mosquirix for active immunisation of children aged 6 weeks up to 17 months against malaria caused by *Plasmodium falciparum* and against hepatitis B is favourable.

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

The CHMP recommends that batch compliance control of individual batches be performed before release on the market in third countries.

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Conditions and requirements of the scientific opinion

Periodic Safety Update Reports

The scientific opinion holder shall submit the first periodic safety update report for this product within 90 calendar days after the data lock point of 04/03/2016. Subsequently, the scientific opinion holder shall submit periodic safety update reports for this product every year until otherwise agreed by the CHMP.

Conditions or restrictions with regard to the safe and effective use of the medicinal product Risk Management Plan (RMP)

The scientific opinion holder shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Scientific Opinion application and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

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