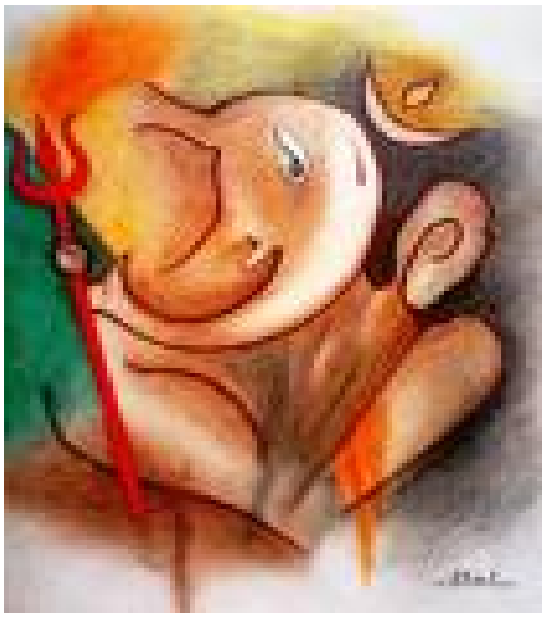
UNIVERSITAT DE BARCELONA

TESIS DOCTORAL

"Clinical development of RTS,S as a vaccine for the prevention of malaria in Mozambican children"

"Desarrollo clínico de la RTS,S como vacuna para la prevención de la malaria en niños Mozambiqueños"



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UNIVERSITAT DE BARCELONA

FACULTAT DE MEDICINA

"PROGRAMA DE DOCTORAT DE MEDICINA"

GRUP DE RECERCA: CENTRE DE RECERCA EN SALUT INTERNACIONAL DE BARCELONA – CRESIB

Thesis presented by **JAHIT SACARLAL** to aspire to the degree of Doctor in Medicine and Surgery by the University of Barcelona, under the direction of Professor **Dr. Pedro L. Alonso Fernandez** and Professor **Dr Carlos Ascaso Terrén.**

Tesis presentado por **JAHIT SACARLAL** para optar al grado de Doctor en Medicina y Cirurgia per la Universidad de Barcelona

Línea de investigación: Agresión biológica y mecanismos de la respuesta

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DEDICATED TO:

My parents

My brothers and their families

My sons and my wife

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OM, SREE GANESH !!!

PRESENTATION

The present doctoral thesis is presented followed the University of Barcelona recommendations on presentation of doctoral thesis by compendium of publications.

The thesis is presented as a collection of five articles and one editorial published in peer reviewed international journals on work conducted at the Barcelona Centre for International Health Research (CRESIB) and at the Centro de Investigação em Saúde de Manhiça (CISM), in Mozambique. Five articles are related to results of the RTS,S malaria vaccine candidate and one further article uses verbal autopsies to describe cause of death in children living in a rural area of Mozambique.

PUBLICATIONS THAT CONTRIBUTE TO THE DOCTORAL THESIS

1. **Sacarlal J,** Nhacolo AQ, Sigauque B, Nhalungo DA, Abacassamo F, Sacoor CN, Aide P, Machevo S, Nhampossa T, Macete EV, Bassat Q, David C, Bardaji A, Letang E, Saute F, Aponte JJ, Thompson R, Alonso PL.; A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique.; **BMC Public Health. 2009** Feb 24;9(1):67, PMID: 19236726

   Impact factor: 1.633

2. Alonso PL, **Sacarlal J,** Aponte JJ, Leach A, Macete E, Milman J, Mandomando I, Spiessens B, Guinovart C, Espasa M, Bassat Q, Aide P, Ofori-Anyinam O, Navia MM, Corachan S, Ceuppens M, Dubois MC, Demoitie MA, Dubovsky F, Menendez C, Tornieporth N, Ballou WR, Thompson R, Cohen J; Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children randomised controlled trial; **Lancet. 2004** Oct 16-22;364(9443):1411-20. PMID: 15488216

   Impact factor: 28.638

3. Alonso PL, **Sacarlal J**, Aponte JJ, Leach A, Macete E, Aide P, Sigauque B, Milman J, Mandomando I, Bassat Q, Guinovart C, Espasa M, Corachan S, Lievens M, Navia MM, Dubois MC, Menendez C, Dubovsky F, Cohen J, Thompson R, Ballou WR; Duration of protection with RTS,S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. **Lancet. 2005** Dec 10;366(9502):2012-8. PMID: 16338450

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4. **Sacarlal J,** Aponte JJ, Aide P, Mandomando I, Bassat Q, Guinovart C, Leach A, Milman J, Macete E, Espasa M, Ofori-Anyinam O, Thonnard J, Corachan S, Dubois MC, Lievens M, Dubovsky F, Ballou WR, Cohen J, Alonso PL; Safety of the RTS,S/AS02A malaria vaccine in Mozambican children during a Phase IIb trial. **Vaccine. 2008** Jan 10;26(2):174-84. PMID: 18069097

Impact factor: 3.377

5. **Sacarlal J,** Aide P, Aponte JJ, Renom M, Leach A, Mandomando I, Lievens M, Bassat Q, Lafuente S, Macete E, Vekemans J, Guinovart C, Sigaúque B, Sillman M, Milman J, Dubois MC, Demoitié MA, Thonnard J, Menéndez C, Ballou RW, Cohen J, Alonso PL; Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children, **JID, 2009 – in press**

Impact factor: 6.035

6. **Sacarlal J,** Lafuente S, Macete E, Alonso PL. Últimos avances en el desarrollo de una vacuna de la malaria. **Evid Pediatr.** 2008 Marzo, 4:2

Impact factor: unknown

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LIST OF ACRONYMS

ALRI	Acute lower respiratory infection
AMA	Apical membrane antigen
CISM	Centro de Investigação em Saúde de Manhiça
CMI	Cell mediated immunity
CMM	Crude maternal mortality
CSP	Circumsporozoite protein
DDT	Dichlorodiphenyltrichloroethane
DSMB	Data and safety monitoring board
DSS	Demographic Surveillance Sites
EPI	Expanded Programme on Immunisation
GBD	Global Burden Disease
GDP	Gross Domestic Product
GMT	Geometric mean titer
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HCM	Hospital Central de Maputo
HIV	Human immunodeficiency virus
IC	Informed consent
ICD	International Classification of Diseases
IPTi	Intermittent Preventive Treatment in infancy
IRS	Indoor Residual Spray

ITN	Insectsides Treated Nets
LM	Lourenço Marques
MDH	Manhiça District Hospital
MPL	Monophosphoryl lipid
MSP	Merozoite surface protein
MVI	PATH Malaria Vaccine Initiative
NANP	Tetrapeptide repeat motif
NMCP	National Malaria Control Program
OPD	Outpatient department
PATH	Programme for Appropriate Technology in Health
PE	Pre-eritrocytic
QS21	"*Quillaja saponária 21*": a triterpene glycoside purifield from the bark of Quillaj saponária.
RTS,S	Particulate antigen, containing both RTS and S proteins
SAE	Severe Adverse Events
SP	Sulphadoxine-Pyrimethamine
TBV	Transmission-blocking vaccines
VA	Verbal Autopsy
WHO	Word Health Organization
WRAIR	Walter Reed Army Institute of Research

TABLE OF CONTENTS

I. SUMMARY

1. Resumen (Castellano)

La malaria es causada por un parásito protozoario del género *Plasmodium*, de la familia *Plasmodium Plasmodiiade*, transmitido a los humanos a través de la picadura de un mosquito *Anófeles.spp* hembra infectado. Es uno de los mayores problemas de salud pública en el África Subsahariana y una de las causas más importantes de muerte en niños pequeños en la región.

La malaria es tanto una causa como una consecuencia de la pobreza. Recientes estimaciones sugieren que la malaria cuesta aproximadamente 12 mil millones de dólares americanos a los países endémicos de África, y por consiguiente, representa un constreñimiento mayor al progreso económico de dichos países.

Actualmente, África continúa a suportar la mayor carga de malaria del mundo, con aproximadamente 350-550 millones de episodios clínicos y entre 700.000 y 1.6 millones de muertes anuales. Así, representa el 80-90% de la malaria en el mundo, la mayoría en niños menores de 5 años y mujeres embarazadas. Las estimaciones de mortalidad por malaria son bastante imprecisas. Sin embargo, describir la contribución de la mortalidad por malaria en menores de 5 anõs, es un objetivo muy importante y pertinente. Ayuda a estimar el peso de la enfermedad, a priorizar los esfuerzos de control y de evaluar el impacto de la misma.

Sin embargo, en áreas rurales de África, como Mozambique, muchos niños nacen y mueren sin ser nunca registrados, y una proporción significativa de muertes suceden fuera de las estructuras de salud. En estos escenarios la única manera de estimar la causa de una muerte es a través de la entrevista a un testigo presente en el momento final de la enfermedad. Esto es llamado autopsia verbal (AV).

Las últimas décadas han sido testimonio del establecimiento de una red de centros a lo largo del continente, que incluye a vigilancia demográfica continuada de poblaciones definidas, los llamados Sistemas de Vigilancia Demográficos (DSS). Los DSS han sido creados para registrar prospectivamente informaciones demográficas, incluyendo los nacimientos y las muertes, para investigar los determinantes epidemiológicos y sociales, y para proveer con una plataforma en la cual se puedan llevar a cabo ensayos de intervención comunitaria a gran escala.

El primer artículo de esta tesis cuenta con datos provenientes del DSS de Manhiça en los últimos 10 años y describe las causas más frecuentes de mortalidad en niños menores de 15 años, entre 1997 y 2006. Durante este periodo, fueran registradas 10037 muertes, de las cuales 3730 fueron en niños menores de 15 años. Fueran conducidas 3002 entrevistas, correspondente al 80.4% del total de niños fallecidos en la area. Según los entrevistados, 54% de las muertes ocurrieron fuera de un centro de salud. En general, la malaria fue la responsable del 22% de

total de las muertes. Sin embargo, la frecuencia relativa era más alta en niños de 1 a 4 años de edad, contabilizando un 34% de todas las muertes lo que corresponde a una tasa de mortalidad de 6.1 muertes/1000 pyrs.

Esta última década ha presenciado un esfuerzo renovado en el estudio y el control de esta enfermedad. Nuevas herramientas están siendo disponibles, y el desarrollo de una vacuna es considerado un componente potencialmente importante para un mejor control.

RTS,S/AS02A GlaxoSmithKline (GSK) Biological es actualmente la vacuna candidata contra la malaria más avanzada en el mundo. RTS,S/AS02A tiene como objetivo específico la actuación en el estado pre-eritrocítrico del *P.falciparum* y ha mostrado conferir protección contra la infección experimental por *P.falciparum*, administrada via mosquitos infectados criados en el laboratorio, en voluntarios nunca expuestos previamente y contra la infección natural en adultos semi inmunes y niños inmunes.

Como parte del plan de desarrollo clínico, realizamos un estudio prueba de concepto, aleatorizado, controlado, de fase IIb de RTS,S/AS02A en 2022 niños Mozambiqueños de 1 a 4 años de edad viviendo en una área rural endémica. La fase inicial de doble ciego incluyó los meses de estudio de 0 al 8.5 y la fase de ciego simple del mes 8.5 al 21. La fase abierta incluyó desde el mes 21 al 45. Los niños fueron aleatorizados en una proporción de 1:1 de recibir RTS,S/AS02A o las vacunas control. La RTS,S/AS02A era administrada intramuscularmente en

la región deltoidea según un programa de 0,1 y dos meses. Los niños de 24 meses o mayores en el grupo control recibieron tres dosis pediátricas (0,5 ml) de Engerix BT™. Los niños de menos de 24 meses recibieron dos dosis pediátricas de Prevnar ™, en la primera y tercera vacunación y una de Hiberix ™ en la segunda vacunación. Los resultados confirmaron un buen perfil de seguridad y una buena respuesta inmunitaria. Durante el periodo de vigilancia, la eficacia de la vacuna (VE) $_{(2.5\text{-}45)}$ contra un primer o único episodio de malaria clínica fue del 30.5% (95% CI 18.9–40.4; p=<0.001) y contra malaria severa fue del 38.3% (95% CI 3.4 - 61.3; p=0.045).

Estos resultados resaltan la viabilidad del desarrollo de una vacuna contra la malaria y pueda ser, por consiguiente, un componente útil entre las estrategias para mejorar su controle.

2. Summary (English)

Malaria is caused by protozoan parasites of the genus *Plasmodium, Plasmodiidae family*, transmitted to humans through the bite of infected female *Anopheles*.spp mosquitoes. It is one of the major global public health problems and an important cause of death in young children in Subsaharina Africa.

Malaria is both a cause and a consequence of poverty. It best represents the paradigm of the vicious circle of disease and poverty. Recent estimates suggest that malaria alone costs about 12 billion US dollars to the endemic countries of Africa, and therefore represents a major constraint to economic progress.

Today, Africa continues to carry the brunt of the global malaria burden with around 350-550 million clinical episodes and between 700.000 and 1.6 million deaths annually, representing 80-90% of the all malaria deaths in the world, mostly in children younger than five years and pregnant women. Estimates of malaria mortality are rather imprecise. However, describing the contribution of malaria to under five mortality is a very important and relevant objective. It improves the precision of our burden estimate, and helps prioritize and guide control efforts, as well as evaluate its impact.

However in rural areas of Africa such as Mozambique, many children are born and die without ever being registered, and a significant proportion of all deaths take place outside health facilities. In this

scenario, the only way of estimating the likely cause of death is through an interview of a witness of the final illness. This is called a verbal autopsy (VA)

The last decades have witnessed the establishment of a network of centres throughout the continent that include continuous demographic surveillance of defined populations, the so called Demographic Surveillance Sites (DSS). The DSS have been established to record prospectively demographic information, including births and deaths, to investigate epidemiological and social determinants, and to provide a platform on which to undertake large-scale community interventions trials.

The first article of this thesis describes 10 year data from the Manhiça DSS on the most frequent causes of mortality in children under 15 years of age, between 1997 and 2006. During this period, 10037 deaths were recorded, of which 3730 were recorded in children under 15 years old. Verbal autopsy interviews were conducted for 3002 (80.4%) of these deaths. According to respondents, 54% of deaths occurred outside a health facility. Overall, malaria accounted for 22% of all deaths, but the relative frequency was highest in children 1 to 4 years of age, accounting for 34% of all deaths corresponding to a mortality rate of 6.1 deaths/1000 pyrs.

The last decade has witnessed a renewed effort in the study and control of this disease. New tools are becoming available, and the

development of a vaccine is considered a potentially key component for improved control.

GlaxoSmithKline (GSK) Biological's RTS,S,AS02A is a currently the world's most clinically-advanced malaria vaccine candidate. RTS,S/AS02A specifically targets the pre-erythrocytic stage of *P. falciparum*, and has been shown to confer protection against *P. falciparum* infection, delivered via laboratory-reared infected mosquitoes, in immunised malaria naive volunteers and against natural infection in semi-immune adult and immune children.

As part of the clinical development plan, we conducted a Proof of Concept randomised, controlled, phase IIb trial of RTS,S/AS02A in 2022 Mozambican children aged 1-4 years living in a rural endemic area of Mozambique. The initial double blind phase included study months 0 to 8.5 and the single blind phase from study months 8.5 to 21. The open phase included study months 21 to 45. Children were randomised in a 1:1 ratio to receive RTS,S/AS02A or the control vaccines. The RTS,S/AS02A was administered intramuscularly in the deltoid region according to a 0, 1, and 2 month schedule. Children aged 24 months and older in the control group received three paediatric doses (0.5 ml) of Engerix-B™. Children under 24 months received two paediatric doses of Prevenar™, administered at the first and third vaccinations and one dose of Hiberix™, at the second vaccination. Good safety profile and immunogenicity has been confirmed. Considering the entire follow up period of 45 months,

Vaccine Efficacy (VE)(2.5–45) against first or only episode of clinical malaria disease was 30.5% (95% CI 18.9–40.4; p=<0.001), and VE against severe malaria was 38.3% (95% CI 3.4 to 61.3; p=0.045).

These results highlight the feasibility of developing a malaria vaccine that may protect children against malaria, and may therefore be a useful component of the strategies to improve malaria control.

II. INTRODUCTION

1. History of malaria

Malaria is one of the ancient diseases afflicting maintain. Malaria seems to have been known for almost 5,000 years. Writings from the Nei Ching (The Canon of Medicine – 2,700 B.C.), Indus valley in northern India, (Vedic period 1500-800 B.C.) and Brahmanic (800 BC-100 A.C.) contain many references to repeated paroxysmal fevers associated with enlarged spleens, known as the "king of disease". This suggests the occurrence of tertian malaria (*P. vivax*) and quartan malaria (*P. malariae*) infection in India.(1). Solely much later time, around 1000 B.C *P. falciparum* had reached India.

The works attributed to Hippocrates (460 to 377 B.C.), leave no doubt about the presence of malaria in Greece in its benign tertian (*P. vivax*), quartan (*P. malariae*), and malignant subtertian (*P. falciparum*) forms. He recognized the seasonal pattern on its occurrence; late summer and autumn, and that the quartan fever was the less dangerous.

Malaria seems not to have reached mainland Italy until the second century B.C. Romans describe an association between cyclic fevers and vicinity to marsh areas and stagnant waters. However, under the prosperity of the Roman Empire (around 50 B.C. to 400 A.D.), by drainage, husbandry, and building development, malaria was excluded for several centuries from the Roman Campagna itself. Then, as the Empire declined

and the campagna fell into ruin, largely uninhabitable marshes known and feared by later generations.

These would have been hotbeds of *P. vivax* and especially *P. falciparum*. These long episodes of absence and then presence of malaria have been associated with corresponding rising and falling agricultural and economic prosperity. They reflect, yet again, the dependence of malaria on prevailing human activity and life-style.

The term "malaria", derived from the Italian word for "bad air", was adopted in the 18th century and it believed to be caused by gases emanating from swamps and stagnant waters. Later in some Century was introduced the term "paludisme" from French "palud" (marshes).

By the beginning of the Christian era, malaria was widespread around the shores of the Mediterranean, in southern and northern Europe, across the Arabian peninsular, and in Central, South, and Southeast Asia, China, Manchuria, Ukraine, Russia, Korea, and Japan up to 40° of northern latitude.

From the time of the voyages of Columbus until the mid-19thcentury, European trade and colonization in the tropics were marked by enormous losses of life from infectious disease. More deaths were registered as recognisably due to malaria than to any other disease, although in specific circumstances and locations the tolls from yellow fever and dysentery were often greater (2). In the worst locations, as on the coasts

of West Africa, mortality rates often exceeding 50% of a company per year of contact were the norm (3) .

In 1820 Palletier and Caventou isolated two active alkaloids, quinine and chichonine. From then on quinine sulphate, extracted from the bark of the Peruan chichonine tree, was commercialized worldwide (4).

Overall mortality rates fell rapidly to less than one-quarter of those before the introduction of quinine. It showed the extent to which the previous mortality had, indeed, been the result mainly of malaria.

The *Plasmodium* parasite was first discovered in human blood and described by Alphonse Laveran in Algeria in 1880. Later, in 1894 Patrick Manson suggested that malaria was transmitted from person to person by mosquitoes. In 1897 Ronald Ross, working in India, discovered a developing form of the parasite in a mosquito (5). In 1899 Grassi finally described the life cycle of malaria parasite in Anopheles mosquitoes (sporogonic phase), which was then confirmed by Manson in 1900 after doing experiments in human volunteers. With studies of Marchiafava and Bignami, culminate with description of all process of malaria transmission cycle, and the conditions responsible for disease dissemination.

At some time during the 19th century, malaria reached its global limits. In absolute numbers and in the proportion of the humanity now affected, malaria was exacting its highest ever toll of sickness and death. Well over one-half of the world's population was at significant risk from malaria. Of those directly affected by malaria at least 1 in 10 could

expect to die from it. The prosperity and well-being of all who lived within its reach were reduced deeply and usually catastrophically. Then, from toward the end of the 19th century and beginning of the 20th Century, throughout North America and most of the European parts, malaria entered an inevitable decline toward its present extinction in these regions due human health and rural environments and living conditions, and especially housing, were beginning to improve rapidly, so that contact between humans and vector mosquitoes was in decline.

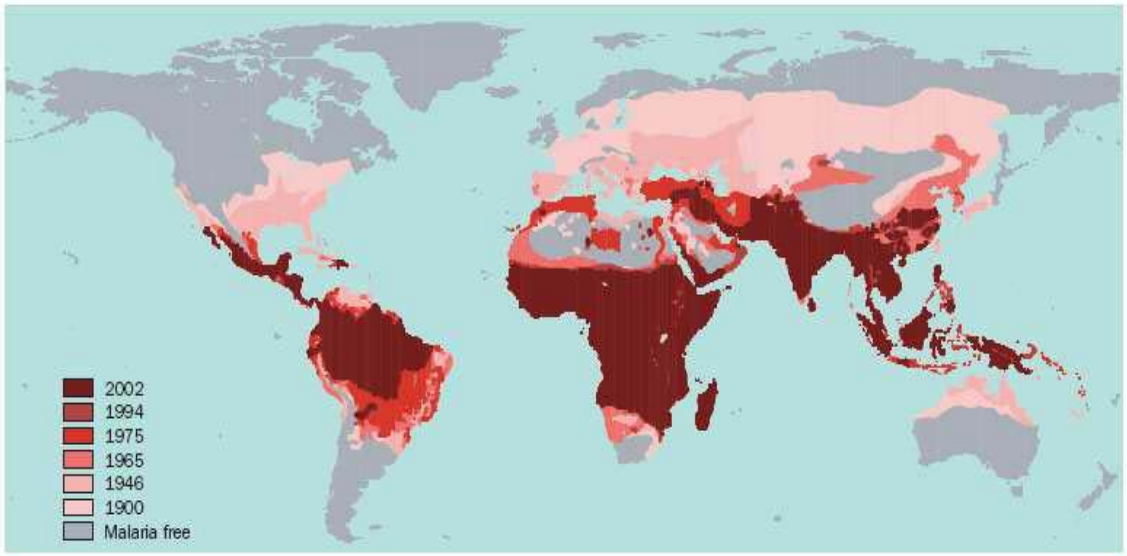

Figure 1 show the historical evolution of the global distribution of malaria (Hay 2004)

In the early 20th century, repeated untreated infections of *P. vivax*, and prolonged infections of *P. malariae,* would have contributed significantly to the mortality due to malaria in Asia and in parts of the Americas and Europe.

The development of the synthetic antimalarial drugs as chloroquine, proguanil, amodiaquine, primaquine and pyrimenthamine were began after the First Word War. Chloroquine, an effective, cheap and safe antimalarial, has played a major role in the control of malaria. It was widely used for decades in all malaria-endemic areas and was key to decrease malaria mortality in the second half of the 20th Century.

In sub-Saharan Africa the malaria control before Word War II, had little success, except in its southern and most marginal zones of transmission. Indeed, the malaria problems of Africa were, and are, of an altogether different type from those confronted anywhere else, both in human terms and in the biological factors that underlie African malaria transmission. Above all, the stability and intensity of malaria transmission in Africa presented two huge, actual or potential, problems.

The early 1950´s were characterized by hope and optimism for malaria eradication worldwide. The first large-scale "Malaria Eradication Program", was carried out by WHO during 1955-1969. The main goal was to eradicate malaria in vast areas across the word by vector control. Eradication efforts focused mainly on insecticide residual spraying with DDT, antimalarial drug treatment, and surveillance. (6)

By the end of the 1960s, malaria had largely disappeared from Europe and North America and had been formally declared malaria free by the World Health Organization(3).

However, in spite of this powerfully delivered effort, the anticipated goal, the eradication of malaria, was not achieved in many country of the word. The prohibitive economic and political costs of operating the Malaria Eradication Program were not sustainable. This combined with emerging resistance of the parasites and their vectors to the chemicals used to attack them, led, from the early 1970s, to resurgence of malaria transmission throughout southern Asia, Africa and the Western Pacific. Most damaging was the emergence of multidrug resistant *P. falciparum,* including total resistance to chloroquine. Since the mid-1960s, chloroquine resistance has spread inexorably outward across the tropics of Asia and the Western Pacific and into Africa in 1978 from a focus of origin in Southeast Asia (first reported in 1957). Despite high levels of resistance, chloroquine was used as first line-treatment in most countries until recently, and some authors claim that this was the cause of a rise in malaria mortality in Africa during the 80´s and 90´s decades (7).

National malaria control organizations were nevertheless operational in many African countries by the 1950s. It must soon have become clear, however, that whatever may have been being achieved elsewhere by reducing malaria transmission using DDT, rather little effect was served by this approach in sub-Saharan Africa, except in certain limited circumstances and mainly in its southernmost parts. Nevertheless, a determined optimism reigned among the advocates of "global malaria eradication program" and a policy of "intent to tackle the problem in

Africa upon an eradication basis" seems to have persisted until at least 1996. In the end, however, and as the goal of "malaria eradication" collapsed in most other malaria-endemic regions of the world, this aspiration for Africa also, and inevitably, died (6).

Overall, however, Africa had benefited during the era of "malaria eradication." It has benefited from a new availability of antimalarial drugs, especially chloroquine. Although drug distribution and access to treatment were relatively poor and largely uncontrolled, the effects during this period were real and evident. Malaria-related deaths in Africa showed evidence of relative (per head of population) decline from the 1950s to the early 1980s (8). But then, from some time in the 1980s, the downward trend in malaria-related mortality appears to have reversed. In relation to total population and in relation to deaths from other causes, the numbers of childhood deaths from malaria in tropical Africa are almost certainly rising again (8). The most likely cause is the spread throughout Africa of chloroquine resistant *P. falciparum* (8-10).

However In the last decade a significant increase in resources has been devoted to the development of new control tools, including drugs and vaccines, and an increased international commitment as the Bill and Melinda Gates Foundation and other partnerships and consequently decreasing of the malaria cases in last 5 years across Africa.

2. Epidemiology and burden of malaria

During the 20th century, economic and social development, together with anti-malarial campaigns has resulted in the eradication of malaria from large swathes of the planet, reducing the world's malaria-prone area from 50% to 27%. Nonetheless, given expected population growth, it is projected that by 2010, half of the world's population, nearly 3.5 billion people, will be living in areas where malaria is transmitted (11). The recent publication estimates that in 2007 2.37 billion people lived in areas at risk of *P. falciparum* transmission in 87 countries (12). Figure 2 show the global distribution of malaria.

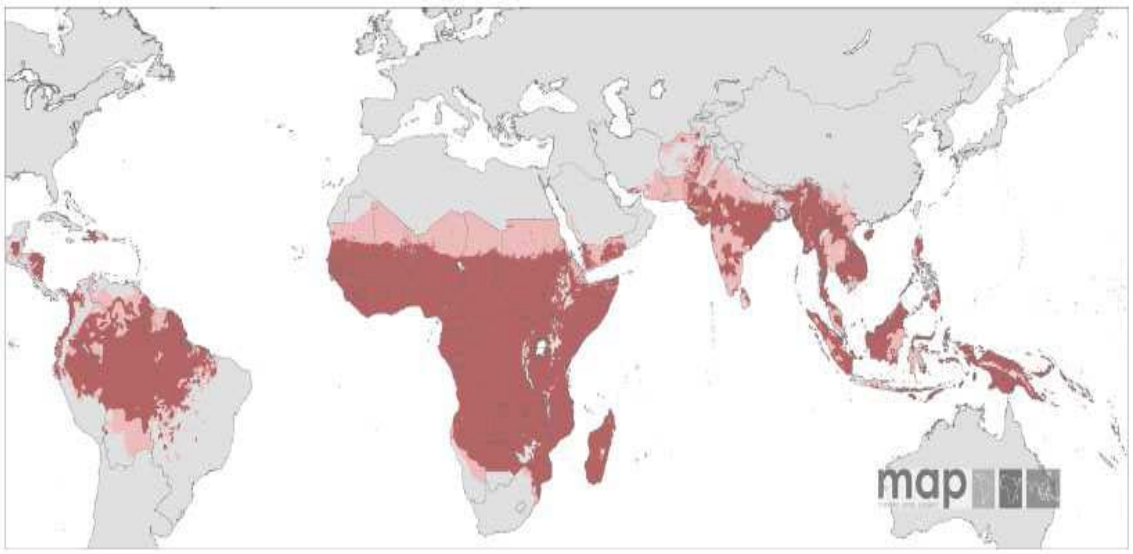

Figure 2. Map with the distribution of *P. falciparum* malaria risk. Dark red areas represent areas with stable malaria transmission, pink areas unstable transmission and grey areas no transmission (12).

The high-risk groups for severe malaria include pregnant women, young children, non-immune travellers, refugees, displaced persons and labourers entering endemic areas. Epidemics are often associated with

political upheavals, economics constrains and environmental problems (13, 14).

The intensity of malaria transmission fluctuate largely along with variations on geophysical characteristics, climatic, environment conditions, malaria mosquito vectors and parasite species and the socio-economic status, behaviour and distribution of human populations (15).

The methods commonly used to estimate the levels of malaria endemicity are based on the proportion of palpable enlarged spleens and the relative degree of splenomegaly that is "Spleen Rate", or based on the results of blood smear examination for malaria parasite presence, the "Parasite Rate". To estimate the degree of exposure and the intensity of the malaria infection transmission was estimated in indigenous population aged between 2 and 10 years old (15).

The method of enlarged spleen examination, was first introduced in India by Dempster in 1848, it is not an accurate measurement of the degree of exposure to the malaria infection; enlarged spleens are seen also in other parasitic disease, for instance intestinal leishmaniasis or Manson´s schistosomiasis.

Malaria endemicity levels classified as i) Hypoendemic, where the intensity of transmission is little, impact on the general population is unimportant and spleen rate (proportion of enlarged spleen in children aged 2-9 years) or parasite rate do not exceed 10%.

ii) Mesoendemic, usually find in subtropical areas, where of intensity of transmission varies depending to the local conditions and the spleen rate or parasite rate oscillation is between 11% and 50%. Normally the incidence is high in childhood and adolescence.

iii) Hyperendemic, were the intensity of transmission is high and seasonal, and the spleen rate or parasite rate is constantly over 50%. The spleen rate in adults is also high (over 25%). The malaria infection occurs in late infancy or early childhood age.

iv) Holoendemic, where an intense and perennial transmission occurs, resulting in the acquisition in early infancy a considerable immunity against severe malaria, resulting in substantial degree, particularly in the adults. The spleen rate or parasite rate is constantly over 75%, but the spleen rate in adults is low.

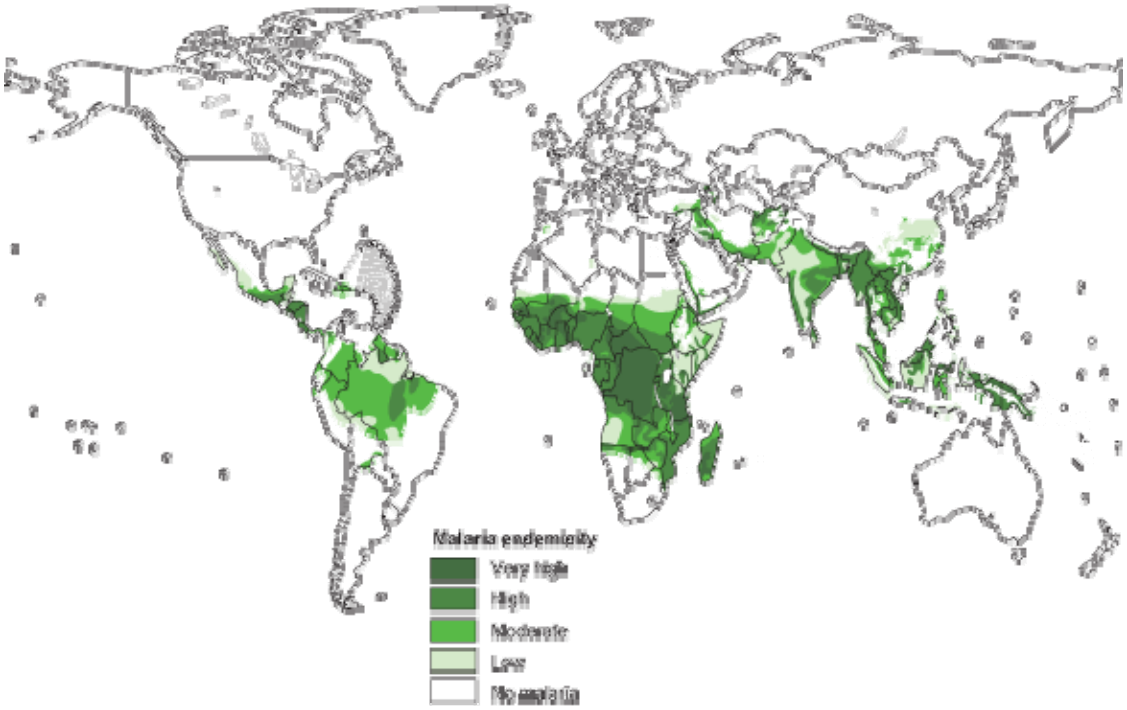

Figure 3: Distribution of malaria due endemicity

Quantifying malaria burden is a challenge because the disease may be asymptomatic, appear in an acute cerebral or deadly forms or some forms in between. Inadequate diagnosis and incomplete reporting make the estimates more imprecise in additional to the fact that most febrile patients and many deaths often in children take place at home without having a contact with the formal heath sector especially in Sub-Saharan Africa.

Manhiça data provides by the VA, estimate around 54% of the deaths occurred outside a health facility (16). Furthermore, many diseases in malaria endemic areas have symptoms that are similar to malaria or coexist with malaria.

The malaria burden is determined by a variety of factors related to the host, the parasite, the mosquito, the environment, the societal behavioural and political context, the economy, and the interventions to fight disease. However, there is an overall agreement that malaria is an actual leading cause of morbidity and mortality in South of Saharan Africa (17).

Today, Africa continues to absorb the brunt of the disease's impact with around 350-550 million clinical episodes and between 700.000 and 1.6 million deaths annually, mostly children younger than five years (11, 18-20). Globally, 9% of all deaths in children younger than 5 years are attributed to malaria, this proportion increasing to around 20% in sub-Saharan Africa

(21, 22). Figure 4 shows a map with the distribution of deaths from *P. falciparum* malaria in 2006.

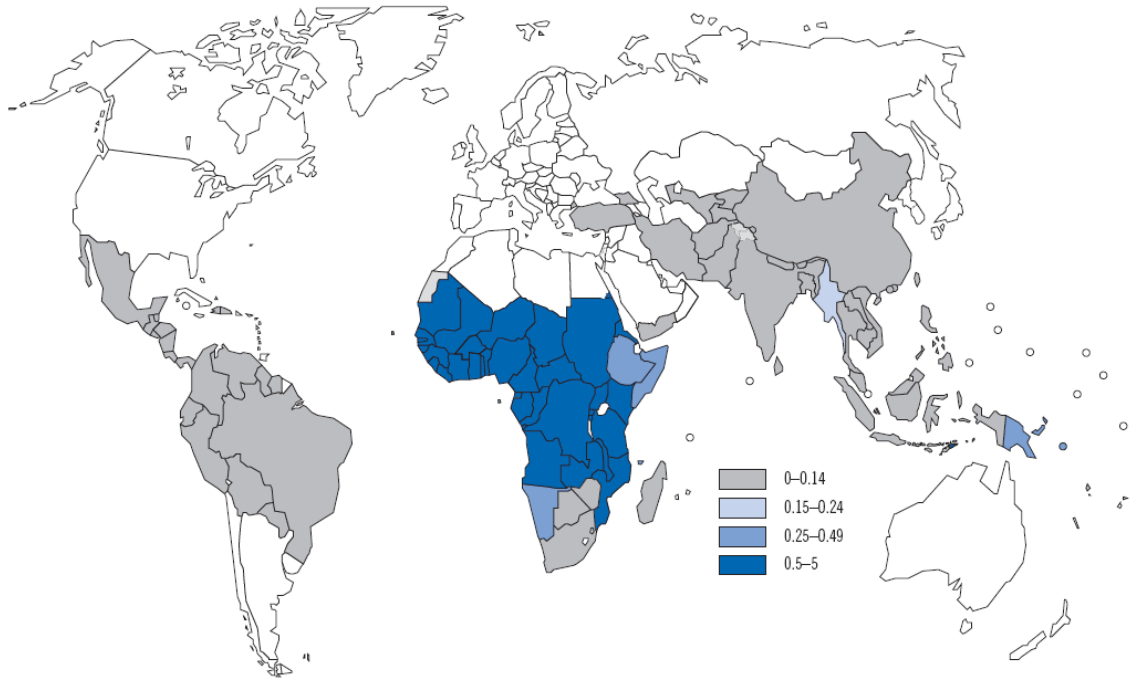

Figure 4: Estimated deaths from malaria per 1000 population, 2006

Verbal autopsy data from demographic surveillance undertaken between 1931 and 1997 have already been used to obtain a median estimate of malaria mortality across Africa of 7.3 per 1000 child-years, or about 800.000 deaths among children in 1995 (17).

Current estimates of the malaria mortality burden are largely based on observation made in Demographic Surveillance Sites (DSS) across different countries in Africa. The DSS have been established to record prospectively information of deaths, to investigate epidemiological determinants, and to provide a platform on which to undertake large-scale community interventions trials.

Recently, data from 1999-2002 were analyzed across 12 Demographic Sites in sub-Saharan Africa and Bangladesh to find cause-specific and age-specific mortality rates, using verbal autopsies. Causes of death in the African sites differ strongly from those in Bangladesh, where there is some evidence of a health transition from communicable to noncommunicable diseases, and little malaria.

Seven of the 12 sites have stable endemic malaria, which appears as the most of Africa, with exceptions being the two South African sites, where it is well controlled, and Butajira in Ethiopia. The Butajira site is an area of unstable malaria (both *P. falciparum* and *P. vivax*), with wide variations in transmission intensity associated with variations in altitude. The malaria rates we have provided for stable endemic areas are inflated by the inclusion of fevers of unknown origin. In the Butajira site, there were few deaths from malaria (0.6 deaths per 1000 person-years), malaria is seasonal and occurs in low-lying areas, and the disease could thus be excluded as a likely cause of most of the deaths from fever of unknown origin (FUO).

The death rate for FUO from Butajira (0.8 per 1000 person-years, included in the category "other") thus provides an estimate of the true rate of non-malaria FUO mortality in the areas with stable endemic *P. falciparum*. This procedure suggests that there is only a small inflation of the malaria mortality rates in these areas. The two South African sites,

which could also be used to provide estimate of non-malaria fever mortality, had negligible numbers of FUO deaths.

Most estimates tend to leave out indirect effects of malaria such as anemia, hypoglycemia, respiratory distress, low birth weight, and other complications. Recent estimates put the figure of indirect effects of malaria at 750.00-2.000.000 infant's deaths per year in Africa with anemia and low birth weight alone contributing up to 50% of the malaria morbidity and mortality among children under the age of 5 in Africa. More than 15% of the survivors are left with severe sequaelae and brain or neurological damage that may impair their development and learning capacity.

The incidence of malaria in much of Africa is increasing after collapse of "Malaria Eradication Program" for a variety of reasons: changes in agricultural practices, armed conflicts, migration of refugees, increasing drug resistance to conventional anti-malarial drugs, and insecticide resistance of the *anopheline* mosquito vectors. It is estimated that the number of cases of clinical malaria will more than double over the next 20 years without effective control.

However in last 5 years, with more effective malaria control tools are mainly based on the rapid diagnosis and prompt treatment of cases with effective drugs, the reduction of host-vector contact through Insecticide Treated Nets (ITNs), and vector control with Indoor Residual Spraying (IRS) , showed decrease of the malaria cases in many areas of Africa .

The burden of malaria at the country level correlates closely with the rate of economic development even after adjustment for confounding factors, indicating that malaria is an important constraint on economic progress (18).

3. The epidemiology and burden of malaria in Mozambique

Malaria is one of the diseases afflicting the Mozambican inhabitants due to a multitude of factors such as climatotological/ environmenta (favourable temperatures and rain patterns, abundant breeding sites) and socio-economical (poverty related improper housing/shelter, unaffordable preventive means).

Malaria is endemic across the country, varying between mesoendemic and hyperendemic areas. Transmission is perennial, with peaks during and after rainy seasons (January to April). In last then years, the number of malaria cases has increased throughout the country, particularly in the rural regions, where approximately 73% of the Mozambican population lives.

The first description of malaria in Mozambique, carried out in 1846, by Jacques de Salis entitled "Draft on the illness of the African eastern cost"(23). In this document, the author describes the relationship between the illnesses and environment conditions and type of soil. When describing the region´s climate, the author emphasized the alternation between dry and rainy seasons, hot temperatures, predominant southern winds and

the lower altitude that are typical characteristics of the Mozambican costal line. Also referred that fevers were the most important illness and believed to come from swamps. Treatment of fevers consisted of infusions, including lemon syrup, cooked barley and other seasonings containing sulphured quinine.

Malaria control is nowadays mostly based on three strategies, a) drugs for treatment of cases and prophylaxis, b) reducing human-vector contact and c) vector control (24).

In Mozambique and as other countries in word the history of malaria control began during the early 1900s, with the discovery of synthetic antimalarial drugs and the implementation of anti-larval activities.

Later, the discovery of the insecticidal effect of DDT and dieldrin has increased malaria control activities worldwide.

However, some statistics compiled in the main hospital in the city of Lourenço Marques (LM) during the period between 1900 and 1909, showed an increased number of fever cases admitted to the hospital and consequently deaths due the treatment of fever was palliative. Malaria and malaria-related anaemia were the main causes. (25)

In May 1907, in Lourenço Marques city the first antimalarial activities were initiated and consisted of elimination of breeding places and application of larvacides such as residual oils.(26)

Between January 1937 and March 1938, was carried out in Maputo region southern of the Umbeluzi River, the first epidemiological study to

categorize the endemicity levels. Children less than 15 years age and born in the area included in this study. For determine the endemicity levels of malaria infection in the Maputo region was the methodology used as microscopy examination of blood-slide for Plasmodium species identification, density determination and enlarged spleen rates.

In 1960, the WHO met with the Portuguese government in order to design a pre-eradication plan. This comprised an area of the Save River, with the following objectives: to block malaria and develop an inter-governmental malaria control plan with neighbouring countries. From 1961 to 1973 house-to-house spraying with DDT was carried out.

Presently malaria accounts for an estimated 44% of all outpatient attendance, 57% of paediatric admissions and 29% of all hospital deaths in rural and provincial hospitals. Malaria also contributes to maternal mortality; the crude maternal mortality (CMM) ratio was vary between 995 to 849/100,000 live births in 2001 to 2003, respectively at the Maputo Central Hospital (HCM) (27). *P. falciparum* is responsible for over 90% of all malaria cases (National Malaria Control Program - NMCP, 2002).

The last comprehensive characterize the malaria transmission intensities and to estimate the disease burden in Mozambique was carried out in 24 districts randomly selected between February 2002 and April 2003. A total of 8,816 children ageds below 10 years old were enrolled a house-to-house survey. Finger prick and blood collection were performed to prepare thick and thin films for malaria parasite species identification,

density and haemoglobin concentration. Axillary temperature was also measured. Prevalence of infection, parasite density and anaemia were estimated for age groups category in each region/stratum. Malaria parasite prevalence was 58.9% (5.190/8.816), the majority of blood smears 52.4% (4,616/8,816) were due to *P. falciparum* and geometric mean parasite density was 1,211 parasites/µl (95% CI, 1,141 – 1.286). Gametocytes prevalence, only for *P. falciparum* was 5.6% (518/8,816). The burden was highest in the northern regions and in the coastal stratum. Parasite infection and geometric mean parasite density peaked during the second year of life and thereafter decreased with increasing age. Mean haemoglobin concentrations was 9.9 g/dl (95% CI 9.5 – 10.2). Anaemia prevalence was 69.8% (6.257/8.816) and among anaemic children 11.5% (743/6.257) were severely anaemic (28).

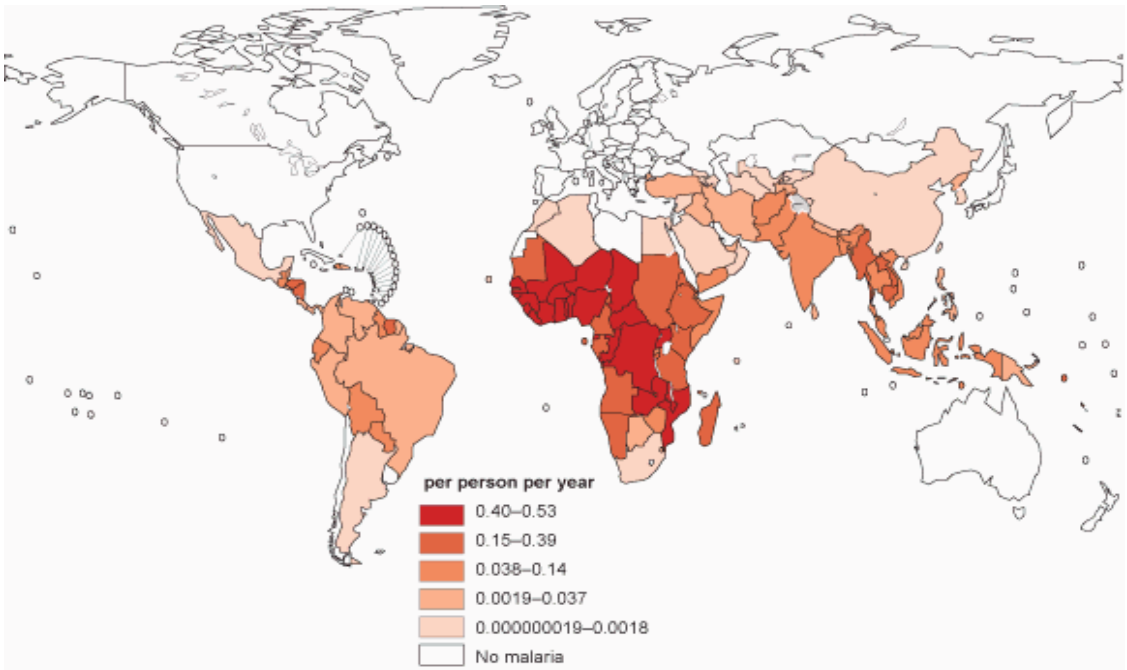

Figure 5: Estimated incidence of clinical malaria episodes caused by any species resulting from local transmission, country level averages, 2004
Source: http://www.rbm.who.int/wmr2005/html/map3.htm

4. The economic and social burden of malaria

Malaria is both a cause and a consequence of poverty. It best represents the paradigm of the vicious circle of disease and poverty. Malaria is most prevalent in Africa south of the Sahara and in Southern Asia with 58% of malaria deaths occurring in the poorest 20% of the word population. Reaching the poor with effective health interventions, including malaria, is a challenge.

For the most endemic countries in the region, the high burden of malaria is responsible for an estimated average annual reduction of 1.3% in economic growth, US$12 billions loss, and serious social disruptions.

Recent studies to estimate the economic burden of malaria in endemic countries showed that the direct cost of a single episode of malaria to a household was US$ 6.87 in Ghana, US$ 4.8 in Uganda and US$ 4.5 in Mali. In Nigeria, it cost about US$ 1 to treat a malaria episode by self-medication, and about US$ 10 to treat it by the use of orthodox health care provider when admission is not involved.

Estimates of the burden of malaria on the overall economies of Ghana, Mali, Nigeria and Uganda reveal that malaria impedes economic growth in the countries ranging from 0.067% in Uganda to as much as 3.8% in Nigeria. The Gross Domestic Product loss due to malaria in Uganda in 2003 was equivalent to US$ 11 million, which is a very substantives loss to the economy for a country like Uganda. This estimated GDP loss due to

malaria translates into US$ 0.43 per capita, which is about 5% of health per capita expenditure.

The same studies in Ghana, Nigeria, Mali, Uganda and Guinea found that indirect cost make up more than 70% of total household malaria costs. These studies found that sick adults last 1 to 7 days per malaria episode, depending of severity. The value of days lost due to malaria is estimated at US$ 8.92 and US$ 8.84 Uganda.

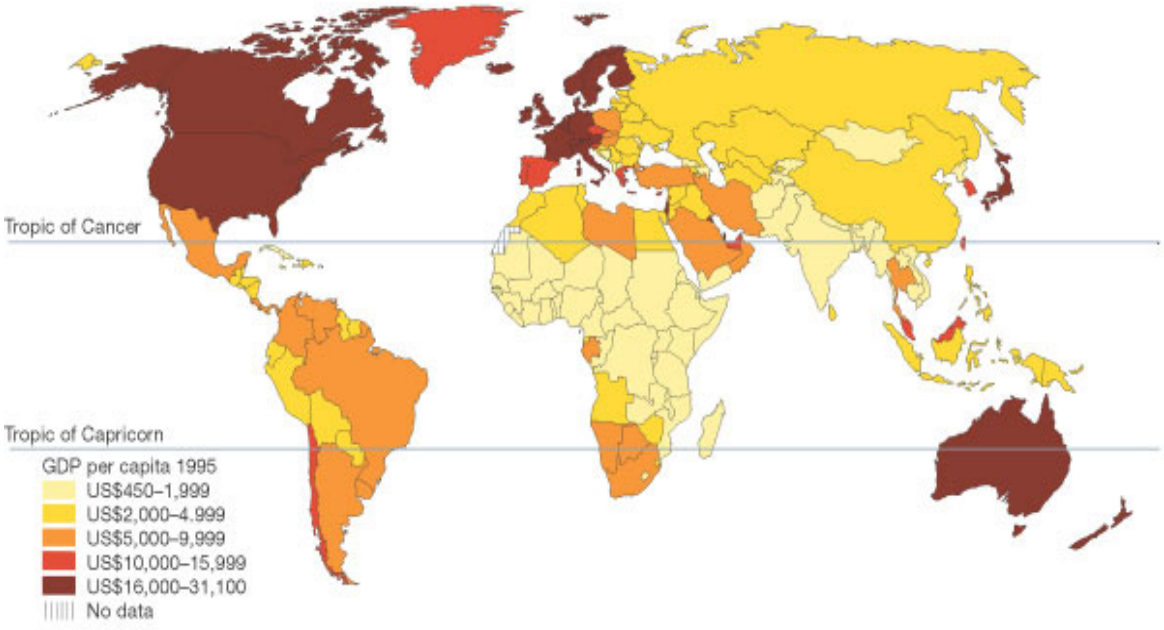

Figure 6: GDP per capita in 1995

5. The malaria parasite

Malaria is caused by protozoan parasites of the genus *Plasmodium, Plasmodiidae family*, which are transmitted to humans through the bite of infected female *Anopheles* spp mosquitoes. There are four species of *Plasmodium* that affect humans, *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Of these four parasites, *P. falciparum* is the major cause of severe morbidity and mortality.

Today, *P. malariae* has lost whatever predominance it may once have had and *P. vivax* and *P. falciparum* are the most commonly encountered malaria parasites.

P. vivax is still found sporadically in some temperate regions, where it was widely prevalent but the clinical presentation is usually mild and rarely results in death. It remains, however, very common throughout much of the tropics and subtropics. Because of the temperature limitations on its transmission by its mosquito vectors,

P. falciparum is normally present only in tropical, subtropical, and warm temperate regions. In the tropics today, *P. falciparum* remains widely prevalent. Is the most virulent of the four and is responsible for most of the severe morbidity and mortality worldwide.

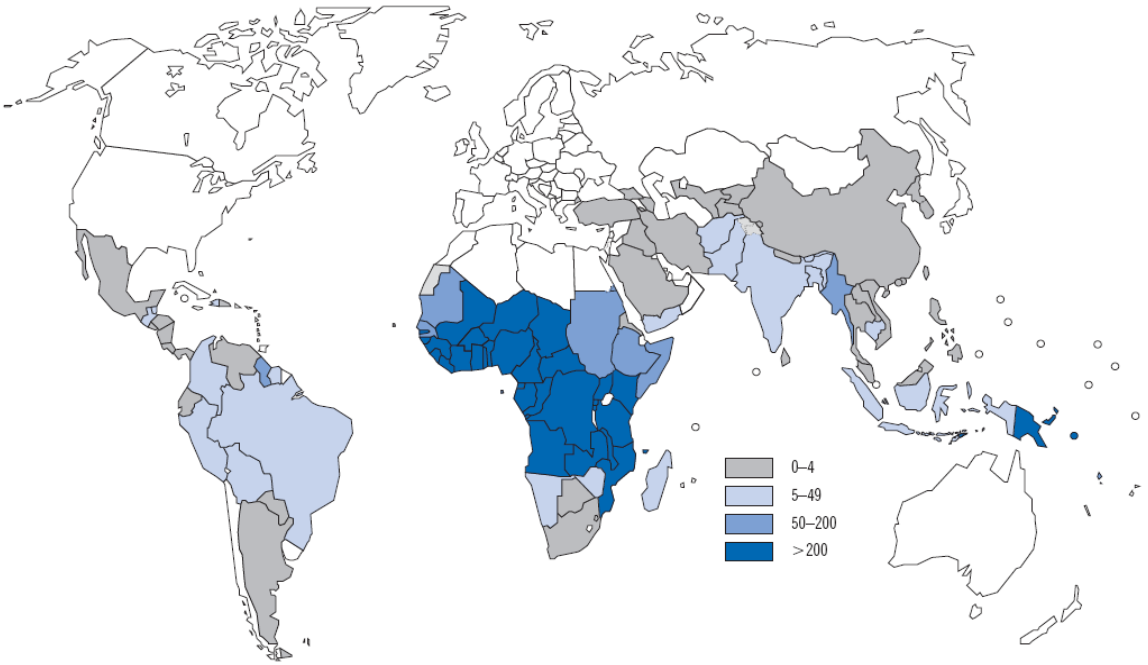

Figure 7: Estimated incidence of malaria per 1000 population, 2006

The fourth human malaria parasite is *P. ovale*, which, like *P. vivax*, is the agent of tertian malaria and which, also like *P. vivax* malaria today, carries a very low risk of fatal outcome. *P. ovale* has the most limited distribution of all the malaria parasites of humans. While it is prevalent throughout most of sub-Saharan Africa, it is otherwise known to be endemic only in New Guinea and the Philippines (29)

P. knowlesi, a species affecting long-tailed macaque monkeys, was detected for the first time in humans in Malaysia several years ago (30-32). It was already known that *P. knowlesi* was infectious to humans by inoculation of infected blood, but this was the first time a naturally acquired infection was detected in humans.

5.1. The life Cycle of the *P. falciparum* Parasite

The life cycle of parasites of the family *Plasmodidae* is characterized by two multiplication´s phases. The *schizogony,* an asexual phase, occurring in the vertebrate host, and *sporogony* a single sexual multiplication taking place in the invertebrate host, a mosquito of *Anopheles* species. The life cycle is illustrated in figure 8.

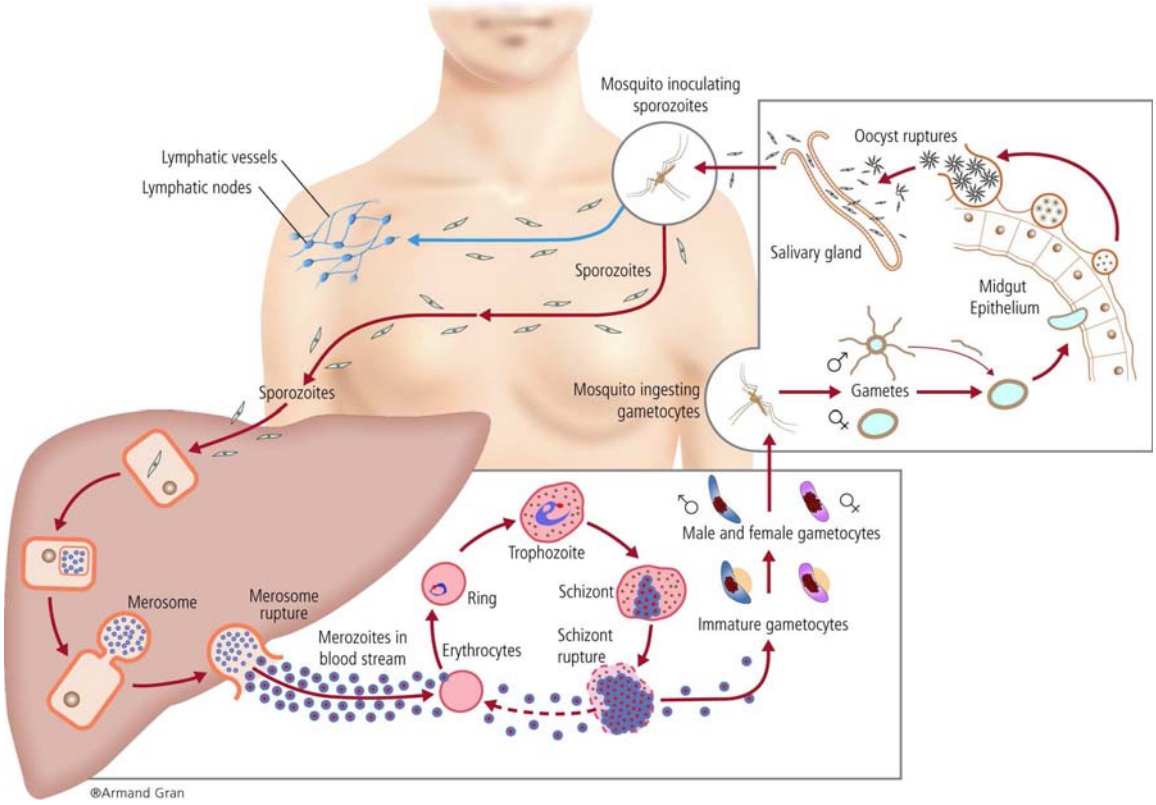

Figure 8: Life cycle of *P. falciparum*

5.1.1. In the Human

When an infected female *Anopheles spp.* mosquito bytes a human, approximately 100 Plasmodia sporozoites contained in its salivary glands are injected into subcutaneous tissue.

After circulating in the blood stream for less than an hour, they infect the hepatocytes. The mecanisms by which the sporozoites enter the hepatic cells are not clear, although it has been suggested that hepatic cells receptors to the principal surface protein on the sporozoite, play an important role. Here they grow, multiply and develop directly into schizonts. The schizonts in the liver are referred to as pre-eritrocytic (PE) schizonts.

New studies have shown that sporozoites are injected into the skin, from where a proportion are drained by lymphatics to lymph nodes, where most sporozoites are degraded, and the rest enter blood capillaries and reach the liver (33).

After one to two weeks, when mature these and liver cells rupture and free tens of thousands merozoites to the blood stream (each sporozoite produces 10 000 to 30 000 merozoites). In order to survive, these must enter red cells (erythrocytes) within few minutes of being released from schizont. Most of them enter red cells in the sinusoids of the liver. A proportion is phagocytosed and destroyed.

To infect the red cells, the merozoites attach to the red cells by special organelles, which bind to specific glycophorin protein receptors on the red cell membrane. The membrane becomes indented and the parasite enters. This starts a cycle in blood (erytrocytic cycle) which takes 36-48 hours to complete. In this phase, the merozoites develop into a trophozoite within a vacuole formed by the internal membrane of the host

red cell. The trophozoites are concave disk, they appear ring-shaped in stained preparations because the centre of the disk is very thin and does not stain. The ring of cytoplasm contains the organelles. The nucleus is clearly visible as a single or sometimes double chromatin dot. The trophozoite feeds on haemoglobin by ingesting small amount of red cells cytoplasm. The end product of haemoglobin breakdown produces malaria pigment (haemozoin). It accumulates as brow-black granules in the trophozoites. As the trophozoite grows it becomes globular in form and the vacuole membrane enlarges. Red cells containing late stage thophozoites and schizontes of *P. falciparum* develop small knobs on their surface (visible by electron microscopy). When the trophozoite is fully developed, its nucleus begins to divide, followed by a division of cytoplasm. This process (schizogony) takes place in capillaries of internal organs and results in the formation of schizont. A mature *P. falciparum* schizont (containing 8-32 merozoites and malaria pigment) ruptures from the red cells, releasing merozoites, malaria pigment and toxins into the plasma. The entry of toxin metabolites into the blood circulation of the host causes fever and a "malaria attack".

The merozoites that scape destruction by the host´s immune system invade new red cells. They develop into trophozoites and schizonts and so cause further red cells to be destroyed. After several erytrocytic cycles, some of merozoites enter red cells and instead of developing into schizonts they follow a different path and become gametocytes. These

are thought to form in response to developing immunity (raise antibody levels), lack of nutrients, or an accumulation of metabolites or parasitic debris, and their presence in the blood is not proof of an active infection.

5.1.2. In the Mosquito

For the lifecycle to be continued, the gametocytes need to be ingested by a female Anopheles mosquito. In the stomach of the mosquito, gametocytes rapidly divide into a number of male gametes each with a flagellum (exflagelation). These become free and following contact and entry into a female gamete, fertilization occurs. The male nucleus fuses with the female nucleus and zygote is formed. The latter develops into a motile ookinete, which penetrates the stomach wall of the mosquito, forming an oocyst. Inside the oocyst and spread to all parts of the mosquito, particularly to the salivary glands, ready to be transmitted when the next insect takes a blood meal. The development of *P.falciparum* in *Anopheles* mosquito (sporogony) takes one to two weeks.

The Anopheles gambiae complex and Anopheles funestus group, include the most efficient mosquito vectors of human malaria, implicated in malaria infection transmission across several regions of tropical Africa (34-36).

6. Clinical Manifestations of Malaria

The presentation of clinical manifestations of malaria is wide and depends on multiple factors that age, intensity of malaria transmission as well as pregnancy(37). This can vary from an asymptomatic infection to mild clinical malaria to severe malaria to death, depending on host and parasite characteristics and social and environmental factors and immune status acquired in different epidemiological settings (37, 38). The other factor is the rapid seeking of treatment and the availability of effective antimalarial drugs. Host genetics also play a role, as some polymorphisms protect against severe disease, being thalassemia, sickle cell trait, glucose-6-phosphate dehydrogenase deficiency, haemoglobin C, ovalocytosis and red blood cell duffy-negativity the most prevalent ones.

The first symptoms of malaria are nonspecific and similar to the symptoms of a minor systemic viral illness. They comprise: headache, lassitude, fatigue, abdominal discomfort and muscle and joint aches, followed by fever, chills, perspiration, anorexia, vomiting and worsening malaise. This is the typical picture of uncomplicated malaria. Residents of endemic areas are often familiar with this combination of symptoms, and frequently self-diagnose.

Malaria is therefore frequently overdiagnosed on the basis of symptoms alone. Infection with P. vivax and P. ovale, more than with other species, can be associated with well-defined malarial paroxysms, in

which fever spikes, chills and rigors occur at regular intervals. At this stage, with no evidence of vital organ dysfunction, the case-fatality rate is low (circa 0.1% for P. falciparum infections – the other human malarias are rarely fatal) provided prompt and effective treatment is given. But if ineffective drugs are given or treatment is delayed in falciparum malaria, the parasite burden continues to increase and severe malaria may ensue. A patient may progress from having minor symptoms to having severe disease within a few hours. This usually manifests with one or more of the following: coma (cerebral malaria), metabolic acidosis, severe anaemia, hypoglycaemia and, in adults, acute renal failure or acute pulmonary oedema. By this stage, mortality in people receiving treatment has risen to 15–20%. If untreated, severe malaria is almost always fatal.

The nature of the clinical disease depends very much on the pattern and intensity of malaria transmission in the area of residence, which determines the degree of protective immunity acquired and, in turn, the clinical disease profile.

Where malaria transmission is "stable" – meaning where populations are continuously exposed to a fairly constant rate of malarial inoculations – and if the inoculation rates are high – entomological inoculation rate (EIR) >10/year –, then partial immunity to the clinical disease and to its severe manifestations is acquired early in childhood. In such situations, which prevail in much of sub-Saharan Africa and parts of Oceania, the acute clinical disease described above is almost always confined to

young children who suffer high parasite densities and acute clinical disease. If untreated, this can progress very rapidly to severe malaria. Adults may develop mild symptoms.

In stable and high-transmission areas, adolescents and adults are partially immune and rarely suffer clinical disease, although they continue to harbour low blood-parasite densities. Severe malaria illness is common amongst younger children, between 6 months and 2 to 3 years of age, becoming however, less frequent in older children (37, 39, 40). Immunity is reduced in pregnancy, and can be lost when individuals move out of the transmission zone.

In areas of unstable malaria, the situation prevailing in much of Asia and Latin America and the remaining parts of the world where malaria is endemic, the rates of inoculation fluctuate greatly over seasons and years. EIRs are usually <5/year and often <1/year. This retards the acquisition of immunity and results in people of all ages, adults and children alike, suffering acute clinical malaria, with a high risk of progression to severe malaria if untreated (37).

Epidemics may occur in areas of unstable malaria when inoculation rates increase rapidly. Epidemics manifest as a very high incidence of malaria in all age groups and can overwhelm health services. Severe malaria is common if effective treatment is not made widely available.

Thus in areas of high transmission, it is children who are at risk of severe malaria and death, whereas in areas of low or unstable transmission, all age groups are at risk.

7. Malaria control strategies and recommendation

The strategy for malaria control consists of three basic technical elements: (I)To provide early diagnosis, prompt and effective treatment, through health care services. (II)Implementation of selective preventive measures to reduce man-vector contact.Insecticide residual spraying is the backbone of the vector control interventions. (III)Community health education and social mobilization; to improve heath awareness in the community.

Implementation of such a control strategies was loaded by various problematic situations which eventually led to the breakdown of the heath services. Furthermore, the widespread of parasites resistant strains to the available anti-malarial drugs, resulted in a deficient case management, particularly at the periphery of the heath systems where a significant proportion of people are exposed to malaria parasites.

In addition, the widespread use of insecticides (in malaria control, agriculture and other pest control) led to a selection of resistant mosquito vectors, which became a challenge to the vector control interventions, especially insecticide residual spraying.

The complexity of the dynamic of malaria transmission warrants the development and deployment of specific control strategies for specific

ecological settings. The interventions should focus on the most afflicted groups (children, pregnant women, immuno-deficient and elderly), in areas of risk, mainly rural areas.

Community heath education and social mobilization were the basic elements to improve good heath practices. However, to reach remote communities in rural areas with heath information and education materials is still a challenging task.

Integrated vector management approach should include indoor residual spraying, insecticide treated nets and environmental management.

Expansion of the health network, trained heath workers for an accurate diagnosis and effective anti-malarial drugs for prompt treatment is crucial.

Monitoring and evaluation are important tools to inform planning and advocacy process. Adequate and persistent funding is an important component for the success of any malaria control interventions.

In Mozambique, the malaria cases were treated according to the national guidelines. At the time of the studies (2003-2007), Sulphadoxine-Pyrimethamine (SP) plus Amodiaquine was the first line treatment for ambulatory patients and Co-Artem® the second line treatment. Patients who were admitted to the day-care unit and later to the wards received parenteral quinine, followed by SP.

8. Immune mechanisms against malaria

Natural acquired immunity to malaria is complex and not yet been completely elucidated. Usually naturally acquired immunity has been divided into "anti-disease immunity" and "antiparasite immunity" (41). Anti-disease immunity develops more quickly, decreases the frequency of clinical episodes, protects against severe disease and diminishes susceptibility to parasitaemia.

Anti-parasite immunity takes longer to develop and decreases the density and frequency of parasitaemia. However, clinical malaria and parasitaemia density follow the same age pattern, with parasitaemia density decreasing as the incidence of clinical malaria goes down, even if parasitaemia prevalence declines later. This argues and supports the idea that actually these two immunities are the same and develop at the same time (42).

The velocity at which the naturally acquired immunity is developed is correlated with the transmission intensity. Children younger than two years experience the highest incidences of clinical malaria in stable and intense transmission areas, decreasing rapidly with age. In areas with a unstable or low transmission the age pattern is shifted to older ages, and cerebral malaria is more likely to develop with high mortality rates.

Children born in an endemic area acquire a natural immunity that, although it is never sterilizing, is quickly developed against death and the severe forms of the disease and progressively protects against non-severe

disease and infection. It has been proposed that protection against non-cerebral life-threatening malaria is achieved after only one or two infections (43).

Individuals with naturally acquired immunity will have asymptomatic malaria parasitaemia or mild clinical malaria episodes, but relatively few will develop severe disease. To maintain the level of naturally acquired immunity, continuous exposure to the parasite is needed, although it is not clear to what level immunity decreases after periods without exposure (44).

During pregnancy this naturally acquired immunity is partially lost, pregnant women being more susceptible to infection and severity than before pregnancy. Moreover, there seems to be a pregnancy-specific immunity acquired through consecutive pregnancies, as primigravidae have a higher risk of malaria which decreases with increasing parity, especially in high transmission areas (45, 46).

Immune responses to the parasite are species and stage-specific but do not seem to be strain or variant-specific. Some authors argue that the slow acquisition of naturally acquired immunity is explained by the need to develop specific immune responses to a wide spectrum of antigenically distinct parasites before effective immunity is achieved. However, strain-transcending protection seems a better explanation for the development of naturally acquired immunity, supported by the evidence from challenge studies, in which humans and animals

developed strain-transcending immunity after a single exposure, and malariatherapy syphilis patients, who developed solid clinical immunity after a few challenges with homologous strains. Furthermore, naïve-adults who moved to a high transmission area showed rapid acquisition of naturally acquired immunity and, in young children from high transmission areas protection against the severe forms of the disease is acquired relatively quickly, also arguing against the cumulative strain-specific immunity model (47).

Antibodies against some parasite antigens have been correlated with a lower incidence of clinical malaria, but no surrogate marker of protection has yet been identified. This makes the vaccine design difficult, as there is little evidence of which antigens have to be included in a candidate vaccine.

9. Malaria Vaccine Development

9.1. General review

Recent years have witnessed an intensified investment in malaria vaccine development (48, 49), and first generation a malaria vaccine is close to being registration. The history of the attempts to induce protective immunity and the intention to develop malaria vaccine are long. In 1910, Sergent brothers attempted to develop a malaria vaccine using sporozoites that were extracted from the salivary glands of mosquitoes infected with the avian *Plasmodium relictum* in Algiers.

However, recently evidences of protective immunity to malaria came from experiments using attenuated X-irradiated sporozoites in monkeys (50), and later in human volunteers (51, 52)

During the later 1980´s decade, the results of the vaccine Spf66 generated much enthusiasm and optimism on vaccine development (53). But initial field trials showed little low long-term protection in areas of high transmission, and efficacy was limited to certain point of time (54) and did not reduce reduce the risk of clinical malaria among study population (55).

At present there are around 100 candidate malaria vaccines in development (56, 57), however, most of them are based on a small number of *P. falciparum* antigens (half of them are based on MSP-1, CSP and AMA-1), and only a few have entered clinical trials. A high percentage of the candidates are discarded along the malaria vaccine development pipeline and only a few have reached advanced clinical trials in humans.

9.2. Vaccine strategies and targets

In theory, the *plasmodium* life cycle can be interrupted by blocking sporozoite entry into the liver or preventing the growth of the tissue phase in the liver (pre-erythrocitic). Interrupting asexual development in the erythrocytes (Asexual stage vaccine), and eliminating the gametocytes or

interfering with their infectivity to the mosquito (transmission blocking vaccines). See Figure 9

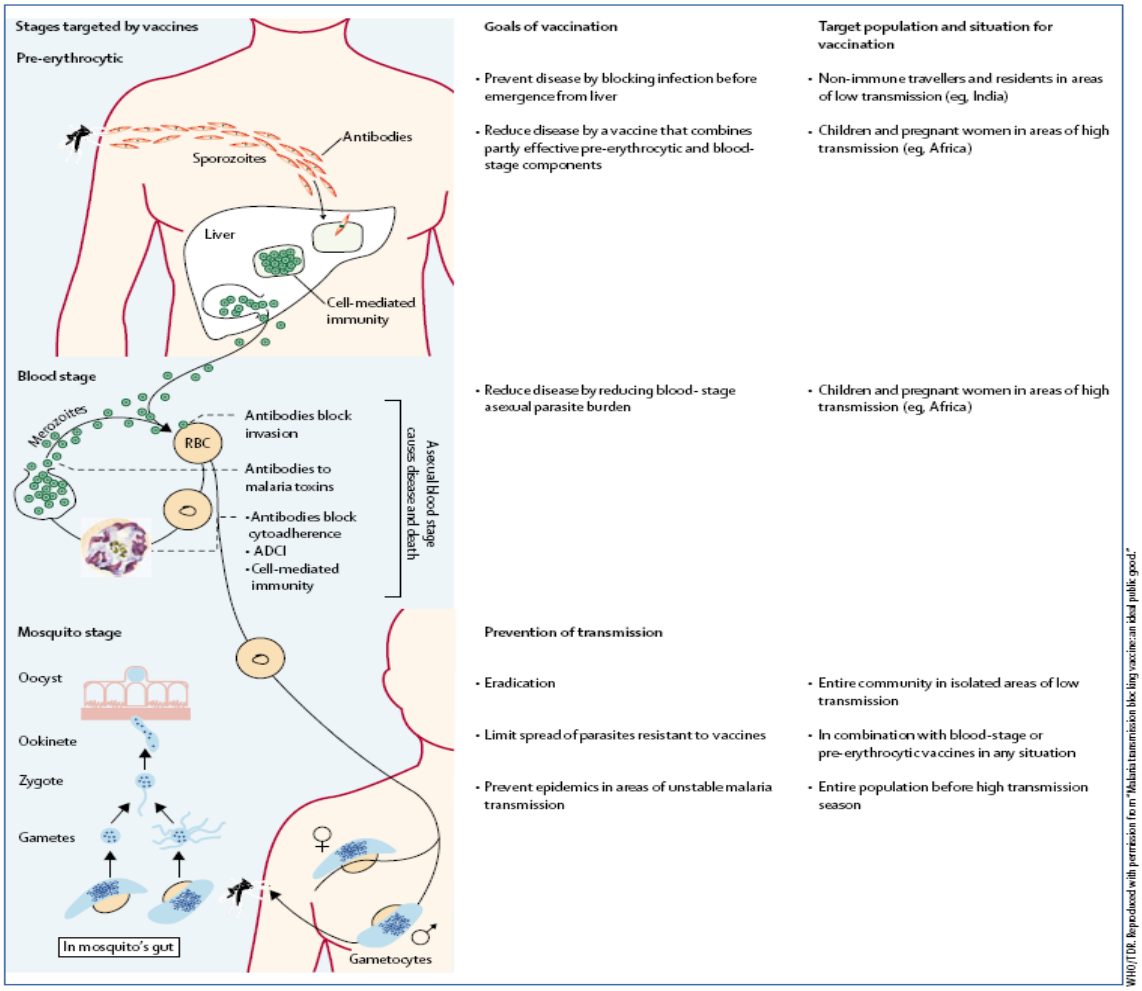

Figure: **Targets and goals of malaria vaccine candidates**[3]
RBC=red blood cell, ADCS=antibody-dependent cellular immunity.

Figure 9: Targets and goals of malaria vaccine candidates

9.2.1. Pre-erytrocytic vaccines

Pre-erytrocytic vaccines are designed to target both sporozoites before invasion of hepatocytes or schizont-infected hepatocytes and thus prevent the release of primary merozoites from infected hepatocytes in

the blood stream. Interrupting the parasite cycle at this stage would prevent all clinical symptoms of clinical disease and parasitaemia.

Evidence from pre-clinical trials with irradiated sporozoites have shown to induce sterilizing immunity in vaccinated volunteers, although protection was short and only lasted a few weeks. (52)

The target antigen of the malaria parasite in the pre-erythrocytic stage is the circumsporozoite (CS) protein. Many studies to target this stage were entered in clinical trial in several settings across Africa. Most advanced malaria vaccine is RTS,S/AS, described below.

9.2.2. Blood stage vaccines

An affective blood-stage vaccine is one that would prevent the invasion of erythrocytes by merozoites. Therefore the development of a blood-stage malaria vaccine is to target immune responses against the asexual stage (blood stage) of the parasites. The rational for development of blood-stage vaccines is based on observation that: the majority of individuals living in malaria-endemic areas acquire the ability to control parasite replication to levels below those that result in clinical disease, and hyperimmune globulin prepared form the sera of individuals chronically infected with malaria enhance clearance of parasitized red blood cells from infected individuals (58, 59), consequently reducing clinical symptoms and severity of disease.

During invasion, the membranes of both parasite and red blood cell fuse, to allow parasite invasion without damaging the red blood cell. This complex process involves a number of parasite proteins that are located on the surface of the merozoites which become temporarily accessible to circulating antibodies.

The major obstacle in the development of asexual blood –stage vaccine is that many blood-stage antigens are highly variable making the selection of targets more difficult as they have to be relatively conserved. Most of the development of blood-stage vaccines has been focused on targeting the surface protein 1 (MSP), a protein synthesized during the development of the schizont and present on the surface of the merozoites as a complex of proteolytic fragments (60-63). The most well characterized surface antigens are: MSP-1, MSP-2, MSP-3, and apical membrane antigen 1(AMA-1).

The first asexual malaria vaccine was the SPf66, a synthetic protein vaccine containing aminoacid sequences derived from pre-erythrocytic and asexual blood-stage proteins of *P. falciparum* (64). Results from initial clinical trials showed the vaccine was protective against malaria (65-68), but successive trials conducted in different ages and epidemiological settings by different groups showed borderline or no protective efficacy in children and infants (54, 55, 69, 70), resulting in the halting of the development of this candidate in 1996.

9.2.3. Transmission blocking vaccine

Transmission-blocking vaccines (TBV) are directed against antigens of the sexual stages of the parasite with the objective of inhibiting the sexual reproduction of the parasite in the mosquito and thus breaking the transmission cycle (71, 72). These vaccines if discovered, their administration would benefit the vaccinated community but not individual. In this case will make more importance in the context of the new malaria eradication campaign, especially if they are combined with PE or ABS antigens.

PvS25 is the most advanced transmission-blocking vaccine, which has already gone through a phase 1a trial.

9.3. Malaria vaccine development plan

In the last decade, the mobilisation and awareness of international organizations from both the public and private sectors has increased. One example is the Bill & Melinda Gates Foundation's challenge through the PATH Malaria Vaccine Initiative (MVI). Due to this funders mobilization, a part of RTS,S candidate vaccine, currently there are many other candidates products being assessed in different phases.

For the first time in malaria vaccine development there is a robust pipeline of candidate vaccines to be evaluated in clinical trials. Even RTS,S results remain encouraging and other candidates that are moving forward could also have clinical impact in the field (see the WHO portfolio

of candidate malaria vaccines currently in development, August 2005 (http://www.who.int/vaccine_research/documents/en/malaria_table.pdf).

However, while malaria vaccines are still in trial phase, alternative methods of malaria control including, case management, prophylaxis for no immune (travellers), insectsides Treated Nets (ITNs), intermittent Preventive Treatment in infancy (IPTi) and Indoor Residual Spray (IRS), are still very important.

In order to be considered for widespread implementation, Malaria vaccines will need to be more clinically or cost effective than existing, alternative control strategies, or be able to provide substantial added value such as having the ability immunity to blocks infection, preventing pathology or blocking transmission of parasites. Consequently, some research groups are trying to combine vaccines or products in order to achieve different targets using one or more *plasmodium falciparum* antigens such as a combination between circumsporozoite (CS) and SSP2/TRAP, LSA-1, EXP1, and LSA-3 (59)

Early clinical development of the RTS,S malaria candidate vaccine was initiated in studies in malaria-naïve adults in collaboration with the Walter Reed Army Institute of Research (WRAIR), WRMAL-003 *in which confirmation of the efficacy, safety and immunogenicity of the RTS,S/AS02A vaccine formulation was demonstrated (73, 74). Two doses of RTS,S/AS02A (0.5 mL) provided protection to 37.8% healthy non-immune*

volunteers against homologous sporozoite challenge (pooled results for WRMAL-004 & -005); 3 doses demonstrated protection of 43.2% of subjects (pooled results for WRMAL-004 & Malaria-012). In subjects not protected, the prepatent period was significantly prolonged in the RTS,S/AS02A group compared to control (WRMAL-004, WRMAL-005 & Malaria-012). Protective efficacy was low following re-challenge six months after Dose 3 of RTS,S/AS02A, but a statistically significant difference between prepatent period for vaccines compared to infectivity control was observed (WRMAL-004, WRMAL-005 & Malaria-012). A strong humoral immune response to the RTS,S/AS02A vaccine in terms of anti-CS and anti-HBs antibodies was demonstrated in all the adult studies in malaria-naïve individuals.

Evaluations of the CMI response showed consistently that administration of RTS,S/AS02A induced strong cellular Th1 T-cell responses, specific to the vaccine (75-77).

The RTS,S/AS02A vaccine progressed to evaluation in subjects under conditions of natural transmission. In adult males from The Gambia, VE against infection adjusted for covariates was 71% (95% CI: 46 to 85; p< 0.001) during the first 2 months and 34% (95% CI: 8.0 to 53, p=0.014) for the entire 15 week surveillance period (Malaria-005) (78). VE, adjusted for covariates, following a booster dose given during a second year malaria season was 47% (95% CI: 3 to 71; p=0.039). Protection was not limited to the NF54 parasite genotype from which the vaccine was derived (79).

Safety surveillance over approximately 5 years showed no safety signal (Malaria-016, -017 & -018). A strong humoral immune response to the RTS,S/AS02A vaccine in terms of anti-CS and anti-HBs antibodies was demonstrated. Overall, the kinetics of the humoral immune response induced by vaccination with RTS,S/AS02A were similar in malaria-naïve and experienced populations, while the absolute GMT values appeared to be higher in malaria-naïve volunteers. The vaccine induced and boosted Th1-like cellular immunity to several T-cell epitopes in a population naturally exposed to malaria (80-82).

The RTS,S/AS02A candidate vaccine progressed to clinical evaluation in children. Two age de-escalation and dose comparison trials (which compared doses with 10 µg, 25 µg and 50 µg of antigen corresponding to volume fractions of RTS,S/AS02A: 0.1 mL, 0.25 mL and 0.5 mL respectively) enrolled a total of 225 children aged 1 to 11 years from The Gambia (Malaria-015 & 020), (78). From these trials, the 0.25 mL dose was selected due to equivalent immunogenicity and slightly less reactogenicity in the 0.25 mL group compared to the 0.5 mL group; immunogenicity was consistently lowest in the 0.1 mL group. The safety and immunogenicity of the RTS,S/AS02A 0.25 mL dose was further confirmed in another study, Malaria-025, conducted in 1 to 4 year old children in Mozambique (83).

Recently two studies demonstrated that the vaccine RTS,S/AS0 a has promising safety profile and efficacy. A phase IIb, randomized,

controlled, double blind trial was conducted at two sites in Tanzania and Kenya with 894 children between the ages of five and 17 months at time of first dose. Participants received either 3 doses of RTS,S/AS01 or a rabies vaccine on a 0, 1, 2-month schedule. Children were followed for an average of 8 months to determine safety and efficacy of RTS,S/AS01 against clinical malaria disease. The trial showed that RTS,S/AS01 reduces episodes of clinical malaria by 53 percent for an eight-month period.(84)

The other Phase IIb single center, double-blind, controlled trial enrolled 340 infants in Tanzania who received either 3 doses of RTS,S/AS02 or a licensed hepatitis B vaccine (Engerix-B™) at 8, 12 and 16 weeks of age in co-administration with a licensed DTPw/Hib vaccine. Infants were then followed for six months to determine safety and efficacy of RTS,S/AS02 against malaria infection and immunogenicity of co-administered vaccines. It also reported 65 percent efficacy against malaria infection in protecting infants from 8 weeks of age over a six-month period. The study was also the first to show that RTS,S/AS is efficacious when coadministered alongside the World Health Organization's schedule for the Expanded Program on Immunization (EPI) and does not compromise the immune response to other vaccines in the current schedule.(85)

A summary of clinical studies of the RTS,S vaccine in children is provided in Table 1.[Adapted from MAL055 protoco])

Table 1: Summary of pediatric clinical trials in Africa *[Adapted from MAL055 protocol])*

Study nº	Syudy design	Study population	Objectives	Study groups	Status (March 2008)
Malaria-015	Double-blind, randomized Controlled	Childre 6-11y The Gambia	Primary: safety & immunogenicity Secondary: humoral responses	RTS,S/AS02A (n=60)**** Rabies vaccine (n=30)	Complete
Malaria-020	Double-blind, randomized Controlled	Childre 1-5y The Gambia	Primary: safety & immunogenicity Secondary: humoral responses	RTS,S/AS02A (n=90)**** Rabies vaccine (n=45)	Complete
Malaria-025	Double-blind, randomized Controlled	Childre 1-4y Mozambique	Primary: safety & immunogenicity Secondary: humoral responses	RTS,S/AS02A (n=30) Engerix B (n=30)	Complete
Malaria-026	Double-blind, randomized Controlled	Childre 1-4y Mozambique	Primary: efficacy against clinical disease Secondary: safety & immunogenicity	Cohort 1: RTS,S/AS02A (n=782) Engerix-B or Prevnar and Hiberix (n=782) Cohort 2: RTS,S/AS02A (n=208) Engerix-B or Prevnar and Hiberix (n=208)	Complete
Malaria-034	Double-blind, randomized Controlled	Childre 3-5y Mozambique	Primary: safety, non inferiority of ab responses to CS Secondary: non-inferiority of ab responses to HBsAg	RTS,S/AS02D (n=100) RTS,S/AS02A (n=100)	Complete
Malaria-038	Double-blind, randomized Controlled	Inafants 6-12w Mozambique	Primary: safety Secondary: immunogenicity	RTS,S/AS02D (n=110)*** Engerix-B (n=110)***	Complete
Malaria-039*	Double-blind, randomized Controlled	Childre 1-4y** Mozambique	Primary: safety Secondary: immunogenicity	n/a	Complete
Malaria-040	Double-blind, randomized Controlled	Infants 6-10w Tanzania	Primary: safety & non-inferiority of EPI antigens Secondary: immunogenicity & efficacy vs infection	RTS,S/AS02D+TETRAActHib (n=170) Engerix-B +TETRAActHib (n=170)	Complete
Malaria-046	Double-blind, randomized Controlled	Childre 18m-4y Gabon	Primary: safety, non inferiority of ab responses to CS Secondary: safety & immunogenicity	RTS,S/AS01E (n=90) RTS,S/AS02D (n=90)	Complete
Malaria-047	Partially-blind, randomized controlled	Childre 5-17m Ghana	Primary: safety Secondary: safety & immunogenicity	RTS,S/AS01E at 0, 1m (n=90) RTS,S/AS02D at 0, 1m (n=90) RTS,S/AS01E at 0,1 2m(n=90) RTS,S/AS02D at 0, 1, 2m (n=45) Rabies vaccine at 0, 1, 2m (n=45) RTS,S/AS01E at 0,1, 7m (n=90) RTS,S/AS02D at 0, 1, 7m(n=90)	Complete
Malaria-049	Double-blind, randomized Controlled	Childre 5-17m Tanzania&Kenya	Primary: efficacy against clinical disease Secondary: safety & immunogenicity	RTS,S/AS01E (n=445) Rabies vaccine (n=445)	Ongoing
Malaria-050	Open, randomized controlled	Childre 6-10w Tanzania, Ghana & Gabon	Primary: safety Secondary: safety & immunogenicity	EPI vaccines alone (n=170) EPI vaccines & RTS,S/AS01E at 0,1,2m (n=170) EPI vaccines & RTS,S/AS01E at 0,1,7m (n=170)	Ongoing

*Malaria-039 is the 2 year follow-up of the Malaria-026 study. No study vaccine were given during the follow-up peiod.
** age at time of primary vaccination
n= number of enrolled (to be enrolled) subjects ab: antibody

*** Vaccine staggered with TETRAActHIb doses
**** A third of subjects enrroled to receive RTS,S/AS02A got a 1/5 dose volume, a third ½ dose volume and a third full dose volume. The ´1/2 dose volume` contained 25 µg antigen and 0.25µL adjuvant

9.4. Formulations of RTS,S

RTS,S has been evaluated to demonstration of field efficacy in the pediatric population with the AS02 adjuvant (proprietary oil-in-water emulsion formulated with MPL® and Stimulon® QS21 immunostimulants). The RTS,S antigen has more recently been evaluated with the AS01 adjuvant (liposome formulation with MPL and QS21 immunostimulants) in a strategy to improve vaccine efficacy (VE) and the duration of efficacy. Both the AS02 and AS01 adjuvant systems have a number of similar key components (Table 02) and therefore safety data collected with RTS,S administered with the AS02 family of adjuvants is supportive of the RTS,S/AS01E development.

Table 2: Formulations of RTS,S [Adapted from MAL055 protocol])

	Freeze-dried fraction	***Liquid fraction***				
Formulation	*RTS,S (µg)*		*MPL (µg)*	*QS21 (µg)*	*Dose Volume*	
RTS,S/AS02A (0,5 mL dose)	*50*	*Oil-in-water emulsion*	*50*	*50*	*0.5 mL*	*Efficacy demonstrated in adults in The Gambia (Malaria-005) (78-81)*
RTS,S/AS02A (0,25 mL dose)	*25*	*Oil-in-water emulsion*	*25*	*25*	*0.25 mL*	*Efficacy against clinical disease in children in Mozambique (Malaria -026)(82, 86-88)*
RTS,S/AS02D	*25*	*Oil-in-water emulsion*	*25*	*25*	*0.5 mL*	*Pediatric formulation (malaria-034, 038, 040) (85, 89, 90)*
RTS,S/AS01B	*50*	*Liposomes*	*50*	*50*	*0.5 mL*	*Efficacy in challenge model, adults (malaria-027), endemic countries (malaria-044)*
RTS,S/AS02E	*25*	*Liposomes*	*25*	*25*	*0.5 mL*	*Pediatric formulation (malaria-046, 047, 049, 050m)(84)*

The numbers of doses of RTS,S-containing vaccines and the number of recipients to date (March 2008) is tabulated in Table 03.

Table 3: Approximate number of doses of RTS,S vaccine administered with number of recipients (up to March 2008) [Adapted from MAL055 protocol])

Candidate Malaria Vaccine	***Subject population***	***Recipients***	***Doses administered***
RTS,S/AS02A	*Malaria-naïve adults**	*302*	*732*
	Adults in sub-Saharan Africa	*424*	*1030*
	Children in sub-Saharan Africa	*1572*	*4562*
RTS,S/AS02D	*Children in sub-Saharan Africa*	*190*	*550*
	Infants in sub-Saharan Africa	*276*	*802*
RTS,S/AS01B	*Malaria naïve adults**	*52*	*146*
	Adults in Sub-Saharan Africa	*85*	*242*

**_a subjects who took part in studies conducted in the USA and Belgium, where there is no naturally occurring transmission of malaria.*

9.5. RTS,S/AS02A

GlaxoSmithKline (GSK), has collaborated with the Walter Reed Army Institute of Research (WRAIR) to develop an adjuvant candidate malaria vaccine based on the RTS,S antigen for the routine immunisation of infants and children living in malaria endemic areas of Africa. Since 2000, trials of this vaccine in children have been conducted under a partnership agreement with the PATH Malaria Vaccine Initiative (MVI) and are guided by a joint MVI/GSK Steering Committee.

This pre-erythrocytic stage vaccine is based on the CS protein of the 3D7 clone of *P. falciparum*. The components of this vaccine are two polypeptides, RTS and S. Both are expressed in Saccharomyces cerevisiae.

RTS,S is a single polypeptide chain corresponding to amino acids 207-395 of the CS protein fused to the amino terminus of the hepatitis B surface antigen (HBsAg). S is a polypeptide of 226 amino acids that corresponds to HBsAg. Each RTS molecule includes 19 copies of the tetrapeptide repeat motif (NANP) fused to the C terminal region of the protein. During purification, both antigens were fused leading a RTS,S particle. The preclinical and early clinical development of RTS,S was conducted by GSKBio in collaboration with the WRAIR (Washington DC), where it was demonstrated that RTS,S formulated with the proprietary adjuvant SBAS2 (now renamed AS02A) was superior in terms of immunogenicity and efficacy against experimental sporozoite challenge than RTS,S formulated with less potent adjuvants (59, 73, 83).

The AS02A adjuvant is mixed just prior to injection. AS02A consists of an oil-in-water emulsion that incorporates the immunostimulants monophosphoryl lipid MPL and the saponin derivative QS21. Adjuvanted vaccines based on RTS,S would offer protection against malaria disease due to *P. falciparum*. This vaccine specifically targets the pre-erythrocytic stage of *Plasmodium falciparum* and confers protection against infection by *P falciparum* sporozoites delivered via laboratory-reared infected mosquitoes in malaria-naive adult volunteers and against natural exposure in semi-immune adults. In addition, studies with the RTS,S/AS02A candidate have shown that the vaccine stimulates the production of high anti-HBsAg antibody titers (73, 74, 78, 91, 92).

Previous trials have shown that this vaccine is safe, tolerable and immunogenic in malaria-naïve (74) semi-immune or malaria-naïve. Pain at the injection site (almost all mild to moderate in intensity), has been the most frequently reported local solicited symptom. Various vaccination schedules (0, 1, 5, 18 months / 0, 1, 6, 13 months / 0, 1, 9 months) have been assessed initially and all show the vaccine to be immunogenic with acceptable reactogenicity in adults.

In 2000, GlaxoSmithKline (GSK) and the Malaria Vaccine Initiative (MVI) Programme for Appropriate Technology in Health (PATH) entered into a partnership to develop RTS,S/AS02A as a vaccine to prevent severe malaria disease in infants immunised in the context of the Expanded Programme on Immunisation (EPI). Consecutive phase I studies undertaken in Gambia in adults and children aged 6–11 years and 1–5 years showed that the vaccine was safe, well tolerated, and immunogenic (78, 91, 93). Subsequently, a paediatric vaccine dose was selected and studied in a phase I trial of Mozambican children aged 1– 4 year. Results showed it was safe, well tolerated and immunogenic (83), These encouraging results paved the way, as part of the clinical development plan, to the phase IIb proof-of-concept efficacy study in children aged 1–4 years living in southern Mozambique, which forms part of this thesis.(86-88) (Sacarlal, J-in press)

Recently we completed a phase I/IIb clinical trial in infants living in a malaria-endemic area of Mozambique. Administration, staggered with EPI

vaccines, showed that RTS,S/AS02A had a good safety profile was well tolerated and immunogenic and vaccine efficacy against new infection was 65.9% (95% CI 42.6%; 79.8%, p <0.001) (90).

Together with recent data showing a favourable safety profile, non-interference with EPI antigens immune responses and an efficacy of 65% in the reduction of the risk of new infections when giving the vaccine to young infants staggered or coadministered with routine EPI vaccines (85, 90) these results strengthen the rationale for advancing toward a phase III trial aiming to register RTS,S/AS as the first malaria vaccine

III. HYPOTHESIS AND OBJECTIVES

1. HYPOTHESIS

1.1. Malaria is a major contributor to under five mortality in children living in rural area in Mozambique.

1.2. The RTS,S/AS02A has an acceptable safety profile.

1.3. The RTS,S/AS02A malaria vaccine reduces the frequency and severity of clinical disease episodes.

1.4. The RTS,S/AS02A malaria vaccine provide protection for only 6 months against clinical and severe malaria.

2. OBJECTIVES

2.1. GENERAL OBJECTIVES

To describe the most frequent causes of mortality in children under 15 years of age in the demographic surveillance area of the Manhiça Health Research Centre, using the verbal autopsy tool and to explore the impact of the intervention studies in reduction of malaria mortality.

To evaluate the safety and efficacy of RTS,S/AS02A candidate malaria vaccine against clinical episodes of *P. falciparum* malaria in children aged 1 to 4 years at first vaccination, over a 4 years surveillance period

2.2. SPECIFIC OBJECTIVES

2.2.1. To describe the evolution of the specific malaria mortality during trial period

2.2.2. To evaluate the safety of RTS,S/AS02A malaria vaccine for a 4 year period start after Dose 1.

2.2.3. To evaluate the reactogenicity of RTS,S/AS02A malaria vaccine from the time of first vaccination until one month after dose 3 (day 90)

2.2.4. To assess persistence of efficacy against clinical malaria disease of RTS,S/AS02A for a 4 year period start after Dose 1.

2.2.5. To asses humoral immune response to the circumsporozoite protein (CS) antigen of the RTS,S/AS02A malaria candidate vaccine

IV. MATERIALS AND METHODS

1. DESCRIPTION OF STUDY SITE

All studies were conducted at the Manhiça Health Research Centre (CISM) at Manhiça District, a rural area located 80Km north in the Maputo province in southern Mozambique.

The CISM is adjacent to the Manhiça District Hospital (MDH), and since 1997 they have jointly operated a round-the-clock surveillance of all paediatric visits to the outpatient department (OPD) and admissions to the wards. MDH a 110-bed referral health facility for Manhiça District, that provides curative and preventive services. It has a round-the-clock emergency room, X-ray department, basic laboratory facilities and outpatient departments (OPD) for adults and children. Currently it also carries out preventive and treatment programmes for HIV. The district health network consists of a further ten peripheral health posts and a rural hospital (Figure 11). Participants in these studies have been recruited from the study area. In case of vaccine study, one group of participants were recruited around the centre and other group living 55km north of Manhiça Village.

The Centro de Investigação em Saúde da Manhiça (CISM) has been running a continuous demographic surveillance system (DSS) in the area since 1996 that currently extends to 500 Km2 and includes 84,000 inhabitants. The district has an estimated population of 140,000 inhabitants (94).

The area has a sub-tropical climate with two distinct seasons: a warm and rainy season between November and April and a cool and dry season during the rest of the year. The mean average annual rainfall is 1100 mm. Malaria transmission, mostly due to *P falciparum*, is perennial with marked seasonality. *Anopheles funestus* is the main vector, and the estimated entomological inoculation rate for 2002 was 38 infective bites per person per year. (87)

A full description of the geographical and socio-demographic characteristics of the study community can be found elsewhere at www.Manhica.org (16, 95, 96).

Figure 10: Map of the Manhiça District

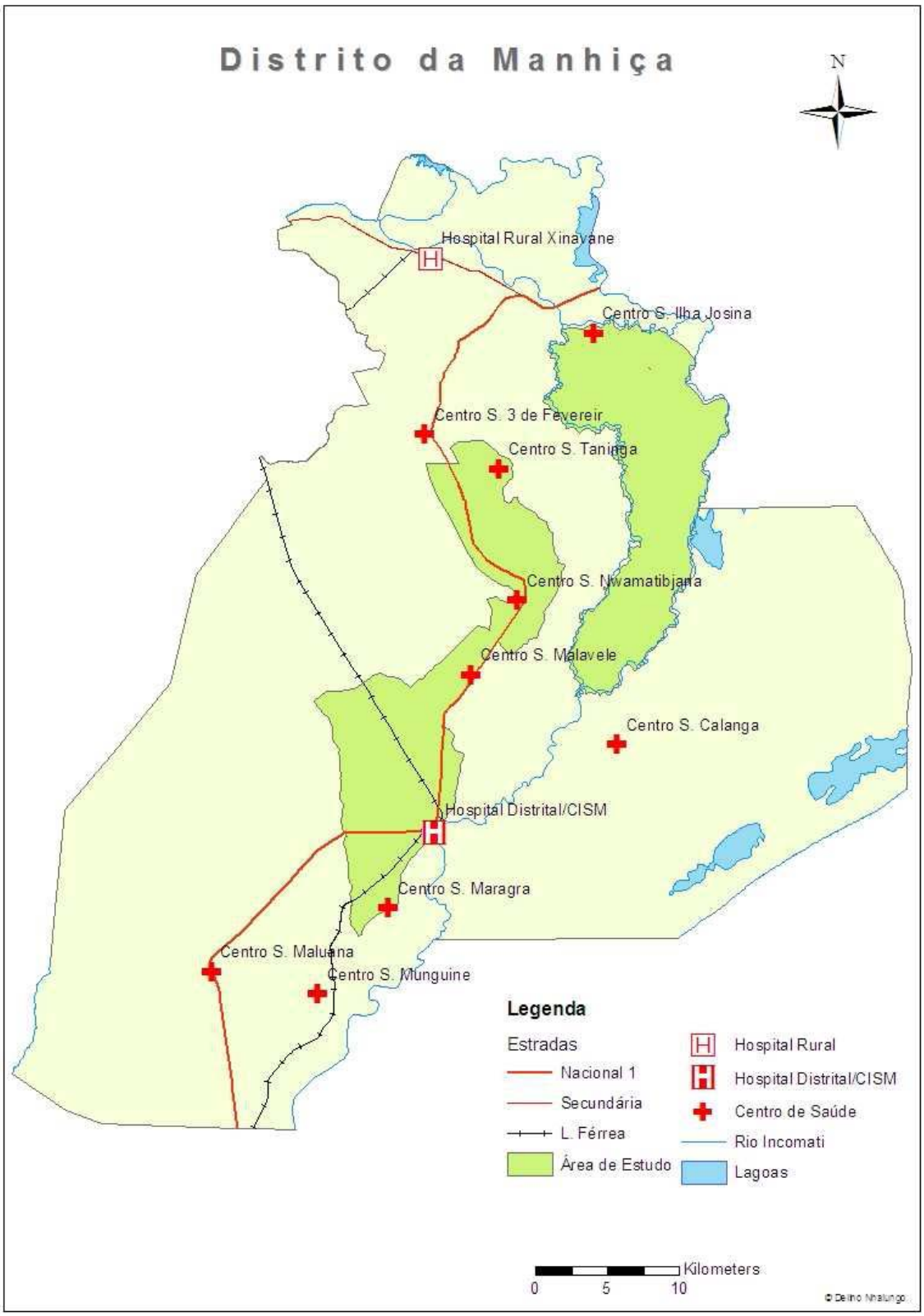

Figure 11: Map of the Manhiça study area (in green)

2. METHODOLOGY OF THE STUDIES

2.1. Article 1

The first article is based analysis of data collected through the demographic surveillance system with use the verbal autopsy. The methodology used in identifying vital events in the study area has been fully described elsewhere (95). Information on deaths comes from one of several sources: (I) household visits twice a year that are conducted to record all deaths and other demographic events that have occurred since the previous visit, (II) daily visits to hospital wards and maternity clinics by supervisors to gather information on all deaths and pregnancy related events that have taken place in the previous 24 hours and (III) weekly reports by local key informants on births, deaths and migrations that might be missed during household census visits by field workers and supervisors. Initially, eight medical students conducted VA interviews in the study area twice a year. The work was supplemented after June 2002 by a lay supervisor and field workers who interviewed key community informants and relatives of the deceased, daily. Between three and six months after a death, a field worker visited the family of the deceased to inquire whether they would accept to participate in a verbal autopsy.

Upon acceptance, an oral consent was obtained from the interviewee and a date for the interview was agreed. On the day of the interview, a signed or fingerprinted informed consent (IC) was sought before the VA took place. To ensure consent within the family, potential

interviewers were given an information sheet with study objectives and procedures during the initial contact, and were encouraged to discuss with family members before proceeding. Interviewers who could not read were free to ask their relatives to read the document for them. The primary informant was, whenever possible, the person who directly took care of the deceased child during the illness or condition that led to death. A demographer was in charge of controlling the data quality through an on-site review of questionnaires. After fieldwork, all questionnaires were checked for consistency and completeness. Questionnaires needing corrections were returned to the field within two weeks of their receipt.

The study used a VA questionnaire standardized from INDEPTH (95)and adapted from the WHO model (97). The standard questionnaire in Manhiça was written in Portuguese. However, the fieldworkers perform an on-site translation of the questions into the local language (Xangana). The questionnaire included questions on the identification of the deceased and the respondent as well as the health seeking behaviour and use of health services by the deceased prior to the death. The questionnaire also had an open-ended section where circumstances surrounding the death of the child, as well as the signs and symptoms presented during the illness preceding death, are recorded. The final section had closed questions on signs and symptoms preceding the death that did not focus on any particular disease.

To assign the cause(s) of death, diagnoses are given using a standardised coding system. Three physicians with experience in tropical diseases independently assigned the cause of death using the International Classification of Diseases (ICD-10)(98). Each physician ascribes a minimum of one and a maximum of 2 causes. When the cause was different among the three reviewers, the final diagnosis was "not consensus", and these deaths were not redistributed to other diagnosis groups. When two final diagnoses were assigned for the same death, each of these was individually mapped onto ICD-10 for a calculated cause-specific rate.

To rank causes of death, we used the GBD tree structures (99). The first level included three mortality groups: Group 1 consisted of deaths attributed to communicable diseases and to maternal, perinatal and nutritional conditions; Group 2 comprised deaths attributed to non-communicable diseases and, Group 3 comprised deaths due to injuries. Each of the three groups was further divided into several major subcategories (second to fourth level).

2.2. Articles 2 to 6

This articles come from the RTS,S malaria vaccine study. Is a phase IIb, randomised controlled trial to assess the efficacy, safety and immunogenicity of three doses of the candidate RTS,S/AS02A malaria vaccine when administered to children aged 1-4 years at moment of the

first vaccine. This study was implemented and conducted between April 2003 and May 2007 and herein includes different follow-up periods. The initial double blind phase included study months 0 to 8.5. During this period, and according to protocol, the investigators were unblinded and a first analysis of safety and efficacy was performed and reported (87). Study participants and case ascertainment mechanisms remained blinded, and follow-up was sustained according to protocol in the single blind phase from study months 8.5 to 21 (86). A subsequent new protocol was developed to expand follow-up for safety and efficacy of the study cohorts from study month 21 to month 45. The present thesis includes the safety and efficacy data for the entire study period from month 0 to month 45.

A total of 2022 healthy children were enrolled to receive either the candidate malaria vaccine or a comparison control vaccine after parents or guardians gave written or thumb-printed informed consent prior to enrolment. Eligibility screening included a brief medical history, a physical examination, and blood sampling by finger-prick for haematology and biochemistry tests. Children were not screened for human immunodeficiency virus (HIV) infection. Hepatitis B surface Antigen (HBsAg) status and anti-HBsAg antibodies were assessed at baseline, but was not an exclusion criterion to the trial.

Children were randomised in a 1:1 ratio to receive RTS,S/AS02A (0.25mL dose) or the control vaccines. The RTS,S/AS02A candidate

vaccine was administered intramuscularly in the deltoid region of alternating arms starting with the left according to a 0, 1, and 2 month schedule. Children aged 24 months and older in the control group received three paediatric doses (0.5 ml) of hepatitis B vaccine (*Engerix-B™*, GSK Biologicals, Rixensart, Belgium). Children under 24 months received two paediatric doses of 7-valent pneumoccocal conjugate vaccine (*Prevenar™*, Wyeth Lederle Vaccines, Madison, NJ, USA), administered at the first and third vaccinations and one dose of *Haemophilus influenzae type b* vaccine (*Hiberix™*, GSK Biologicals, Rixensart, Belgium) at the second vaccination.

Children were enrolled into two cohorts to measure the vaccine efficacy for clinical malaria disease and malaria infection. In cohort 1(Manhiça area), 1605 participants were followed-up using passive surveillance to detect clinical episodes of malaria and safety surveillance until month 45. In cohort 2 (Ilha Josina village), 417 participants were followed-up using active surveillance to detect malaria infection through visits that started 14 days after dose 3 and continued every 2 weeks for 2.5 months, and then monthly for an additional 2 years. At the end of the single blind phase, a new inform consent was obtained to continue the follow-up for more 2 years. This cohort continued under surveillance through a health facility-based passive case detection system to monitor safety.

Vaccines were administered at the Manhiça and Ilha Josina health centres. Vaccine safety was evaluated using active and passive follow-up. Investigators followed-up participants with SAEs until the event had resolved or until month 45. Deaths occurring at home were investigated by a review of all available medical records and through a verbal autopsy.

Analysis for safety was based on intention to treat [ITT] of study participants included in both cohorts 1 and 2 during months 0 to 45. Analyses for efficacy against clinical malaria were based on cohort 1 study participants who were compliant with study procedures (according to protocol [ATP] cohort for analysis) over the period study months 2.5 to 45.

For the efficacy analyses, except for the hospital admissions, the time at risk was calculated considering absences from the study area and antimalarial drug usage. For the analysis of multiple episodes of clinical malaria, a subject was not considered to be susceptible for 28 days after the previous episode. After malaria treatment a child was not considered at risk for an arbitrary period of 28 days after receiving sulfadoxine-pyrimethamine, 7 days after chloroquine alone, 7 days after quinine alone, 7 days after amodiaquine, and 20 days after artemether+lumefantrine.

The studies were implemented under collaboration of different institutions that provide scientific and financial support. All studies were

conducted by CISM and GSK, and both received financial support from PATH-MVI to conduct the work described in this thesis. The initial support to CISM by PATH-MVI was passed through GSK for administrative purposes. CISM is supported by the members of the collaborative programme (Ministry of Health (Mozambique), Universidade Eduardo Mondlane (Mozambique), and Hospital Clínic (Universidad de Barcelona, Spain), and core funding is provided by the Spanish Agency for International Cooperation and Development (AECID – Ministry of Foreign Affairs and Cooperation, Madrid, Spain).

V. ETHICAL ISSUES

The first article was falls within the national ethical clearance granted to the malaria epidemiological studies of the CISM (Ministry of Health/National Institute of Health of Mozambique, 1996). The participation of the respondents during the interview was voluntary and conducted only after the IC procedure described earlier. The interviews were conducted at least one month after death, when the traditional grieving period was over.

For the rest of articles, the trial is registered with the ClinicalTrials.gov (identifier NCT00197041 and NCT00323622). The protocol (CPMS Protocol No 257049/026 and IND number: BB-IND 10514) was approved by the Mozambican National Ethics Review Committee, the Hospital Clínic of Barcelona (University of Barcelona) Ethics Review Committee, and the PATH Human Subjects Protection Committee. The trial was conducted according to the International Conference on Harmonisation of Good Clinical Practice Guidelines, and was monitored by GSK Biologicals. A local safety monitor and a data and safety monitoring board (DSMB) closely reviewed the conduct and safety data of the trial. During the enrolment of the participants, a written informed consent was obtained.

VI. FULL ARTICLES

ARTICLE 1: A 10 year study of the cause of death in children under 15 years in Manhiça, Mozambique. **BMC Public Health 2009**

ARTICLE 2: Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children randomised controlled trial. **Lancet 2004**

ARTICLE 3: Duration of protection with RTS,S/AS02A malaria vaccine in prevention of Plasmodium falciparum disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. **Lancet. 2005**

ARTICLE 4: Safety of the RTS,S/AS02A malaria vaccine in Mozambican children during a Phase IIb trial. **Vaccine. 2008**

ARTICLE 5: Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children – **JID, 2009** - accepted in press

ARTICLE 6: Últimos avances en el desarrollo de una vacuna de la malaria. **Evid Pediatr. 2008**

ARTICLE 1: Sacarlal J, Nhacolo AQ, Sigauque B, Nhalungo DA, Abacassamo F, Sacoor CN, et al. A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique. **BMC Public Health**. 2009;9:67, PMID: 19236726

BMC Public Health

Research article

A 10 year study of the cause of death in children under 15 years in Manhiça, Mozambique

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Abstract

Background: Approximately 46 million of the estimated 60 million deaths that occur in the world each year take place in developing countries. Further, this mortality is highest in Sub-Saharan Africa, although causes of mortality in this region are not well documented. The objective of this study is to describe the most frequent causes of mortality in children under 15 years of age in the demographic surveillance area of the Manhiça Health Research Centre, between 1997 and 2006, using the verbal autopsy tool.

Methods: Verbal autopsy interviews for causes of death in children began in 1997. Each questionnaire was reviewed independently by three physicians with experience in tropical paediatrics, who assigned the cause of death according to the International Classification of Diseases (ICD-10). Each medical doctor attributed a minimum of one and a maximum of 2 causes. A final diagnosis is reached when at least two physicians agreed on the cause of death.

Results: From January 1997 to December 2006, 568499 person-year at risk (pyrs) and 10037 deaths were recorded in the Manhiça DSS. 3730 deaths with 246658 pyrs were recorded for children under 15 years of age. Verbal autopsy interviews were conducted on 3002 (80.4%) of these deaths. 73.6% of deaths were attributed to communicable diseases, non-communicable diseases accounted for 9.5% of the defined causes of death, and injuries for 3.9% of causes of deaths. Malaria

was the single largest cause, accounting for 21.8% of cases. Pneumonia with 9.8% was the second leading cause of death, followed by HIV/AIDS (8.3%) and diarrhoeal diseases with 8%.

Conclusion: The results of this study stand out the big challenges that lie ahead in the fight against infectious diseases in the study area. The pattern of childhood mortality in Manhiça area is typical of developing countries where malaria, pneumonia and HIV/AIDS are important causes of death.

Background

One of the eight Millennium Development Goals (MDG), aims to reduce under five mortality rates by two-thirds, especially in African countries. To accomplish this goal, researchers, programme planners and policy-makers need information on the causes of death occurring in countries [1]. Approximately 46 million of the estimated 60 million deaths that occur in the world each year take place in developing countries [2]. Further, this mortality is highest in Sub-Saharan Africa, although causes of mortality in this region are not well documented [3]. In order to most appropriately allocate resources and to evaluate the impact of individual illnesses and its control, it is important to know the number and proportion of deaths in a community likely to be due to a given condition. In rural areas of developing African countries such as Mozambique, where many children are born and die without ever being registered, and where a significant proportion of all deaths take place outside health facilities, the only way of estimating the likely cause of death is through an interview of a witness of final illness. This is called a verbal autopsy (VA) [3-6].

Methods

Study area

Manhiça district is located in southern Mozambique, in the Maputo Province, about 80 km north of Maputo City. The area has two distinct regions. The first is the fertile lowlands, comprising the Incomati River flood plain running from the northern to the southern district boundary. This area is poorly inhabited and used mainly for sugarcane and fruit plantations. The second area is an escarpment of moderate altitude bordering the west of the river, where the population inhabits an extensive plateau. There are two distinct seasons, a warm and rainy season between November and April and dry and cool season during rest of the year. A full description of the geographical and sociodemographic characteristics of the study area has been presented elsewhere [7,8].

The Manhiça Demographic Surveillance System (DSS) in Manhiça District was established in 1996, and currently covers a 500 square kilometre area. An initial census was carried out in 1996, and vital events registration (births, deaths, pregnancy and, in/out-migration) were conducted on quarterly basis until the year 2000, when this was changed to twice yearly.

Verbal Autopsies (VA) data collection started in 1997 with the aim of generating cause-specific mortality data in the study area. Initially, VA were conducted only in January and July on deaths of children aged less than 15 years reported through the DSS in the previous 6 months. Since the introduction of new questionnaires in June 2002 through the MTIMBA (Malaria Transmission Intensity and Mortality Burden Across Africa) project from INDEPTH, VA interviews are carried out every day by a well-trained lay supervisor and field workers.

Description of the health delivery system

There are two referral health facilities in Manhiça district, the Manhiça District Hospital (MDH), with 110 beds, and the Xinavane Rural Hospital (XRH), with 59 beds. In addition, 10 peripheral health facilities complete the official health facilities network. Most of the government medical services are provided free of charge except for drugs prescribed at the outpatient department that is available for purchase at subsidized prices. Adults pay a symbolic consultation fee of about USD 0.02.

Since 1996 the Manhiça Health Research Center (CISM) has been operating a round-the-clock, hospital-based morbidity surveillance system for children under 15 years of age attending the MDH and three other peripheral health facilities in the study area [8].

Voluntary counselling and testing to prevent mother to child transmission with Niverapina since 2003, and Highly Active Anti Retroviral Therapy (HAART) are available since 2004 for all patients including pregnant women in MDH, according to national policies.

Obstetric services including obstetric emergency care, operation room and morbidity surveillance system were established at the MDH maternity clinic, as a passive case detection system, for all women (pregnant, puerperal and women with gynaecological complaints) attending this clinic with clinical complaints (i.e., not for those attending the routine antenatal clinic).

Selected indicators for the DSS

Between 1997 and 2005, the number of inhabitants living in the study area increased from 32856 to 79783, due to population growth and the extension of the DSS area in August 2002. During these years, the total fertility rate

decreased from 5.2 to 4.8 children per woman. The infant mortality risk in 2005 was 77.5 per 1000 live births, the under five mortality (5q0) rate was 138.6 deaths per 1000 pyrs, and the life expectancy at birth was 40.2 years [9].

Data collection & processing

The methodology used in identifying vital events in the study area has been fully described elsewhere [7]. Information on deaths comes from one of several sources: (I) household visits twice a year that are conducted to record all deaths and other demographic events that have occurred since the previous visit, (II) daily visits to hospital wards and maternity clinics by supervisors to gather information on all deaths and pregnancy related events that have taken place in the previous 24 hours and (III) weekly reports by local key informants on births, deaths and migrations that might be missed during household census visits by field workers and supervisors. Age is ascertained by direct questioning, referral to any existing personal identification documents and, if necessary, an area-specific calendar of events is used. An identification card is issued to all children under 15 years of age to allow identification of patients in the morbidity surveillance system in the MDH.

Initially, eight medical students conducted VA interviews in the study area twice a year. The work was supplemented after June 2002 by a lay supervisor and field workers who interviewed key community informants and relatives of the deceased, daily. Between three and six months after a death, a field worker visited the family of the deceased to inquire whether they would accept to participate in a verbal autopsy. Upon acceptance, an oral consent was obtained from the interviewee and a date for the interview was agreed. On the day of the interview, a signed or fingerprinted informed consent (IC) was sought before the VA took place. To ensure consent within the family, potential interviewers were given an information sheet with study objectives and procedures during the initial contact, and were encouraged to discuss with family members before proceeding. Interviewers who could not read were free to ask their relatives to read the document for them. The primary informant was, whenever possible, the person who directly took care of the deceased child during the illness or condition that led to death. If the primary respondent was absent, information was sought from any other adult, including neighbours, who might have relevant information on the possible cause of death. In order to maintain confidentiality, only the coding physicians and the data entry clerks had access to the assigned causes of death.

A demographer was in charge of controlling the data quality through an on-site review of questionnaires. After fieldwork, all questionnaires were checked for consistency and completeness. Questionnaires needing corrections were returned to the field within two weeks of their receipt.

Nature of the VA tool

The study used a VA questionnaire standardized from INDEPTH [7] and adapted from the WHO model [10]. The standard questionnaire in Manhiça was written in Portuguese. However, the fieldworkers perform an on-site translation of the questions into the local language (Xangana). The questionnaire included questions on the identification of the deceased and the respondent as well as the health seeking behaviour and use of health services by the deceased prior to the death. The questionnaire also had an open-ended section where circumstances surrounding the death of the child, as well as the signs and symptoms presented during the illness preceding death, are recorded. The final section had closed questions on signs and symptoms preceding the death that did not focus on any particular disease.

Assigning the cause of death

To assign the cause(s) of death, diagnoses are given using a standardised coding system. Three physicians with experience in tropical diseases independently assigned the cause of death using the International Classification of Diseases (ICD-10) [11]. Each physician ascribes a minimum of one and a maximum of 2 causes. Conditions should be additive and not alternative. For example, if more than one diagnosis was mentioned, it may be classified as "malaria or pneumonia," but should be stated as "malaria and pneumonia". A final diagnosis was reached when at least two physicians agree on the cause of death. When at least two physicians assigned "unknown" as the cause of death, the final cause of death was considered undetermined. When the cause was different among the three reviewers, the final diagnosis was "not consensus", and these deaths were not redistributed to other diagnosis groups. When two final diagnoses were assigned for the same death, each of these was individually mapped onto ICD-10 for a calculated cause-specific rate.

To rank causes of death, we used the GBD tree structures [12]. The first level included three mortality groups: Group 1 consisted of deaths attributed to communicable diseases and to maternal, perinatal and nutritional conditions; Group 2 comprised deaths attributed to non-communicable diseases and, Group 3 comprised deaths due to injuries. Each of the three groups was further divided into several major subcategories (second to fourth level). Third and fourth levels were used to classify specific causes of death.

Data Management & Analysis

Trained data entry clerks and a data manager ensured data entry into a network of computers under a Windows NT

environment. Double data entry was performed by two clerks using a modified version of The Household Registration System (HRS) [13]. Inconsistencies, if any, were corrected after counter-checking with the original questionnaires. Questionnaires with errors that could not be reconciled were returned to the field for correction. The database with the VA data was linked to other DSS databases. Data management, cleaning and statistical analysis were performed using STATA (Stata Corporation 2005, Stata Statistical Software: Release 9.2 College Station, TX: StataCorp LP, USA).

Calculation of mortality rates

Time at risk of disease was calculated for each individual registered in the demographic surveillance system, subtracting periods of absence due to migration. All-cause mortality rates were calculated by dividing the number of deaths in an age group by the time at risk, and expressed as deaths per 1000 person-years at risk. We calculated cause-specific death rates for each age group by multiplying the all-cause mortality rate by the proportion of deaths assigned to each cause.

Ethical considerations

The study falls within the national ethical clearance granted to the malaria epidemiological studies of the CISM (Ministry of Health/National Institute of Health of Mozambique, 1996). The participation of the respondents during the interview was voluntary and conducted only after the IC procedure described earlier. The interviews were conducted at least one month after death, when the traditional grieving period was over.

Results

Population size and characteristics

From January 1997 to December 2006, 568499 person-year at risk (pyrs) and 10037 deaths were recorded in the Manhiça DSS. 3730 deaths with 246658 pyrs were recorded in children under 15 years old. Verbal autopsy interviews were conducted for 3002 (80.4%) of these deaths. Non-completion was due to family out-migration (9.8%), prolonged absence of the relatives of the deceased (3.9%) or refusals (3.2%). Forty seven percent of the interviews were conducted within a period of 6 months and 83.9% within 1 year from the time of death. The median time was 8 months. According to respondents, 54% of deaths occurred outside a health facility. However, medical and other assisted care during the terminal illness was sought by 81.9% of those who died. Sources of care included: health centers and hospitals (67.8%), traditional healers (8.3%), religious leaders (1.8%), friends and family (1.1%) and others (20.9%).

Age and sex distribution of deaths

Most of the paediatric deaths occurred in children aged 1–4 years (41.3%), followed by infants aged 29 days – 1 year (30.6%). Overall, males constituted 54.7% of the total children analyzed (Table 1).

Main causes and mortality rate of registered death

Table 2 summarizes the distribution of 3696 causes by group for the 3002 deaths. Communicable diseases were responsible for most deaths (73.6%), non-communicable diseases accounted for 9.5%, and injuries for 3.9%. Among communicable disease, the most frequent diagnosis was infectious and parasitic diseases (60.0%), followed by perinatal disease (17.4%). Anaemia was a very common diagnosis with 54.8% of total causes of death among non-communicable diseases. Injury, poisoning drowning and certain other consequences of external causes were also common in group 3.

The more frequent double death causes are malaria and anaemia with 13,5% (94/694) of cases followed by HIV/AIDS and anaemia with 12,1% (84/694) of cases, and malaria and diarrhoea disease with 9,8% (68/694) of cases.

Table 3 shows the twenty leading causes of death and specific crude death rate for different causes as reported from VA for children less than 15 years of age. Malaria was the leading cause, accounted for 21.8% of total diagnosis given physicians and for 3.2 deaths/1000 pyrs. Acute lower respiratory infection (ALRI) including pneumonia,

Table 1: Distribution of deaths and verbal autopsy by age and sex in Manhiça DSS, 1997–2006

	DSS*, MORTALITY				Verbal Autopsy					
Age group	**No deaths**	**%**	**pyar**	**CDR**/1000 pyrs**	**No Male**	**%**	**No Famale**	**%**	**No Total**	**%**
< 1 y	1757	47,1	21003	84	773	47	635	46,7	1308	43,6
1–4 y	1541	41,3	76343	20	671	40,8	568	41,8	1239	41,3
5–9 y	295	7,9	81729	4	136	8,3	103	7,6	239	7,9
10–14 y	137	3,7	67583	2	63	3,8	53	3,9	116	3,9
Total	**3730**		**246658**	**15**	**1643**		**1359**		**3002**	

*_DSS – Demographic Surveillance System
**_CDR-Crude Death Rate

Table 2: Distribution of registered deaths by group and level-two cause in Manhiça DSS, Mozambique, 1997–2006.

Cause of death	No of diagnosis	%
I. Communicable Disease		
Infectious and parasitic disease	1604	58,9
Perinatal disorders	473	17,4
Respiratory infections	361	13,3
Nutritional disorders	240	8,8
Others	43	1,6
Total	**2721**	**73,6**
II. Non-communicable Disease		
Blood disease	193	54,8
Metabolic disorders	49	13,9
Congenital abnormalities	34	9,7
Neuropsychiatric disorders	28	8,0
Digestive disorders	12	3,4
Genitourinary disorders	11	3,1
Respiratory disorders	10	2,8
Cardiovascular disorders	9	2,6
Malignant disorders	3	0,9
Skin disorders	3	0,9
Total	**352**	**9,5**
III. Injuries		
Injury, poisoning and certain other consequences of external causes	86	58,5
External causes of morbidity and mortality	61	41,5
Total	**147**	**4,0**
IV. Undetermined and badly defined symptoms	**106**	**2,9**
V. No consensus	**370**	**10,0**
Total	**3696**	

was the second leading cause of death with 9.8% of total diagnosis and 1.5 deaths/1000 pyrs, followed by HIV/AIDS with 8.3% and 1.3 deaths/1000 pyrs. Diarrheal diseases with 8.0% and 1.2 deaths/1000 pyrs and malnutrition with 6.4% and 0.96 deaths/1000 pyrs were other important conditions.

Respiratory and cardiovascular disorders specific to the perinatal period accounted for 54.4 deaths/1000 pyrs and was the leading neonatal cause in children ≤ 28 days (data not shown). Foetus and newborn diseases affected by maternal related factors (complications of pregnancy, labour and delivery) were responsible for 51.4 deaths/1000 pyrs. Perinatal sepsis was the third principal cause of neonatal deaths with 47.1 deaths/1000 pyrs.

The mortality rate ratio for males compared to females, after controlling for age, during the study period was 1.17, (95% CI 1.10 – 1.25%; $p < 0.001$). This rate ratio was, in general, higher for males than for females in all diagnoses except HIV/AIDS, metabolic diseases and superficial traumatic injuries (table 3). Deaths from external causes of accident (poisoning, falls, animal bitten, drowning and suffocation) were frequent in children male compared to female with relative risk RR = 2.2 (95% CI 1.01–4.87; $p = 0.041$). The same observation occurs in perinatal deaths with respiratory and cardiovascular disease RR = 1.6 (95% CI 1.08–2.47; $p = 0.019$)

Table 4 presents mortality rates over the entire study period in children aged more than 29 days to 15 years of age. In the age group between 29 days to 1 year, malaria (12.5 deaths/1000 pyrs), ARLI (10.6 deaths/1000 pyrs) and HIV/AIDS (6.5 deaths/1000 pyrs) accounted for 37% of infant mortality.

Malaria was very common in children 1 to 4 years of age, with 34.4% of the total diagnoses and a mortality rate of 6.1 deaths/1000 pyrs. Malaria was followed by diarrhoea disease, HIV/AIDS and malnutrition with 2.3, 2.1 and 2.1 deaths/1000 pyrs, respectively.

Figure 1 presents the cause-specific death rates during the entire study. Overall mortality increased until 2001 in the

Table 3: The 20 leading causes of verbal autopsy deaths and mortality rate by sex and relative risk in Manhiça DSS, Mozambique, 1997–2006

	Cause of death (block-ICD 10)	No of diagnosis	%	CDR Male	CDR Female	CDR Total	RR all age	pvalue	95% IC
1	Malaria (B50-B54)	805	21,8	3,4	3,1	3,2	1,07	0,367	0,93–1,22
2	ALRI (Pneumonia) (J10-J18)	361	9,8	1,5	1,4	1,5	1,08	0,494	0,87–1,32
3	HIV/AIDS (B20-B24)	307	8,3	1,3	1,2	1,2	0,99	0,953	0,79–1,24
4	Diarrhoeal diseases (A00-A09)	297	8,0	1,3	1,1	1,2	1,07	0,548	0,85–1,35
5	Malnutrition (E40-E46)	238	6,4	1,0	0,9	1,0	1,02	0,893	0,79–1,31
6	Anaemia (D55-D59)	186	5,0	0,8	0,7	0,8	1,12	0,426	0,84–1,50
7	Inflammatory diseases of the central nervous system (G00-G09)	129	3,5	0,5	0,5	0,5	1,02	0,896	0,73–1,45
8	Respiratory and cardiovascular perinatal disease (P20-P29)	103	2,8	0,5	0,3	0,4	1,63	0,019	1,08–2,47
9	Fetus and newborn affected by mother infection (P00-P04)	88	2,4	0,4	0,3	0,3	1,01	0,967	0,66–1,54
10	Infection specific to the perinatal period (Sepsis perinatal) (P35-P39)	87	2,4	0,4	0,3	0,3	1,14	0,550	0,74–1,76
11	Other bacterial disease (A30-A49)	86	2,3	0,4	0,3	0,3	1,33	0,199	0,86–2,05
12	Tuberculosis (A15-A19)	64	1,7	0,3	0,2	0,3	1,44	0,154	0,87–2,36
13	Disorders related to length of gestation and fetal growth (P05-P08)	61	1,7	0,3	0,2	0,2	1,59	0,076	0,95–2,67
14	Metabolic disorders (E70-E90)	46	1,2	0,2	0,2	0,2	0,90	0,711	0,50–1,60
15	Burns (T20-T31)	37	1,0	0,2	0,1	0,2	1,44	0,270	0,75–2,78
16	Other disorders originating in perinatal period (P90-P96)	34	0,9	0,2	0,1	0,1	1,38	0,358	0,70–2,72
17	Other external causes of accident (W00-X59)	31	0,8	0,2	0,1	0,1	2,22	0,041	1,01–4,87
18	Transport accident (V01-v99)	28	0,8	0,1	0,1	0,1	1,70	0,178	0,78–3,71
19	Injuries involving multiple body region (T00-T07)	28	0,8	0,1	0,1	0,1	0,93	0,847	0,44–1,98
20	Other	204	5,5			0,8			
	Undetermined and Unspecific symptoms	106	2,9			0,4			
	No consensus	370	10,0			1,5			
	Total of diagnosis	**3696**	**100**	**12,8**	**11,4**	**14,8**	**1,17**	**0,000**	**1,10–1,25**

study area and then decreased during the second period between 2001 and 2006. Malaria and pneumonia largely predominated among causes of death, accounting for 30% of the mortality during the study. The first period (1997 to 2001), was marked by an increase in death rates attributed to malaria, pneumonia, diarrhoeal disease and HIV/AIDS, but not malnutrition. Deaths attributed to malaria almost triplicate between 1997 and 2001, increasing from 2.2 deaths/1000 pyrs to 7.7 deaths/1000 pyrs, respectively, and then declined substantially between 2001 and 2006 to 2.8 deaths/1000 pyrs. During the second period all main mortality causes, declined in the study area.

Discussion

This study sought to identify the causes of death in our study area based on the verbal autopsy technique. Variability in recall period, a frequently cited limitation of VA is reported in our study. The median time was 8 months, about double that reported in other studies [14]. This is due to the data collection period used in our study that was only twice a year during the first six years of the study. However despite this limitation, interviews well recorded the signs and symptoms presented during the illness period preceding death. Other possible limitations included the relatively low specificity and sensitivity of the VA tool for detecting major causes of childhood death and the need to validate the diagnosis. In validation studies conducted in Maputo in children under 5 years old, verbal autopsy was judged to be appropriate to detect measles (sensitivities 75%, specificity 98,6%), ALRI (sensitivities, 66,7%, specificity 85,4%) and malaria (sensitivities 62,8%, specificity 90,3%) but it performed poorly for meningitis (sensitivities 33,3%, specificity 98,6%) and

Table 4: Mortality rate in children by age group in Manhiça DSS, Mozambique 1997–2006

	Cause of death (block-ICD 10)	CDR * 28-1 y	CDR* 1–5 y	CDR* 5–10 y	CDR* 10–15 y
1	Malaria (B50-B54)	12,5	6,1	0,7	0,4
2	ALRI (Pneumonia) (J10-J18)	10,6	1,5	0,2	0,1
3	HIV/AIDS (B20-B24)	6,5	2,1	0,1	0,1
4	Diarrhoeal diseases (A00-A09)	4,9	2,3	0,2	0,1
5	Malnutrition (E40-E46)	3,4	2,1	0,1	0,0
6	Anaemia (D55-D59)	2,7	1,5	0,6	0,1
7	Inflamatory Central nervous System (G00-G09)	2,5	0,7	0,2	0,1
8	Other bacterial disease (A30-A49)	1,9	0,2	0,0	0,0
9	Tuberculosis (A15-A19)	1,1	0,3	0,1	0,1
10	Metabolic disorders (E70-E90)	0,5	0,4	0,0	0,0
11	Burns (T20-T31)	0,3	0,3	0,1	0,1
12	Other external causes of accident (W00-X59)	0,1	0,1	0,2	0,1
13	Transport accident (V01-v99)	0,0	0,1	0,2	0,1
14	Injuries involving multiple body region (T00-T07)	0,1	0,1	0,2	0,1
	Total of diagnosis	**46,9**	**17,7**	**2,8**	**1,4**

* CDR – Crude Death Rate (per 1000 persons year at risk)

anaemia (sensitivities 51,9%, specificity 84,9%)[15]. These results are comparable with the results of other validation studies made in Africa, including reported by Snow et al [3], Chandramohan et al [5], Kahn et al [16] and Philip WS et al [17].

Whether to use a single or a multiple diagnostic cause is arguable particularly in the regions where patients present more than one pathology [4], as it was observed in the current study. A single diagnosis of cause of death can be used, with the assumption that other causes are ignored in the analysis. An alternative type of analysis when more than one cause of death is considered has been described by Adjuik et al [18]. He has allocated percentages of a death to the codes assigned, in proportion to the number of coders who offered a diagnosis. This methodology may underestimate individual rates per cause. For us, many times it is difficult to assign a single cause of death because the process that leads to death is complex and several diseases are involved. We try to solve this question giving the same weight for each cause of death. However it is possible that our methodology may overestimate the individual rates per cause.

In our study about 54% of all deaths took place outside a health facility. This percentage is slightly lower than that found in another study carried out in Manhiça district between 1994–96 (59,9%) [19], and is a strong reminder that access to the health facilities is not just a matter of distance, as other factors may be even more important in defining the pattern of health seeking behaviour in the area. Even when taking into account all the limitations described above, the tool may be useful in monitoring changes in mortality patterns over time.

In children under 15 years of age, mortality decreased about 30% during the last 5 years in the Manhiça study area, but the same decrease has not been observed in adults (Nhacolo A, unpublished). A main factor not only in Mozambique but in other neighbouring countries is the rapid growth of AIDS cases in adults [9].

The crude mortality rates found in this study are similar to those reported by Adjuik M, for sites as Africa Center (ACDIS) in South Africa (16.5/1000 pyrs) and Navrongo in Ghana(15.6/1000 pyrs) [18] and by Korenromp EL, for sites in Hai district (16.6/1000 pyrs) and Dar es Salam (26/1000 pyr) in Tanzania [20]. The Manhiça study area has a similar mortality pattern and high rate of deaths due to infectious disease as other African countries. The pattern of child deaths found in Manhiça is typical of developing countries [21,22].

Malaria due to *Plasmodium falciparum* was the main killer among children between 28 days to 4 years living in Manhiça area. Overall, one in four deaths was due to malaria infection. The spread of resistance to antimalarial drugs, especially chloroquine, has probably contributed substantially to this increase before 2001. After 2001, malaria cases and malaria mortality rapidly declined in the study area [23]. This may be due to several reasons such as use of more effective antimalarial drugs for treatment of non severe malaria cases such as sulfadoxine-pyrimethamine (SP) plus chloroquine initially in 2000 and SP plus amo-

Figure 1
Cause-specific death rates per 1000 person-years in children under 15 years of age, Manhiça, Mozambique.

diaquine that began in 2002. In addition, intervention studies including a malaria vaccine candidate trial for children aged 1 to 4 years, [24-26] or an the intermittent preventive treatment trial in infants using SP [27] and the distribution bed nets to pregnant mother during the last 5 years may be contributed to drop in the malaria death rate.

Pneumonia was the second overall largest cause of death in children, and was the first cause in the neonatal group. This finding is not surprising [28] and highlights the importance of some pathogens, particularly the *Streptococcus pneumoniae* and *Haemophilus Influenzae*, as major health threats to African children. The observed age pattern of pneumonia deaths, whereby neonatal infants experience the highest burden, is confirmed by morbidity data from this area [8,29]. The specific death rates decreased after 2002, particularly in children less than 5 years, when surveillance of pneumococcal disease was established.

The high ranking of HIV/AIDS in children between 29 days and 1 year explained by the growing HIV epidemic in the study area. Many HIV/AIDS deaths were probably related to malnutrition, pneumonia, malaria and diarrhoea. Given the 23.6% HIV maternal seroprevalence detected at the antenatal clinics between August 2003 and April 2005 (Menendez C – in press), we might have expected more deaths from AIDS than the reported 8.3%. It is probable that many deaths registered as diarrhoeal disease and malnutrition also had AIDS as the underlying cause, but was not reported as such. The crude death rate began decreasing later 2004, just after the implementation of antiretroviral treatment in MDH.

Diarrhoea related deaths accounted for 8% of all deaths and remains as another main contributor to child mortality in Manhiça. A similar results has been reported by Dgedge et al in children from 0 to 14 years of age in Maputo City where up to 10% of paediatric deaths are attributed to diarrhoeal [21]. In sub-Saharan Africa, hos-

pital-based mortality from acute diarrhoea varies from 1.9% of all deaths in The Gambia to 37% in Nigeria, with most of deaths occurring during the first year of life [30]. Even though morbidity caused by diarrhoea is still high, mortality has been decreasing worldwide, also in Manhiça, mainly because of improved management and community education [31-33].

Malnutrition constitutes an important cause of child death in Africa [34]. In Manhiça the specific rate decreased during the study period due to an effective malnutrition programme in MDH that included improved detection, treatment and community follow-up at home of children after discharge from hospital. However several other factors such as poor socio-economic conditions, increasing prevalence of HIV/AIDS and tuberculosis, and the migration of the adult male population to Maputo capital and South Africa [9] may have all contributed to maintaining a high prevalence of this disease in the study area.

In Manhiça the crude mortality rate for diarrhoeal diseases, decreased at the same pace as malaria and malnutrition deaths. These related patterns suggest the relationship and possible misclassification of cause of death among these three diseases.

Finally, these results confirm that most causes of death in children are preventable. Research and programs that enable mothers to identify malaria, acute respiratory infections (particularly pneumonia) and diarrhoea, and that encourage prompt care-seeking behaviour. Strengthening case management at the primary health care facilities are important priorities. Morbidity and mortality related to prenatal causes, including asphyxia, can be reduced if staff is well-trained. Mothers should be encouraged to seek early for antepartum and intrapartum care for adequate attendance. The quality of neonatal care, with a focus on preventing infection needs to be improved.

Conclusion

In conclusion results of this study highlight the big challenge that lies ahead in the fight against preventable infectious diseases in developing countries.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JS, AQN, DN and CNS were responsible for field data collection and quality control of questionnaires. JS with BS, FA, PA, SM, TN, EVM, QB, CD, AB, EL and FS, have assigned causes of death on VA. JS, JJA and PLA were involved in the data analysis and interpretation. RT, JS and PLA participated in the design of the study and the preparation of the manuscript. JS wrote the manuscript with collaboration from all authors. All authors read and approved the final manuscript.

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Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial

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See Comment

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Summary

Background **Development of an effective malaria vaccine could greatly contribute to disease control. RTS,S/AS02A is a pre-erythrocytic vaccine candidate based on *Plasmodium falciparum* circumsporozoite surface antigen. We aimed to assess vaccine efficacy, immunogenicity, and safety in young African children.**

Methods **We did a double-blind, phase IIb, randomised controlled trial in Mozambique in 2022 children aged 1–4 years. The study included two cohorts of children living in two separate areas which underwent different follow-up schemes. Participants were randomly allocated three doses of either RTS,S/AS02A candidate malaria vaccine or control vaccines. The primary endpoint, determined in cohort 1 (n=1605), was time to first clinical episode of *P falciparum* malaria (axillary temperature ⩾37·5°C and *P falciparum* asexual parasitaemia >2500 per μL) over a 6-month surveillance period. Efficacy for prevention of new infections was determined in cohort 2 (n=417). Analysis was per protocol.**

Findings **115 children in cohort 1 and 50 in cohort 2 did not receive all three doses and were excluded from the per-protocol analysis. Vaccine efficacy for the first clinical episodes was 29·9% (95% CI 11·0–44·8; p=0·004). At the end of the 6-month observation period, prevalence of *P falciparum* infection was 37% lower in the RTS,S/AS02A group compared with the control group (11·9% *vs* 18·9%; p=0·0003). Vaccine efficacy for severe malaria was 57·7% (95% CI 16·2–80·6; p=0·019). In cohort 2, vaccine efficacy for extending time to first infection was 45·0% (31·4–55·9; p<0·0001).**

Interpretation **The RTS,S/AS02A vaccine was safe, well tolerated, and immunogenic. Our results show development of an effective vaccine against malaria is feasible.**

Introduction

During the 20th century, economic and social development and antimalarial campaigns have resulted in eradication of malaria from large swathes of the planet, reducing the world's malarious surface from 50% to 27%. Nonetheless, in view of expected population growth, by 2010, half the world's population—nearly 3·5 billion people—will be living in areas where malaria is transmitted.[1] Current estimates suggest that more than 1 million deaths are attributable to malaria every year, and the economic costs for Africa alone are equivalent to US$12 billion annually.[2] These figures highlight the desperate global malaria crisis and the profound challenges to the international health community and countries of sub-Saharan Africa. The reasons for this crisis are many and include emergence of widespread resistance to available, affordable, and previously highly effective drugs, breakdown and inadequacy of health systems, and lack of resources. Unless we find ways to control this disease, global efforts to improve health and child survival, reduce poverty, increase security, and strengthen the most vulnerable societies will fail.

At the beginning of the 21st century, emerging scientific knowledge and new impetus for malaria research provide opportunities to develop an effective malaria vaccine that could greatly contribute to control of this devastating disease.[3] RTS,S/AS02A is one of the most advanced malaria vaccine candidates in development.[4] This vaccine specifically targets the pre-erythrocytic stage of *Plasmodium falciparum* and confers protection against infection by *P falciparum* sporozoites delivered via laboratory-reared infected mosquitoes in malaria-naive adult volunteers and against natural exposure in semi-immune adults.[5–7]

In 2000, GlaxoSmithKline (GSK) and the Malaria Vaccine Initiative (MVI) Programme for Appropriate Technology in Health (PATH) entered into a partnership to develop RTS,S/AS02A as a vaccine to prevent severe malaria disease in infants immunised in the context of the Expanded Programme on Immunisation (EPI). Consecutive phase I studies undertaken in The Gambia of children aged 6–11 years and 1–5 years showed that the vaccine was safe, well tolerated, and immunogenic (Bojang KA, unpublished data). Subsequently, a paediatric vaccine dose was selected and studied in a phase I trial of Mozambican children aged 1–4 years, in which it was found to be safe, well tolerated, and immunogenic (Macete E, unpublished data).

These encouraging results paved the way, as part of the clinical development plan, to the phase IIb proof-of-concept efficacy study in children aged 1–4 years living in southern Mozambique, which we report here. Our aim was to assess the efficacy, safety, and immunogenicity of the RTS,S/AS02A vaccine.

Participants and methods

Study design

The trial was done at the Centro de Investigação em Saúde da Manhiça (CISM; Manhiça Health Research Centre), in Manhiça District (Maputo Province), in southern Mozambique between April, 2003, and May, 2004. The characteristics of the area have been described in detail elsewhere.[8] The climate is subtropical with two distinct seasons: a warm and rainy season from November to April; and a generally cool and dry season during the rest of the year. During 2003, annual rainfall was 1286 mm. Perennial malaria transmission with pronounced seasonality is mostly attributable to *P falciparum*. *Anopheles funestus* is the main vector, and the estimated entomological inoculation rate for 2002 was 38 infective bites per person per year. Combination therapy based on amodiaquine and sulfadoxine-pyrimethamine is the first-line treatment for uncomplicated malaria and is readily available at health facilities in Mozambique. Adjacent to CISM is the Manhiça Health Centre, a 110-bed referral health facility. The district health network consists of a further eight peripheral health posts and a rural hospital.

The study was a phase IIb, double-blind, randomised controlled trial to assess the safety, immunogenicity, and efficacy of RTS,S/AS02A malaria vaccine. The primary objective was to estimate the vaccine's efficacy against clinical episodes of *P falciparum* malaria in children age 1–4 years at first vaccination over a 6-month surveillance period starting 14 days after dose three.

We designed the trial to examine the efficacy of the vaccine at two points in the life cycle and pathogenesis of malaria: infection and clinical disease. These two endpoints were measured simultaneously in two cohorts based at different sites (figure 1). Cohort 1, recruited from an area of 10 km radius around Manhiça, contributed to the assessment of the primary endpoint of protection against clinical disease, determined through passive case-detection at the Manhiça Health Centre and the Maragra Health Post. Cohort 2 was recruited in Ilha Josina, an agricultural and marshy lowland area 55 km north of Manhiça, and was followed up to detect new infections through a combination of active and passive surveillance.

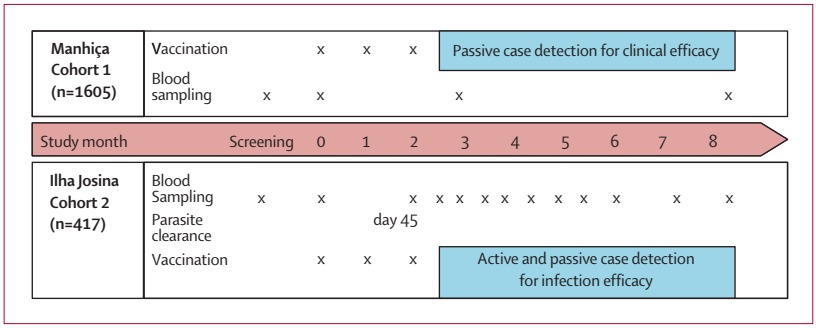

Figure 1: Study design

For cohort 1, 704 assessable children per group were needed to have 80% power to detect a lower confidence limit of vaccine efficacy of 15%, assuming a clinical *P falciparum* attack rate over the surveillance period of 11% in the control group and vaccine efficacy of 50%. For cohort 2, 116 assessable children per group were needed to provide 86% power to detect a vaccine efficacy of 50% in the prevention of new infections with a lower confidence limit of 20%, assuming a rate of new infections of 50% over the surveillance period.

The protocol was approved by the national Mozambican ethics review committee, the Hospital Clinic of Barcelona ethics review committee, and the PATH human subjects protection committee. The trial was undertaken according to the International Conference on Harmonisation Good Clinical Practice guidelines and was monitored by GSK Biologicals. A local safety monitor and a data and safety monitoring board closely reviewed the conduct and results of the trial.

Participants

CISM runs a demographic surveillance system in the study area.[8] Lists of potentially eligible resident children were produced from this census. We visited them at home, read information sheets to parents or guardians, and checked criteria for recruitment. These included confirmed residency in the study area and full immunisation with EPI vaccines. We invited interested parents or guardians to the Manhiça Health Centre or the Ilha Josina Health Post. At first visit, the information sheet was again read and explained to groups of parents and guardians by specially trained staff. We sought individual consent only after the parent or guardian passed an individual oral comprehension test designed to check understanding of the information. They were then invited to sign (or thumbprint if not literate) the informed consent document. A member of the community acted as an impartial witness and countersigned the consent form. Screening included a brief medical history and examination and blood sampling by finger-prick for haematology and biochemistry tests.

We excluded children from participation if they had a history of allergic disease, packed-cell volume less than 25%, were malnourished (weight for height $\leq$3 Z score), had clinically significant chronic or acute disease, or had abnormal haematology or biochemical variables. We enrolled eligible children into the study on the first day of vaccination and gave them a unique study number and individual photographic identification card.

Procedures

We randomly allocated children to receive three doses of either RTS,S/AS02A candidate malaria vaccine or a control vaccine at Manhiça Health Centre or Ilha Josina Health Post. Block randomisation was done at GSK Biologicals with SAS software version 8 (1/1 ratio, block size six). The code was released to the investigators once databases had been monitored, cleaned, and locked after completion of follow-up.

RTS,S consists of a hybrid molecule recombinantly expressed in yeast, in which the circumsporozoite protein,[9] central tandem repeat, and carboxyl-terminal regions are fused to the N terminal of the S antigen of hepatitis B virus (HBsAg) in a particle that also includes the unfused S antigen. A full adult dose of RTS,S/AS02A (GSK Biologicals, Rixensart, Belgium) contains 50 μg of lyophilised RTS,S antigen reconstituted in 500 μL of AS02A adjuvant (proprietary oil in water emulsion with the immunostimulants monophosphoryl lipid A [MPL; Corixa, Seattle, WA, USA] and *Quillaja saponaria* fraction 21 [QS21; Antigenics, New York, NY, USA]). We used half the adult dose in this trial—ie, a 250 μL dose containing 25 μg of RTS,S antigen in 250 μL AS02A adjuvant.

Because routine hepatitis B vaccination was introduced into the EPI schedule of Mozambique in July, 2001, children aged 12–24 months had already received hepatitis B immunisation. Accordingly, children younger than 24 months received as control vaccines two doses of the seven-valent pneumococcal conjugate vaccine (Prevnar Wyeth Lederle Vaccines, Madison, NJ, USA) at the first and third vaccination and one dose of *Haemophilus influenzae* type b vaccine (GSK Biologicals) at the second vaccination. For children older than 24 months, the control vaccine was the paediatric hepatitis B vaccine (GSK Biologicals).

We administered both RTS,S/AS02A and control vaccines intramuscularly in the deltoid region of alternating arms according to a 0, 1, 2 month vaccination schedule. Since the vaccines used are of distinct appearance and volume, special precautions were taken to ensure the double-blind nature of the trial. A vaccination team prepared the vaccine and masked the contents of the syringe with opaque tape before immunisation. This team was not involved in any other study procedures, including surveillance for endpoints.

After every vaccination, we observed study participants for at least 1 h. Trained field workers visited the children at home every day for the following 3 days to record any adverse event. We noted solicited local and general adverse events over this period.[10] Unsolicited adverse events were recorded for 30 days after every dose through the hospital morbidity surveillance system. We detected serious adverse events in a similar way and recorded them throughout the study. Study children were visited at home once a month, starting 60 days after the third dose. During the visit, we checked residence status and unreported serious adverse events were documented. We monitored haematological and biochemical variables for all participants, complete blood count at 1 month after dose three, and creatinine, alanine transaminase, and bilirubin at 1 and 6·5 months after dose three.

We established HBsAg status in all participants before the first dose. Antibodies against circumsporozoite protein were measured before dose one and 30 days and 6·5 months after dose three in cohort 1, and we assessed antibodies against HBsAg at these same timepoints in cohort 2. Indirect fluorescent antibody test was done in both cohorts at screening.

A health-facility based morbidity surveillance system has been established at Manhiça Health Centre and the Health Posts at Maragra and Ilha Josina since 1997.[11] In all three facilities, project medical staff are available 24 h a day to identify study participants through the personal identification card and to ensure standardised documentation and appropriate medical management.

We obtained blood from all children reporting fever within the preceding 24 h or with a documented fever (axillary temperature ⩾37·5°C) for determination of malaria parasites in duplicate thin and thick blood smears and for the packed-cell volume. Children with clinical conditions warranting hospitalisation were admitted to the Manhiça Health Centre. On admission, a more detailed clinical history and medical examination was done and noted on standardised forms by a doctor. Results of laboratory investigations and the final diagnosis were recorded on discharge. Clinical management was undertaken according to standard national guidelines.

We undertook active detection of infection in cohort 2. 4 weeks before the start of surveillance for malaria infection, asymptomatic parasitaemia was cleared presumptively with a combination of amodiaquine (10 mg/kg orally for 3 days) and sulfadoxine-pyrimethamine (one oral dose of sulfadoxine 25 mg/kg and pyrimethamine 1·25 mg/kg). We checked for absence of parasitaemia 2 weeks later and treated positive cases with second-line treatment and excluded them from further assessment for active detection of infection. Surveillance started 14 days after dose three and was done every 2 weeks for the following 2·5 months and then monthly for a further 2 months (figure 1). At every visit, a field worker visited the child at home, completed a brief morbidity questionnaire, and recorded the axillary temperature. If the child was afebrile, blood was obtained by fingerprick onto slides and filter paper. If the child was found to have fever or a history of fever they were accompanied to the Health Post, at which he or she was examined and blood slides collected. All children with a positive slide from the active detection of infection were treated irrespective of symptoms.

We did a cross-sectional survey 6·5 months after dose three in both cohorts. During that visit, axillary temperature and spleen size (Hackett's scale) were measured and a blood slide prepared.

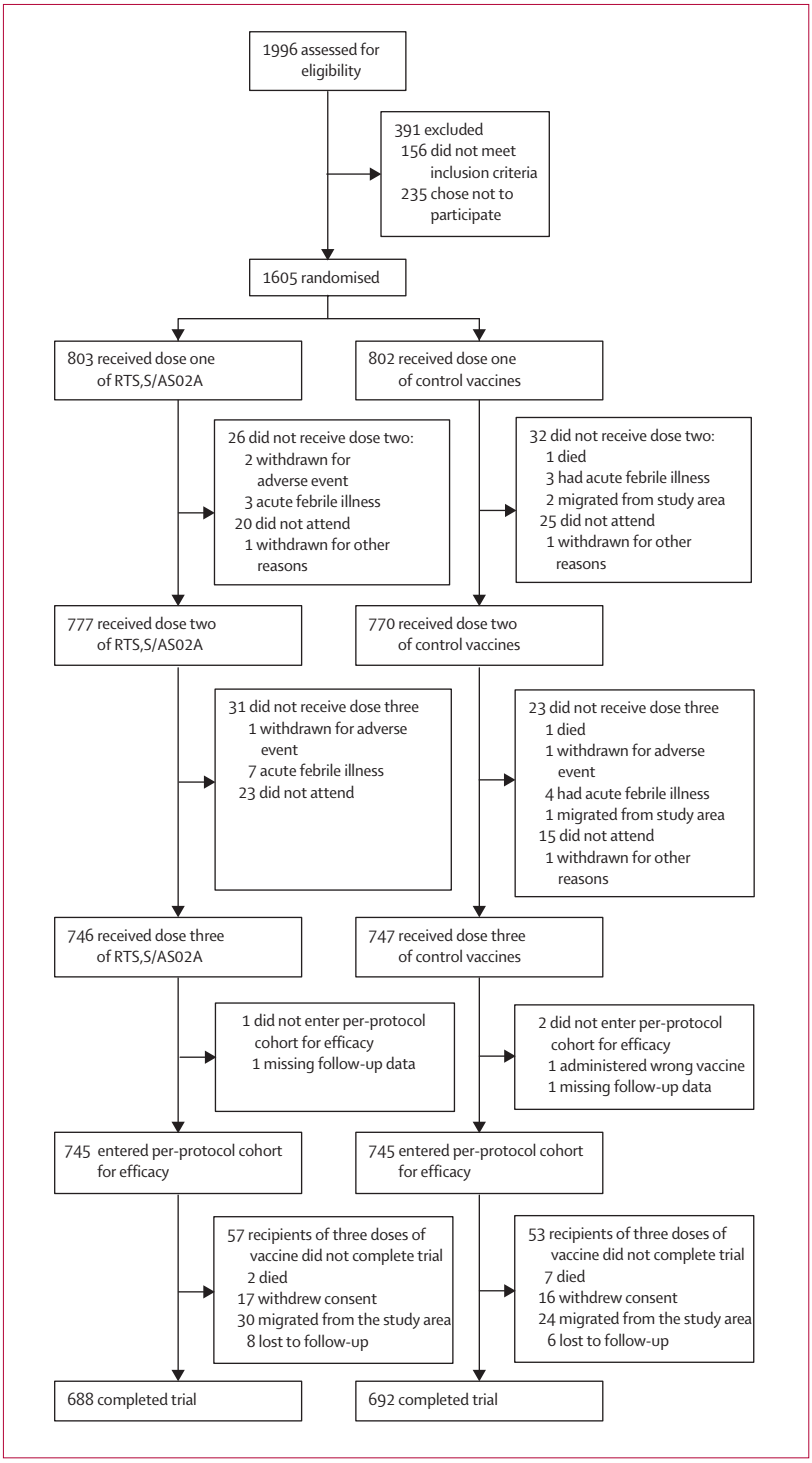

Figure 2: **Trial profile for cohort 1**

To establish parasite presence and density of *P falciparum* asexual stages, Giemsa-stained blood slides were read following standard quality-controlled procedures.[12] External validation was done at the Hospital Clinic of Barcelona, Spain. We measured biochemical variables with a dry biochemistry photometer VITROS DT II (OrthoClinical Diagnostics, Johnson and Johnson, Rochester, NY, USA). We did haematological tests with a Sysmex KX-21N cell counter (Kobe, Japan). We measured packed-cell volume in heparinised microcapillary tubes with a Hawksley haematocrit reader after centrifugation with a microhaematocrit centrifuge.

Antibodies specific for the circumsporozoite protein tandem repeat epitope were assessed by a standard ELISA with plates adsorbed with the recombinant antigen R32LR that contains the sequence [NVDP(NANP)15]2LR with a standard serum as a reference.[13] We established the presence of HBsAg antibody by ELISA with a commercial kit and measured anti-HBsAg concentrations by ELISA with a commercial kit. For the indirect fluorescent antibody test, we incubated 25 μL of test sera (two-fold serial dilutions up to 1/81 920) with *P falciparum* infected red-blood cells fixed onto a slide. Positive reactions were revealed with fluorescein isothiocyanate-labelled secondary antibody Evans blue. The highest dilution giving positive fluorescence under an ultraviolet light microscope was scored.

Statistical analysis

Analysis of this trial strictly adhered to a detailed report and analysis plan established before unmasking. The primary endpoint, assessed in cohort 1, was time to first clinical episode of symptomatic *P falciparum* malaria. We defined a clinical episode as a child who presented to a health facility with an axillary temperature of 37·5°C or more and presence of *P falciparum* asexual parasitaemia greater than 2500 per μL. This case definition was established at the time of study design, before the start of the trial, based on previous background data from the site, and has been estimated to be 91% specific and 95% sensitive.[14] Secondary and tertiary endpoints included estimation of vaccine efficacy for different definitions of clinical malaria and including multiple episodes, prevalence of infection and anaemia, and time to first *P falciparum* infection in cohort 2. Further exploratory analyses included vaccine efficacy estimates for admissions and severe malaria.

All hospital admissions were independently reviewed by two groups of clinicians to establish a final diagnosis, and discrepancies were resolved by consensus before unmasking. We defined malaria needing admission as *P falciparum* asexual parasitaemia judged to be the sole cause of illness or an important contributing factor. The case definition of severe malaria was derived from WHO's guide to clinical practice.[15] All cases of severe malaria were required to have asexual *P falciparum* parasitaemia and no other more-probable cause of illness. The definition was a composite of severe malaria anaemia (packed-cell volume <15%), cerebral malaria (Blantyre coma score <2), and severe disease of other body systems: multiple seizures (at least two or more generalised convulsions in the previous 24 h),

prostration (defined as inability to sit unaided), hypoglycaemia (<22 mmol/L), clinically suspected acidosis, or circulatory collapse.

Because we undertook a proof-of-concept efficacy trial, we based our primary efficacy assessment on a per-protocol analysis, which included children who met all eligibility criteria, completed the vaccination course, and contributed to the efficacy surveillance. Apart from estimates for all-cause admissions, time at risk was adjusted for absences from the study area and antimalarial drug use. A child did not contribute to time at risk during an absence from the study area of at least 2 weeks. After malaria treatment the child was not at risk for an arbitrary period of 28 days after sulfadoxine-pyrimethamine, 7 days after chloroquine alone, 7 days after quinine alone, 7 days after amodiaquine, and 20 days after artemether+lumefantrine. If a combination of drugs was given, the longest period was used. For the analysis of multiple episodes of clinical malaria, we did not judge a child to be susceptible for 28 days after the previous episode.

For the time to first clinical malaria episode or malaria infection, we assessed vaccine efficacy with Cox regression models and defined it as 1 minus the hazard ratio. We adjusted vaccine efficacy for predefined covariates of age, bednet use, geographical area (administrative divisions), and distance from health centre (as determined by geopositioning of every household with a handheld global positioning system with differential correction). The proportional hazards assumption was investigated graphically, using a test based on the Schoenfeld residuals[16] and time-dependent Cox models.[17] For multiple episodes of clinical malaria and admissions, we assessed the group effect by Poisson regression models with normal random intercepts, including the time at risk as an offset variable. Vaccine efficacy was defined as 1 minus the rate ratio. We report the adjusted vaccine efficacy.

Further exploratory analyses in cohort 1 included malaria requiring admission and severe malaria, for which we compared the difference in proportions of children with at least one episode, using Fisher's exact test. We calculated vaccine efficacy as 1 minus the risk ratio, with exact 95% CI, using StatXact PROCs for SAS (version 6; Cambridge, MA, USA). The difference in anaemia prevalence (packed-cell volume <25%) and the proportion of positive parasite densities at 8·5 months were assessed with Fisher's exact test. We measured the effect of treatment on packed-cell volume and the geometric mean of the positive densities with the non-parametric Wilcoxon test.

We used similar methodology in an intention-to-treat analysis. Time at risk, started from dose one, was not adjusted for absences from the study or drug use, and the estimate of effect was not adjusted for covariates.

We summarised anti-circumsporozoite and anti-HBsAg data by geometric mean titres with 95% CI. Seropositivity rates were calculated for anti-circumsporozite titres (defined as >0·5 EU/mL). We measured seroprotection rates for anti-HBsAg titres (defined as ⩾10 IU/mL). Analyses were done with SAS version 8 (Cary, NC, USA) and STATA version 8.0 (College Station, TX, USA).

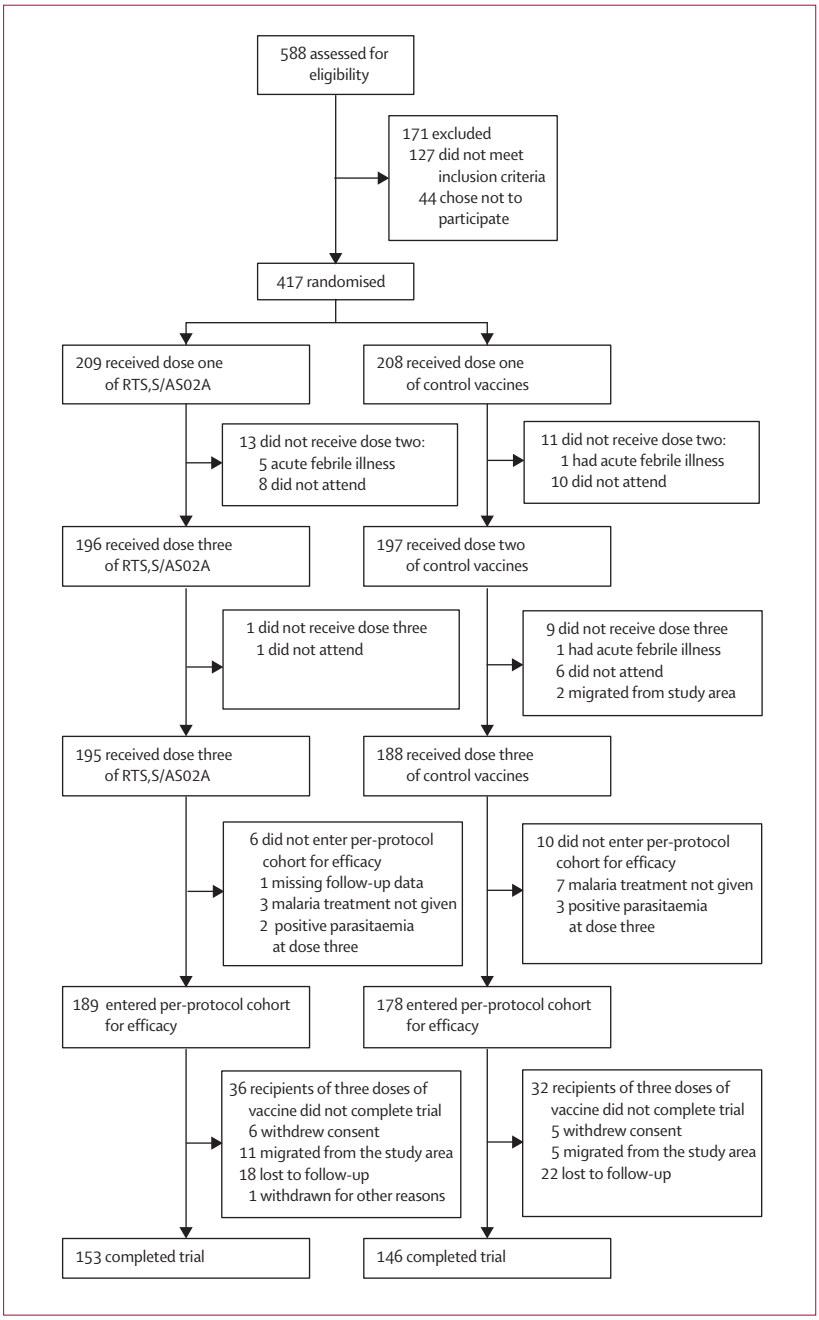

Figure 3: **Trial profile for cohort 2**

Role of the funding source

GSK and CISM both received financial support to undertake the work described in this report from MVI, who have been involved in all aspects of study design (as per authorship guidelines).

	Cohort 1		Cohort 2	
	Control vaccine (n=802)	RTS,S/AS02A (n=803)	Control vaccine (n=208)	RTS,S/AS02A (n=209)
Age at first dose (months)	35·9 (13·4)	35·6 (14·0)	34·9 (13·1)	35·9 (13·0)
Distance to health facility (km)	1·8 (1·1)	1·8 (1·1)	1·8 (1·1)	1·9 (1·2)
Weight for height Z score	0·0 (1·2)	−0·1 (1·3)	0·1 (1·1)	0·1 (1·1)
Geometric mean (95% CI) indirect fluorescent antibody test*	2490 (2084–2976)	2449 (2107–2964)	25 623 (21 360–30 737)	27 496 (22 520–33 571)
Bednet use				
Yes	35 (4%)	37 (5%)	42 (20%)	49 (23%)
No	767 (96%)	766 (95%)	166 (80%)	160 (77%)
HBsAg serostatus				
Positive	39 (5%)	22 (3%)	10 (5%)	9 (4%)
Negative	762 (95%)	776 (97%)	198 (95%)	200 (96%)
No data	1	5 (1%)	0	0
Splenomegaly (Hackett score)				
0	734 (92%)	740 (92%)	159 (76%)	163 (78%)
1	42 (5%)	34 (4%)	27 (13%)	26 (12%)
2–5	25 (3%)	29 (4%)	22 (11%)	18 (9%)
No data	1	0	0	2 (1%)

Data are mean (SD) or number of children (%), unless otherwise indicated. *No data for one person in the control group and two people in the RTS,S/AS02A group in cohort 1 and ten people in the control group and nine in the RTS,S/AS02A group in cohort 2.

Table 1: **Baseline characteristics**

Results

2022 children age 1–4 years were recruited and randomised. Figures 2 and 3 shows the trial profiles for cohorts 1 and 2. Within each cohort, randomisation generated comparable groups of children (table 1). All indicators suggest that malaria transmission intensity was higher in the study area of cohort 2 than cohort 1.

RTS,S/AS02A and control vaccines were safe and well tolerated; more than 92% of children in both groups received all three doses. Local and general solicited adverse events were of short duration and were mostly mild or moderate in intensity. Grade 3 local or general adverse events were uncommon and of short duration. Local injection-site pain that limited arm motion arose after seven (0·2%) doses in the RTS,S/AS02A group and after one (0·03%) dose in the control vaccine group, and injection-site swelling of more than 20 mm happened after 224 (7·7%) and 14 (0·5%) doses, respectively. General solicited adverse events (fever, irritability, drowsiness, anorexia) that prevented normal activities arose after 55 (1·9%) doses in the RTS,S/AS02A group and 23 (0·8%) doses in the control group. At least one unsolicited adverse event was reported by 653 (64·5%) children in the RTS,S/AS02A group and 597 (59·1%) in the control group. Safety laboratory values remained unchanged from baseline over the course of the trial.

429 serious adverse events were reported: 180 (17·8%) in the RTS,S/AS02A group and 249 (24·7%) in the control group. 15 children died during the study, five (0·6%) in the RTS,S/AS02A group and ten (1·2%) in the control group. Four of those who died had malaria as a significant contributing factor and all four were in the control group. No serious adverse event or death was judged to be related to vaccination.

Prevaccination anti-circumsporozoite antibody titres were low in the study children. The vaccine was immunogenic, inducing specific antibody levels after dose three, decaying over 6 months to about a quarter of the initial level, but remaining well above baseline values. Antibody levels in the control group remained low over the follow-up period. The vaccine also induced high levels of antibodies against HBsAg (>97% seroprotection; table 2). For both circumsporozoite and HBsAg, immunogenicity of the vaccine was greater in children younger than 24 months of age.

In the per-protocol analysis done in cohort 1, 282 children had first clinical episodes meeting the primary case definition (123 in the RTS,S/AS02A group and 159 in the control group), yielding a crude vaccine efficacy estimate of 26·9% (95% CI 7·4–42·2; p=0·009; figure 4)

	Control vaccine		RTS,S/AS02A	
	n	Geometric mean titre (95% CI)	n	Geometric mean titre (95% CI)
Anti-circumsporozoite				
In children younger than 24 months				
Baseline	130	0·3 (0·3–0·3)	144	0·3 (0·3–0·3)
30 days after dose three	130	0·3 (0·3–0·3)	144	274 (229–328)
180 days after dose three	130	0·3 (0·3–0·3)	144	52 (43–63)
In children older than 24 months				
Baseline	454	0·3 (0·3–0·3)	457	0·3 (0·3–0·3)
30 days after dose three	454	0·3 (0·3–0·4)	457	158 (142–176)
180 days after dose three	454	0·3 (0·3–0·4)	457	40 (36–45)
Anti-HBsAg				
In children younger than 24 months				
Baseline	42	92 (47–181)	44	63 (37–105)
30 days after dose three	33	68 (34–135)	41	51 035 (27 919–93 292)
180 days after dose three	31	40 (21–76)	33	13 642 (7342–25 347)
In children older than 24 months				
Baseline	142	9 (7–11)	148	9 (7–11)
30 days after dose three	118	350 (237–517)	134	11 369 (8519–15 172)
180 days after dose three	115	153 (111–213)	121	4556 (3500–5932)

Anti-circumsporozoite titres measured in cohort 1. Anti-HBsAg titres measured in cohort 2.

Table 2: **Geometric mean titres (95% CI) for antibodies against circumsporozoite and HbsAg**

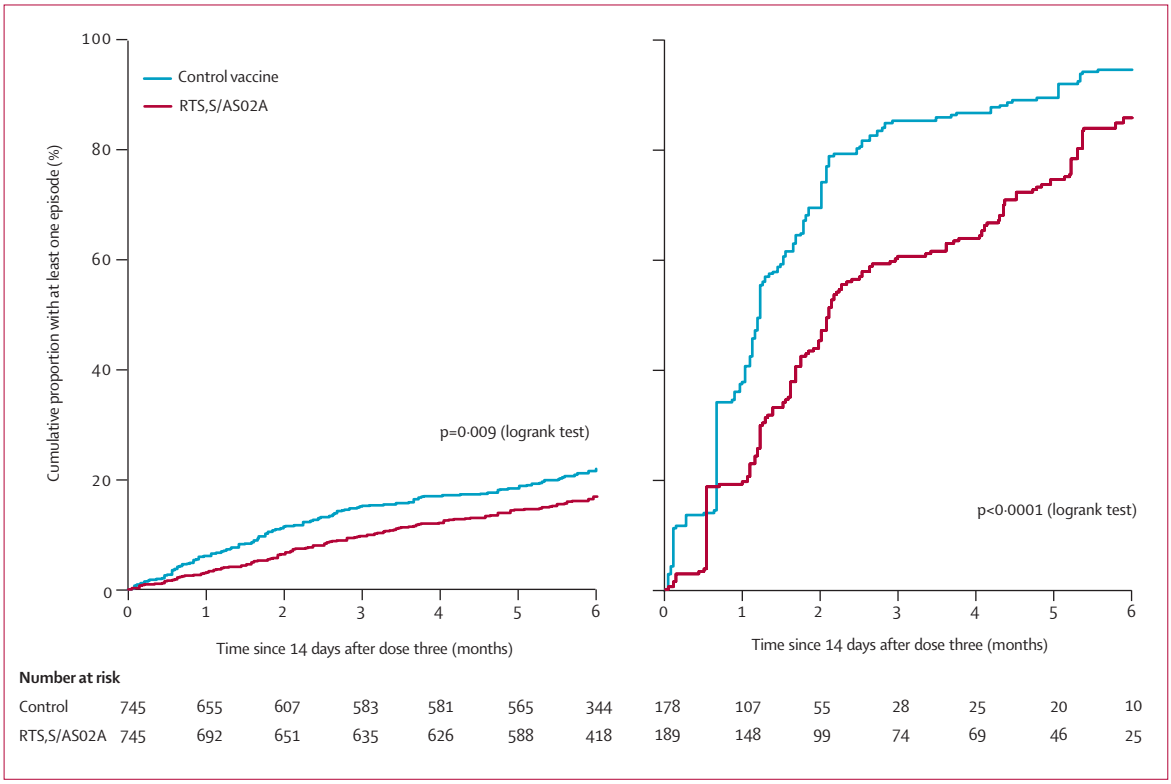

Figure 4: **Kaplan-Meier curves for cumulative proportion with at least one episode of clinical malaria (left) or malaria infection (right)**

and an adjusted estimate of 29·9% (11·0–44·8; p=0·004; table 3). The density of asexual-stage parasites in children with a first episode of clinical malaria was not affected by vaccination because the geometric mean densities at time of presentation were 43 522 per μL for the RTS,S/AS02A group and 41 867 per μL for the control group (p=0·915).

Waning efficacy over the 6-month observation period was not noted for the primary endpoint when analysed by different methods (test for proportionality of hazards with Schoenfeld residuals, p=0·139). Consistent with these data, at the cross-sectional survey 6·5 months after dose three, prevalence of parasitaemia in RTS,S/AS02A recipients was 37% lower than in the control group (11·9% in RTS,S/AS02A *vs* 18·9% in controls, p=0·0003). Parasite densities in these children were similar between RTS,S/AS02A recipients and controls (geometric mean density 2271 *vs* 2513; p=0·699).

Few children had more than one episode of malaria, and vaccine efficacy including all clinical episodes was 27·4% (95% CI 6·2–43·8; p=0·014). The vaccine efficacy estimate did not significantly change for different case definitions based on parasite density cutoffs (table 3). An intention-to-treat analysis of time to clinical disease starting from dose one yielded a vaccine efficacy of 30·2% (14·4–43·0; p=0·0005). All other estimates based on the intention-to-treat analysis were very similar to those derived from the per-protocol cohort. In the per-protocol analysis, 26 incident episodes of anaemia happened

	Control vaccine			RTS,S/AS02A			Vaccine efficacy (95% CI)	p
	Events	PYAR	Rate	Events	PYAR	Rate		
Clinical malaria (cohort 1)								
First episode of fever and parasitaemia >2500 per μL	159	302·9	0·52	123	321·6	0·38	29·9% (11·0 to 44·8)	0·004
First episode of fever and parasitaemia >0 per μL	176	300·5	0·59	137	318·1	0·43	28·6% (10·4 to 43·1)	0·004
First episode of fever or history of fever and parasitaemia >0 per μL	251	284·3	0·88	188	309·0	0·61	33·8% (19·7 to 45·3)	<0·0001
First episode of fever and parasitaemia >15 000 per μL	138	307·8	0·45	104	324·5	0·32	31·7% (11·5 to 47·2)	0·004
First episode of fever and parasitaemia >100 000 per μL	44	324·9	0·14	40	335·3	0·12	16·4% (−29·1 to 45·9)	0·419
Multiple episodes of fever and parasitaemia >2500 per μL	190	330·1	0·58	153	341·0	0·45	27·4% (6·2 to 43·8)	0·014
Malaria infection (cohort 2)								
First episode of parasitaemia >0 per μL	166	25·9	6·42	157	45·0	3·49	45·0% (31·4 to 55·9)	<0·0001

PYAR=person-years at risk. Vaccine efficacy estimates adjusted by age at baseline, bednet use at baseline, distance from health facility, and geographical region.

Table 3: **Vaccine efficacy**

(packed-cell volume <25%) in the RTS,S/AS02A group and 36 in the control group (vaccine efficacy 28·2% [–19·6 to 56·9]; p=0·203). The prevalence of anaemia at month 8·5 was 0·29% (2/692) in the control group versus 0·44% (3/688) in the vaccine group (p=0·686).

In the RTS,S/AS02A group, 11 of 745 children had at least one episode of severe malaria compared with 26 of 745 children in the control group (vaccine efficacy 57·7% [95% CI 16·2–80·6]; p=0·019). In the RTS,S/AS02A group, 42 children with malaria needed admission versus 62 in the control group (vaccine efficacy 32·3% [1·3–53·9]; p=0·053). All-cause admissions were similar between the two groups (79 *vs* 90; vaccine efficacy 14·4% [–19·7 to 38·8]; p=0·362).

Vaccine efficacy in extending time to first infection was determined in cohort 2. 323 children had first episodes of asexual *P falciparum* parasitaemia (157 in the RTS,S/AS02A group and 166 in the control group), yielding a vaccine efficacy estimate of 45·0% (95% CI 31·4–55·9; p<0·0001; figure 4, table 3). The mean density of asexual-stage parasites at the time of first infection was similar for the control and RTS,S/AS02A groups (3950 *vs* 3016 per μL, p=0·354). With the same methods as those used to assess persistence of efficacy for cohort 1, the model with the best fit suggested waning efficacy of the vaccine over time, which stabilised at about 40%. The prevalence of asexual *P falciparum* parasitaemia at the end of follow-up was lower in the RTS,S/AS02A group than in the control group (52·3% *vs* 65·8%; p=0·019), and prevalence of anaemia at month 8·5 was 2·7% in the control group and 0% in the RTS,S/AS02A group (p=0·056).

No interaction was recorded between age and vaccine efficacy, suggesting that efficacy did not change with increasing age. We did, however, do further exploratory subgroup analyses to estimate vaccine efficacy in the youngest children who carry the brunt of malaria disease. In children younger than 24 months of age at the time of dose one, three cases of severe malaria arose in 173 allocated RTS,S/AS02A, whereas 13 cases happened in 173 assigned control vaccines (vaccine efficacy 76·9% [95% CI 27·0–96·9]; p=0·018). Incidence of first episodes of clinical malaria was similarly analysed. 31 episodes of malaria arose in younger children in the RTS,S/AS02A group and 47 in the control group, yielding an incidence of 0·41 episodes per PYAR in the RTS,S/AS02A group and 0·70 episodes per PYAR in the control group (vaccine efficacy 46·7% [14·8–66·7]; p=0·009). Vaccine efficacy against new infections was similar in the older and younger age groups (44·0% *vs* 46·5%).

The relation between circumsporozoite titres and malaria protection was assessed in cohort 1. The hazard ratio per ten-fold increase in circumsporozoite antibody titre was 0·94 (95% CI 0·66–1·33; p=0·708). For the comparison of children in the highest tertile of anti-circumsporozoite response versus those in the lowest tertile, the hazard ratio was 1·38 (0·89–2·12; p=0·150).

Discussion

We have shown that the subunit vaccine RTS,S/AS02A confers protection in young African children against both infection and a range of clinical illness caused by *P falciparum*. This vaccine, based on one pre-erythrocytic antigen that induces partial protection against infection, can reduce morbidity even without a blood-stage component.

Attempts to develop a malaria vaccine go back more than 50 years. Some candidate products have shown promising results and potential.[12,18,19] However, inconsistency with other trials[20,21] and failure of the product to prevent malaria in infants,[22] together with limitations in product availability or reproducibility, resulted in termination of development of these candidate vaccines.[23]

In young African children, RTS,S/AS02A was well tolerated, and its reactogenicity profile was similar to that recorded in previous paediatric trials of this vaccine (Macete E; Bojang KA; unpublished data). Local and general symptoms were more frequent than in the control vaccine group, but did not lead to withdrawals. The vaccine was also safe; children who received RTS,S/AS02A had fewer all-cause serious adverse events, admissions, and severe complications from malaria than did those in the control group. As has been seen in other intervention trials, the mortality rate in our study participants was lower than historical background mortality rates in this population.[8]

Despite high levels of exposure to *P falciparum* sporozoites, naturally occurring circumsporozoite antibody levels in this population were low. The vaccine was highly immunogenic, especially in children younger than 24 months. Antibody levels decayed by about 75% over 6 months, but at the end of the follow-up period they were still well above preimmunisation levels. In RTS,S/AS02A recipients we failed to detect an association between level of circumsporozoite antibody and risk of malaria. However, the high titres achieved by nearly all vaccine recipients and the possibility that a relatively low threshold protective level of immunity might exist potentially constrained this analysis. Also, the vaccine is known to induce cell-mediated responses believed to be involved in protection that were not measured in this study.[24]

The vaccine's efficacy against infection accords with previous vaccine efficacy estimates in both malaria-naive volunteers[7] and Gambian adults[6] and with the known ability of this pre-erythrocytic vaccine to neutralise sporozoites and limit the number of infected hepatocytes or liver-stage merozoites that enter the bloodstream. The vaccine was efficacious against a range of endpoints including infection and protection against mild uncomplicated disease, malaria admissions, and severe malaria. Although efficacy seems to be highest in younger children and for the most severe endpoints, CIs for the different endpoints overlap, and noted differences could be attributable to chance. The recorded protection against different endpoints suggests that the

more easily measured infection endpoint might serve as a surrogate for vaccine efficacy against clinical disease.

We were surprised not to see a difference in cases of anaemia. Although fewer events arose in children allocated RTS,S/AS02A vaccine than in those assigned control, the rates of malaria anaemia during the study were much lower than expected, which limited our ability to detect significant vaccine efficacy for this endpoint. Intense prompting of mothers or guardians to take their children to health facilities early on in the disease process might have ensured prompt treatment of malaria cases and reduced the incidence of anaemia. Furthermore, in November, 2002, Mozambique switched to a more effective first-line treatment for malaria, and children who received these drugs had more rapid clearance of parasites, less recrudescence, and therefore shorter duration of infections than did children who did not receive these new drugs. Each of these interventions could have had an effect on the recorded incidence of anaemia.

The statistical methods we used showed no evidence of waning efficacy against clinical disease and limited waning efficacy against infection. At the last cross-sectional survey a significant difference in prevalence of infection was recorded. The evidence that protection is sustained differs from findings of trials in malaria-naive volunteers or Gambian adults.[6,25] Two main explanations are available for these apparently conflicting results. First, the vaccine was much more immunogenic in this study population than it was in adults, and sustained immune responses might have resulted in persistent protective efficacy. Second, the high level of sporozoite exposure that happened during this trial could have resulted in natural boosting of protective immune responses not revealed by antibody measurements.

Efficacy against severe malaria was 58%, with indications it might be higher in the youngest children. Although the definition of severe malaria is a matter of continuous discussion, little doubt exists that classification of children according to the WHO-based definition identifies children who are very sick and at high-risk of dying. More precise estimates of efficacy against severe malaria in the target population will need to be calculated in larger trials and different settings. This study population remains under surveillance to monitor both long-term safety and duration of vaccine efficacy.

Our results indicate the feasibility of development of an effective vaccine against malaria. They also highlight the potential of modern vaccinology to develop new prophylactic interventions against complex human parasites. Development of an effective malaria vaccine can be accelerated through international partnerships between private and public sectors, including scientific institutions in endemic countries. In combination with existing and other promising new malaria-control measures, malaria vaccines could greatly contribute to reducing the intolerable global burden of this disease.

Contributors

All authors participated in the design, implementation, analysis and interpretation of the study. P Alonso, J Sacarlal, and J J Aponte were involved in all phases of the study. A Leach and N Tornieporth led the clinical team at GSK Biologicals. J J Aponte and B Spiessens led data analysis. J Sacarlal, P Aide, Q Bassat, and E Macete were responsible for field and hospital activities and safety surveillance. J Milman, M M Navia, and C Guinovart were programme managers. I Mandomando and M Espasa coordinated all laboratory work at CISM. S Corachan was the central study coordinator and O Ofori-Anyinam was the clinical development manager at GSK Biologicals. M-C Dubois is the malaria vaccine project manager at GSK Biologicals. M-A Demoitié coordinated the immunology read-out team at GSK Biologicals. M Ceuppens was responsible for safety at GSK Biologicals. R Thompson and C Menéndez contributed to design of the study, implementation, and interpretation. J Cohen heads malaria Vaccine Research and Development at GSK Biologicals. The report was written by P Alonso, R Ballou, and F Dubovsky, with input from all other investigators.

Conflict of interest statement

MVI supports the development and testing of several malaria vaccines that can be seen as competitors. AL, BS, OO-A, SC, M-CD, M-AD, NT, WRB, and JC are employees of GSK Biologicals. MC was employed by GSK Biologicals until April, 2004; he is now employed by Bristol-Myers Squibb. AL, WRB, NT, and JC own shares in GSK. WRB has had the costs of travel paid by healthcare companies other than GSK within the past 3 years. JC and WRB are listed as the inventors of patented malaria vaccines; however, neither individual holds a patent for a malaria vaccine. None of the other authors in this paper have declared conflicts of interest.

Acknowledgments

GSK and CISM both received financial support to undertake the work described in this report from MVI, who have been involved in all aspects of study design (as per authorship guidelines). The initial support to CISM by MVI was passed through GSK for administrative reasons. CISM is supported by members of the collaborative programme (Ministry of Health, Mozambique; Universidad Eduardo Mondlane, Mozambique; and Hospital Clinic, Universidad de Barcelona, Spain) and core funding is provided by the Spanish Agency for International Cooperation (AECI–Ministry of Foreign Affairs, Madrid, Spain). We thank the study children and their parents, and the entire Manhiça community for their continuous support to this work and all other activities that CISM has undertaken since 1996; the staff at CISM and at the Centre for International Health, Hospital Clinic in Barcelona, particularly Antoni Trilla, Prof Joan Rodés, and Gonzalo Vicente; Josep Costa who did the hepatitis B antigen determination; and Carlota Dobaño who did the indirect fluorescent antibody test; the microbiology department at the Hospital Clínic in Barcelona, which undertook quality control of blood slide reading for *P falciparum*; staff of the Malaria Project team at GSK, in particular, Remon Abu Elyazeed, François Beckers, Arthur Berger, Cornelia Bevilacqua, Conor Cahill, Philippe Dehottay, Steve Fitzpatrick, Marguerite Koutsoukos, Gilberte Liebaut, Andreas Pakendorf, Nicholas Perombelon, Nicole Riley, Christine Swysen, Yves Tellier, and Marie Chantal Uwamwezi; Melinda Moree (director of MVI) and Carol Hooks (senior communications officer); Regina Rabinovich (director, Infectious Diseases Program, Bill and Melinda Gates Foundation); Esperança Sevene, who was the local safety monitor for this trial; Prof Malcolm Molyneaux, who was chairman of the data and safety monitoring board; and the District, Provincial, and National Health Authorities, particularly ex-Prime Minister Pascoal Mocumbi and minister Francisco Songane.

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ARTICLE 3: Alonso PL, **Sacarlal J,** Aponte JJ, Leach A, Macete E, Aide P, et al; Duration of protection with RTS,S/AS02A malaria vaccine in prevention of Plasmodium falciparum disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. **Lancet. 2005** Dec 10;366(9502):2012-8. PMID: 16338450

Duration of protection with RTS,S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial

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Summary

Background **RTS,S/AS02A is a pre-erythrocytic stage malaria vaccine that provides partial protection against infection in malaria-naive adult volunteers and hyperimmune adults. A previous report showed that this vaccine reduced risk of clinical malaria, delayed time to new infection, and reduced episodes of severe malaria over 6 months in African children. An important remaining issue is the durability of protection against clinical disease in these children.**

Methods **We did a randomised, controlled, phase IIb trial of RTS,S/AS02A given at 0, 1, and 2 months in 2022 Mozambican children aged 1–4 years. We previously determined vaccine efficacy (VE) against clinical malaria in a double-blind phase that included study months 2·5–8·5 ($VE_{2·5–8·5}$). We now report VE in a single-blind phase up to month 21 ($VE_{8·5–21}$). The primary endpoint was time to first or only clinical episode of *Plasmodium falciparum* malaria (axillary temperature ⩾37·5°C and *P falciparum* asexual parasitaemia >2500 per μL) detected through a passive case detection system. We also determined VE for other case definitions and for episodes of severe malaria. This study is registered with the ClinicalTrials.gov identifier NCT00197041.**

Findings **During the single-blind phase, $VE_{(8·5–21)}$ was 28·9% (95% CI 8·4–44·8; p=0·008). At month 21, prevalence of *P falciparum* infection was 29% lower in the RTS,S/AS02A group than in the control (p=0·017). Considering the entire study period, $VE_{(2·5–21)}$ was 35·3% (95% CI 21·6–46·6; p<0·0001) and $VE_{(2·5–21)}$ for severe malaria was 48·6% (95% CI 12·3–71·0; p=0·02).**

Interpretation **These results show that RTS,S/AS02A confers partial protection in African children aged 1–4 years living in rural endemic areas against a range of clinical disease caused by *P falciparum* for at least 18 months, and confirm the potential of malaria vaccines to become credible control tools for public-health use.**

Introduction

The last two decades of the 20th century generated key scientific knowledge and a new impetus for malaria research that provided unprecedented opportunities to develop a malaria vaccine. As a result, the first years of the 21st century are already witnessing breakthroughs in the global effort to develop a malaria vaccine that could contribute to the control of this devastating disease. Several candidate vaccines are under development and some have reached field testing in endemic areas.[1] RTS,S/AS02A, developed by GlaxoSmithKline (GSK) in collaboration with the Walter Reed Army Institute of Research, targets the pre-erythrocytic stage of *Plasmodium falciparum*, and confers complete or partial protection in most volunteers against infection by sporozoites delivered via laboratory-reared infected mosquitoes in malaria-naive adult volunteers.[2,3]

In 2001, GSK entered into a partnership with the PATH Malaria Vaccine Initiative, to develop RTS,S/AS02A as a vaccine to prevent malaria disease in children. The clinical development plan was designed to lead to immunisation in the context of the Expanded Program on Immunization, and included consecutive phase I and phase IIb studies in The Gambia[4] and Mozambique (Macete E, unpublished). These and other studies showed that the vaccine was safe, well-tolerated, and immunogenic. Selection of a paediatric vaccine dose led to a phase I study and a phase IIb proof-of-concept efficacy study in children aged 1–4 years living in an endemic and rural area of southern Mozambique. During the 6 months' follow-up of this randomised double-blind, controlled trial, immunisation with RTS,S/AS02A was associated with a 29·9% (95% CI 11·0–44·8; p=0·004) reduction in the risk of clinical malaria, delayed time to first infection by 45% (95% CI 31·4–55·9; p<0·0001) and reduced incidence of severe malaria by 57·7% (95% CI 16·2–80·6%; p=0·019).[5] Statistical modelling suggested no significant waning of efficacy. We now report the results of a further 12 months of follow-up in these children.

Methods

Study design

The trial is being done at the Centro de Investigação em Saúde da Manhiça (CISM; Manhiça Health Research Centre), in Manhiça District (Maputo Province), in southern Mozambique. The characteristics of the area have been described in detail elsewhere.[6] The climate is subtropical with two distinct seasons: a warm and rainy season from November to April, and a generally cool and dry season during the rest of the year. Perennial malaria transmission with marked seasonality is mostly due to *P falciparum*. *Anopheles funestus* is the main vector and the estimated entomological inoculation rate for 2002 was 38.

We have previously reported the results of the first 6 months of follow-up of the double-blind phase of the study, where the primary objective was to estimate vaccine efficacy against *P falciparum* malaria in children aged 1–4 years at first vaccination over a 6-month surveillance period starting 14 days after dose 3 (study months 2·5–8·5).[5] The trial involved two cohorts based at two different sites. All vaccine efficacy estimates for clinical malaria endpoints were derived from cohort 1, based at Manhiça and Maragra. According to protocol, during the single-blind phase (study months 8·5–21), cohort 1 has been followed up for an additional 12 months for safety, immunogenicity, and efficacy. Cohort 2, based at Ilha Josina, was drug-treated to clear parasitaemia before dose 3, had follow-up for the detection and treatment of all new infections during study months 2·5–8·5 and subsequently has been followed-up for safety and immunogenicity. Thus, all children have been followed-up during the single-blind phase of this study (study months 8·5–21).

The protocol was approved by the National Mozambican Ethics Review Committee, the Hospital Clinic of Barcelona Ethics Review Committee, and the PATH Human Subjects Protection Committee. The trial was done according to the International Conference on Harmonisation good clinical practice guidelines, and was monitored by GSK Biologicals. A local safety monitor and a data and safety monitoring board closely reviewed the conduct and safety data of the trial.

Screening, randomisation, and immunisation were done as previously reported.[5] 2022 children aged 1–4 years were recruited and randomised to receive three doses of either RTS,S/AS02A candidate malaria vaccine or a control vaccination regimen. Details of the candidate malaria vaccine, the controls, and the trial profile for the double-blind phase have been presented previously.[5] The randomisation code was released to the investigators once databases had been monitored, cleaned, and locked after completion of follow-up. One copy of the code was kept by the head statistician (JJA) and no other members of the investigators team had access to it. From then on, the trial was formally in its single-blind phase. However, all field workers and clinical and laboratory staff involved in the follow-up of study participants remained blinded and had no access to the code. No new immunisations were administered during the single-blind phase.

Procedures

Serious adverse events were reported throughout the study period in both cohorts. 60 days after dose 3, monthly home visits began, during which residence status was checked and unreported serious adverse events documented. Blood was obtained from participants at month 21. Haematological and biochemical variables, including complete blood count, creatinine, alanine aminotransferase, and bilirubin were assessed, as well as antibodies against circumsporozoite protein (cohort 1) and HBsAg (cohort 2).

Efficacy assessment for cohort 1 was based at Manhiça Health Center and Maragra Health Post. All children reporting fever within the preceding 24 h or with a documented fever (axillary temperature $\geqslant$37·5°C) had blood collected for determination of malaria parasites in duplicate thin and thick blood smears as well as a microcapillary tube for determination of the packed-cell volume. Children with clinical conditions warranting hospitalisation were admitted to the Manhiça Health Centre. On admission, a more detailed clinical history and medical examination was done and results recorded on standardised forms by a physician. Results of laboratory investigations and the final diagnosis were recorded on discharge. Clinical management was done following standard national guidelines.

A cross sectional survey was done 21 months after dose 1 in both cohorts. During that visit the axillary temperature was determined, a blood sample was taken, and a blood slide was prepared. All laboratory methods used have been previously described.[5]

Definitions and statistical methods

The analysis of this trial adhered to a detailed report and analysis plan developed and finalised before locking databases and analysing data. A clinical episode was defined as a child who presented to a health facility with an axillary temperature of 37·5ºC or greater and the presence of *P falciparum* asexual parasitaemia of greater than 2500 per μL. Other endpoints included the estimation of vaccine efficacy for different definitions of clinical malaria, including multiple episodes, prevalence of infection, and anaemia. Further exploratory analysis included vaccine efficacy estimates for hospital admissions and severe malaria.

As previously described, all hospital admissions were independently reviewed by two groups of clinicians to establish a final diagnosis. Discrepancies were resolved in a consensus meeting before the database of the single-blind phase was locked. Malaria requiring hospital admission was defined as a child with *P falciparum* asexual parasitaemia where malaria was

judged to be the sole cause of illness or a substantial contributing factor. The case definition of severe malaria has been previously described[5] and was derived from WHO's guide to clinical practice.[7]

Several groups of participants, with different periods of follow-up, were analysed in this study. Vaccine safety was assessed for all study participants (cohort 1 and 2) that received at least one dose, by intention-to-treat (ITT) analysis. $VE_{(8\cdot5-21)}$ was the estimate derived from the according-to-protocol $(ATP)_{(8\cdot5-21)}$ analysis based on participants who met all eligibility criteria, completed the vaccination course, and contributed to the efficacy surveillance from 6 months after dose 3 until month 21 regardless of whether they had an episode of clinical malaria during the double-blind phase. $VE_{(2\cdot5-21)}$ was derived from the $ATP_{(2\cdot5-21)}$ analysis that included participants who met all eligibility criteria, completed the vaccination course, and contributed to the efficacy analysis starting 14 days after dose 3 until month 21.

For the time to first clinical malaria episode, VE was assessed with Cox regression models and was defined as 1 minus the hazard ratio. VE was adjusted for predefined covariates of age, bed-net use, geographical area (administrative divisions) and distance from health centre. For multiple episodes of clinical malaria and hospital admissions, the group effect was assessed with Poisson regression models with normal random intercepts, including the time at risk as an off-set variable. Except for severe malaria and malaria requiring hospital admission, the adjusted VE is reported throughout the text. Except in estimates for all-cause hospital admissions, the time at risk was adjusted for absences from the study area and antimalarial drug use.[5]

Further exploratory analyses in cohort 1 included malaria requiring hospital admission and severe malaria, for which the difference in proportions of children with at least one episode were compared with Fisher's exact test. VE was calculated as 1 minus the risk ratio, with exact 95% CI using StatXact PROCs for SAS (version 6; Cambridge, MA, USA). The difference in prevalence of anaemia (packed cell volume <25%) and the proportion of positive parasite densities at month 21 were evaluated with Fishers exact test. The effect of the treatment on haematocrit values and geometric mean of the positive densities were evaluated with the nonparametric Wilcoxon test.

Similar methods were used to estimate VE in an $ITT_{(0-21)}$ analysis. Time at risk started from dose 1, and was not adjusted for absences from the study area or drug usage. The estimate of effect was not adjusted for covariates.

Concentrations of antibodies against circumsporozoite protein were summarised as geometric mean titres with 95% CI. Immunogenicity against HBsAg was summarised by seroprotection levels (≥10 mIU/mL). Analyses were done with SAS version 8 (Cary, NC, USA) and STATA version 8·0 (College Station, TX, USA).

This study is registered with the ClinicalTrials.gov identifier NCT00197041.

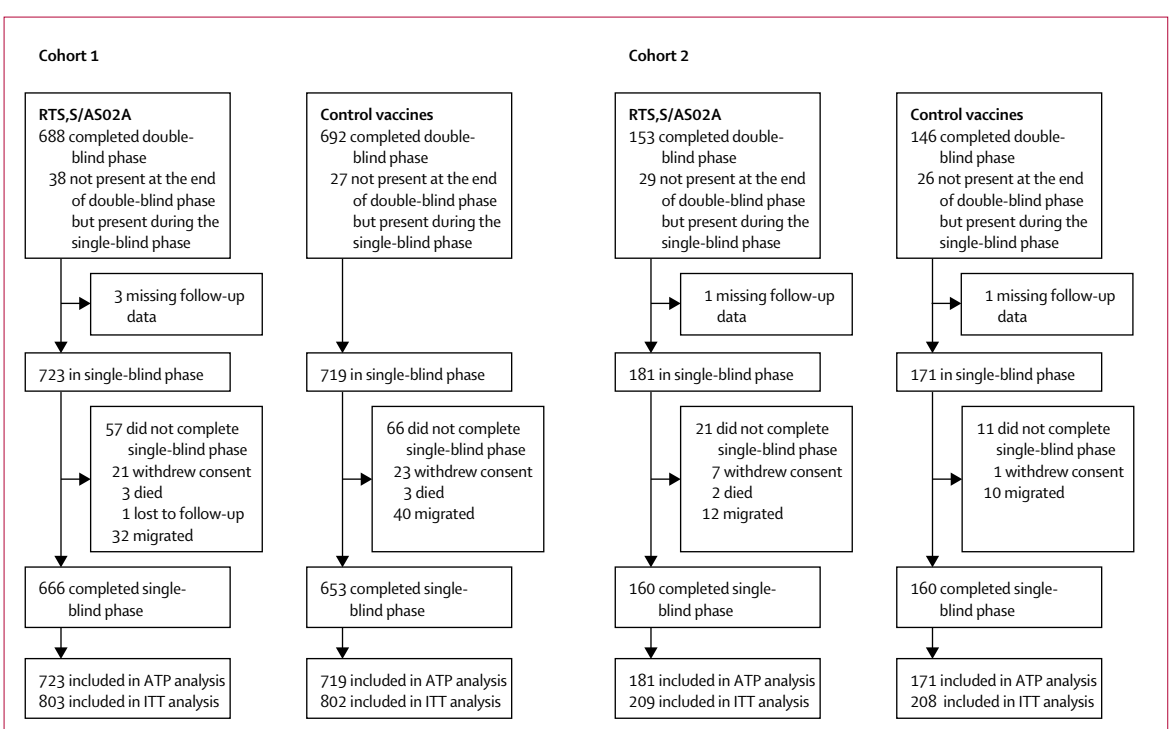

Figure 1: **Trial profile**

	Control vaccine (n=719)			RTS,S/AS02A (n=723)			Vaccine efficacy (95% CI)	p
	Events	PYAR	Rate	Events	PYAR	Rate		
First or only episode of fever and parasitaemia >2500/μL	140	587·5	0·238	110	618·9	0·178	28·9% (8·4–44·8)	0·008
First or only episode of fever and parasitaemia >0/μL	154	581	0·265	131	610·9	0·214	23·3% (2·9–39·4)	0·027
First or only episode of fever or history of fever and parasitaemia >0/μL	229	540·4	0·424	181	581·5	0·311	30·1% (14·9–42·6)	0·0004
First or only episode of fever and parasitaemia >15000/μL	128	592·9	0·216	96	625·7	0·153	31·6% (10·6–47·7)	0·005
Several episodes of fever and parasitaemia >2500/μL	193	642·30	0·301	157	663·5	0·237	28·8% (6·2–45·9)	0·016

PYAR=Person-years at risk. Vaccine efficacy estimates adjusted by age at baseline, bednet use at baseline, distance from health facility, and geographical region.

Table 1: **Vaccine efficacy during single-blind phase ($ATP_{8\text{-}21}$) in cohort 1 by outcome**

Role of the funding source

The sponsors of the study were involved in study design, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The trial profiles for the participants in the single-blind phase are shown in figure 1. Vaccine safety during the single-blind phase ($ITT_{(8\cdot5-21)}$) was similar to that previously reported for the double blind phase. 111 serious adverse events were reported among 5·1% (50 of 974) of the participants in the RTS,S/AS02A group versus 138 among 6·8% (66 of 965) of controls. Most of these events were common childhood diseases in Mozambique. During this time there were eight deaths; five in the RTS,S/AS02A group and three in the control group. Two of these deaths were judged to be related to malaria and both occurred in the RTS,S/AS02A group. No serious adverse event or death was judged to be related to vaccination. Assessment of biochemical and haematological variables showed no imbalance between the RTS,S/AS02A and comparator groups (data not shown).

	Control vaccine (n=719) frequency (%)	RTS,S/AS02A (n=723) frequency (%)
None	579 (80·5%)	613 (84·8%)
1	98 (13·6%)	75 (10·4%)
2	34 (4·7%)	25 (3·5%)
3	5 (0·7%)	8 (1·1%)
4	3 (0·4%)	2 (0·3%)

Table 2: **Frequency of episodes per child in each group during single-blind phase ($ATP_{8\text{-}21}$) in cohort 1**

Concentrations of antibodies against circumsporozoite protein measured in cohort 1 continued to fall during follow up, but at month 21 remained nearly 50 times higher in the RTS,S/AS02A group (geometric mean titre 14·0, 95% CI 12·5–15·6) than in controls (0·3, 0·3–0·3). Concentrations of anti-HBsAg antibody were measured for cohort 2, and in the RTS,S/AS02A group, 173 of 176 (98·3%, 95% CI 95·1–99·6) of participants remained seroprotected at month 21.

In the $ATP_{(8\cdot5-21)}$ analysis of vaccine efficacy, 250 children had first or only clinical episodes meeting the primary case definition. Of these, 110 were in the RTS,S/AS02A group and 140 in the control group, yielding a crude vaccine efficacy estimate of 25·4% (95% CI 4·3–41·9; p=0·021) and an adjusted $VE_{(8\cdot5-21)}$ of 28·9% (8·4–44·8; p=0·008; table 1). The VE estimate did not significantly change for different case definitions based on parasite density cutoffs (table 1). 350 episodes of malaria met the primary case definition, and relatively few children had more than one episode (table 2). The adjusted VE including all clinical episodes was 28·8% (95% CI 6·2—45·9; p=0·016; table 1). In the RTS,S/AS02A group (n=723) there were eight children who had at least one episode of severe malaria; in the control group (n=719) there were 13 children (VE=38·8%, 95% CI –49·2 to 76·6; p=0·282). In the RTS,S/AS02A group, 20 children had malaria that required hospital admission, compared with 28 in the control group (VE=29·0%, 95% CI –25·4 to 60·3; p=0·244). The two groups had similar numbers of all-cause hospital admissions (RTS,S/AS02A group 41, controls 48; VE=18·6%, 95% CI –30·6 to 49·3; p=0·393). We noted no evidence of an interaction between age at first dose and VE, suggesting that efficacy did not significantly change with increasing age (p=0·22 for the interaction test).

	Control Vaccine (n=745)			RTS,S/AS02A (n=745)			Vaccine efficacy (95%CI)	p
	Events	PYAR	Rate	Events	PYAR	Rate		
First or only episode of fever and parasitaemia >2500/μL	251	780·0	0·322	186	857·9	0·217	35·3% (21·6–46·6)	<0·0001
First or only episode of fever and parasitaemia >0/μL	274	761·0	0·360	213	836·6	0·255	31·8% (18·1–43·1)	<0·0001
First or only episode of fever or history of fever and parasitaemia >0/μL	368	672·9	0·547	271	776·1	0·349	38·3% (27·6–47·4)	<0·0001
First or only episode of fever and parasitaemia >15 000/μL	225	804·7	0·280	164	878·7	0·187	36·0% (21·4–47·8)	<0·0001
Several episodes of fever and parasitaemia >2500/μL	384	972·1	0·395	310	1004·5	0·309	29·8% (13·8–42·8)	0·0008

PYAR= Person-years at risk. Vaccine efficacy estimates adjusted by age at baseline, bednet use at baseline, distance from health facility, and geographical region.

Table 3: **Vaccine efficacy during complete follow-up period ($ATP_{2\text{-}21}$) in cohort 1 by outcome**

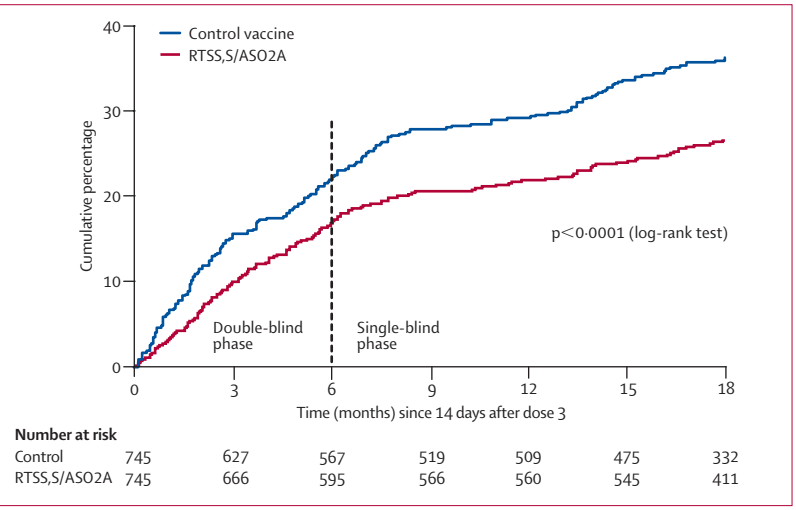

Figure 2: **Kaplan-Meier curves for the cumulative proportion of children with at least one episode of clinical malaria**
Double-blind phase corresponds to study months 2·5–8. Single blind phase corresponds to study months 8–21.

In the $ATP_{(2·5–21)}$ analysis, 437 children had first or only clinical episodes meeting the primary case definition. Of these, 186 were in the RTS,S/AS02A group and 251were in the control group, yielding a $VE_{(2·5–21)}$ of 35·3% (95% CI 21·6–46·6; p<0·0001; table 3). The corresponding Kaplan-Meier curves are shown in figure 2. The VE estimate did not significantly change for different case definitions based on parasite density cutoffs (table 3). 46 incident episodes of anaemia occurred in the RTS,S/AS02A group and 58 in the control group (VE=25·5%; 95% CI –10·5 to 49·7; p=0·143). In the RTS,S/AS02A group (n=745) there were 19 children who had at least one episode of severe malaria, whereas in the control group (n=745) there were 37 children (VE=48·6%; 95% CI 12·3–71·0; p=0·02). In the RTS,S/AS02A group, 57 children had malaria that required hospital admission,compared with 82 in the control group (VE=30·5%, 95% CI 4·1–49·9; p=0·032). Similar numbers of all-cause hospital admissions occurred in the two groups (RTS,S/AS02A group 120, controls 138; VE=19·3%, 95% CI –9·4 to 40·5; p=0·112). We noted no evidence of an interaction between age at first dose and VE, suggesting that efficacy did not significantly change with increasing age (p=0·89 for the interaction test).

In the $ITT_{(0–21)}$ analysis, over the entire course of the study, there were 532 children with first or only clinical episodes meeting the primary case definition. Of these, there were 224 in the RTS,S/AS02A group (n=803) and 308 in the control group (n=802), yielding an $ITT_{(0–21)}$VE estimate of 32·8% (95% CI 20·1–43·4; p<0·0001). During the same period, there were 918 clinical episodes of malaria, of which 391 were in the RTS,S/AS02A group and 527 were in the control group, yielding a VE estimate for multiple episodes of 32·4% (95% CI 17·6–44·5; p<0·0001). At least one episode of severe malaria occurred in 24 children in the RTS,S/AS02A group and 43 children in the control group (VE=44·3%, 95% CI 9·5–66·4; p=0·018).

At the cross-sectional survey in cohort 1 at the end of the single-blind follow-up period (month 21), the prevalence of anaemia was very low (none of 649 children in the RTS,S/AS02A group, two of 663 in the control group; p=0·5). The prevalence of *P falciparum* asexual parasitaemia was 29% lower in the RTS,S/AS02A group (77 of 666, 11·6%) than in the control group (106 of 653, 16·2%; p=0·017). Parasite densities were similar in RTS,S/AS02A recipients and controls (geometric mean density 1940 *vs* 1571; p=0·575). In cohort 2, the prevalence of asexual *P falciparum* parasitaemia was 68·8% (50 of 160) in the RTS,S/AS02A group compared with 69·4% (49 of 160) in the control group (p=1·0).

Discussion

Our results show that the RTS,S/AS02A candidate malaria vaccine confers partial protection for at least 18 months against a range of clinical diseases caused by *P falciparum* in children living in a rural malaria endemic area of African.

This report includes data obtained over 21 months of this controlled trial. Randomisation yielded comparable groups. The initial period of follow-up included 6 months after the third dose. The code was then opened for analysis, and the double-blind nature of the trial was lost. However, no members of the research team other than the senior statistician had access to the code and so to all purposes, both the teams in the field as well as the families of the study participants remained blind as to the allocation to RTS,S/AS02A or comparator. We therefore think that it is reasonable to believe that no bias was introduced.

A meta-analysis of the different vaccine candidates that have undergone clinical trials in the malaria endemic areas has been done by the Cochrane Collaboration.[1] Since its last update in 2003, one more trial has been done. A DNA/MVA ME-TRAP candidate vaccine failed to prevent new infections in Gambian adult male volunteers.[8]

Results from previous trials of RTS,S/AS02A in malaria-naive volunteers or hyperimmune Gambian adults suggested that protection against infection induced by this vaccine might be short-lived.[9,10] Similarly, it has been argued that vaccine efficacy reported for the first 6 months of follow-up of this trial was compatible with a short period of protection covering only 8–12 weeks after the third dose.[11] By contrast, the results of this extended follow-up show that vaccine efficacy did not wane and that protection against clinical malaria lasts for at least 18 months after vaccination with RTS,S/AS02A. These findings are further reinforced by the significant difference between RTS,S/AS02A-vaccinated people and controls in the prevalence of infection seen in this same

cohort at the last cross-sectional survey towards the end of the high transmission season. These results contrast with the duration of protection seen in malaria-naive volunteers in the USA and in Gambian adults. They also refute the notion that protection induced by RTS,S/AS02A is mediated by some undescribed, transient, non-antigen-specific mechanism. No significant difference in the prevalence of infection at month 21 was observed in cohort 2, but this cohort differed from cohort 1 in that participants experienced substantially higher malaria transmission and underwent intensive follow-up for detection and treatment of all new infections during the double-blind phase.

As discussed in the previous report,[5] we have again found a very low incidence of anaemia in children who participated in the study, and although the rate was lower in the RTS,S/AS02A group than among controls, the difference did not reach statistical significance. This low rate of anaemia is probably the result of intense follow-up and early management of disease and the fact that as children get older, they leave the high-risk window when anaemia is a frequent complication of *P falciparum* infection. We also noted that the efficacy estimate for other exploratory endpoints based on different case definitions remained stable at around 30%. We interpret this to indicate that the protocol-defined changes in the specificity of the case definition were sufficiently small as to not bias our estimates substantially downwards. One of the exploratory analyses presented indicates persistent vaccine efficacy against multiple episodes of malaria. We acknowledge the methodological complexity of such an analysis and note there is no consensus on a model that best takes into account how risk changes with previous malaria episodes, antimalarial treatment, and premunition. We therefore have opted to use the more simplistic model based on a Poisson regression with normal random effect at the intercept to take account of the extra-Poisson variation due to the possible lack of independence between episodes within an individual.

The immunological mechanisms that underlie the observed protective efficacy of this vaccine against clinical malaria are probably complex. The RTS,S/AS02A vaccine was developed to induce both humoral and cellular immune responses against circumsporozoite protein, since preclinical data indicated that both were required for protection against infection.[12] In this trial, sustained vaccine efficacy against clinical malaria was observed even though concentrations of antibody against the circumsporozoite repeat region decreased substantially from the peak levels achieved after dose 3. However, nearly two years after having received the first dose of RTS,S/AS02A, antibody concentrations remained nearly 50 times higher in the vaccine group than in controls. The very low levels of naturally occurring anti-circumsporozoite antibodies in the control group confirms the poorly immunogenic nature of native circumsporozoite protein, even with substantial *P falciparum* exposure. In this trial we did not measure cellular immune responses, but their potential role in protection is well supported by data derived from surrogate animal models as well as from a few clinical vaccine trials.[13,14] We suggest that the observed vaccine efficacy results from an interplay between cellular and humoral immune responses induced by the vaccine.[7] Both of these mechanisms might be amenable to natural boosting, and could contribute to sustaining vaccine efficacy. Future trials with this vaccine may offer the opportunity to evaluate and better understand the respective roles of these multiple factors in mediating sustained protection against malaria disease.

The according-to-protocol $ATP_{(2\cdot5-21)}$ analysis for the entire study included 437 episodes of clinical malaria meeting a highly specific case definition, and consequently the adjusted efficacy estimate of 35·3% was quite precise (95% CI 21·6—46·6; $p<0\cdot0001$). Similarly, we documented 56 episodes meeting the case definition for severe malaria and yielding a vaccine estimate of 48·6% (95% CI 12·3–71·0; $p=0\cdot02$). Although the efficacy estimate for severe malaria was higher than that for clinical malaria, this difference still could be due to chance. However, other methods of malaria control, such as insecticide treated nets, that could involve reduction in the infecting dose of sporozoites, have also yielded higher estimates of efficacy for the more severe forms of the disease than for the mild forms.[15] This exciting possibility needs to be further explored in the case of this vaccine.

In conclusion, these findings show that the RTS,S/AS02A vaccine reduces the risk of clinical malaria by 35% and nearly halves the risk of severe malaria over a period of 18 months, with no evidence of waning efficacy. The combination of sustained protection together with substantial prevention of the severe forms of malaria marks RTS,S/AS02A as a promising vaccine candidate and strongly suggests that malaria vaccines have an important role as future public-health instruments. In parallel with these efforts, work is continuing to improve the efficacy of the RTS,S vaccine, including studies of more potent adjuvants, the inclusion of additional pre-erythrocytic and asexual-stage antigens, and further exploration of prime boost strategies.[16] The ongoing clinical development plan for this vaccine will continue and aims to explore its full potential by evaluating its safety, immunogenicity, and efficacy among young infants, and administering this vaccine together with other Expanded Program on Immunization contained antigens. With sustained funding and improved international partnerships, the first two decades of this century are likely to witness vaccines being part of the armoury against malaria in use throughout the endemic areas of Africa.

Contributors

All authors participated in the design, implementation, analysis, and interpretation of the study. P Alonso, J Sacarlal, and J J Aponte were involved in all phases of the study. A Leach led the clinical team at GSK Biologicals. Together with J J Aponte, M Lievens led the data analysis. J Sacarlal, P Aide, Q Bassat, C Guinovart, E Macete, and B Sigaúque were responsible for field and hospital activities and safety surveillance. J Milman and M Navia were the programme managers. I Mandomando and M Espasa coordinated all laboratory work at CISM. S Corachan was the central study coordinator at GSK Biologicals. M-C Dubois is the malaria vaccine project manager at GSK Biologicals. R Thompson and C Menéndez contributed to the design of the study, implementation, and interpretation. J Cohen heads malaria Vaccine Research and Development at GSK Biologicals. The writing of the paper was led by P Alonso, R Ballou, and Filip Dubovsky, with inputs from all other investigators.

Conflict of interest statement

The Malaria Vaccine Initiative supports the development and testing of a number of malaria vaccines that can be seen as competitors. AL, ML, SC, M-CD, WRB, and JC are employees of GSK Biologicals. AL, WRB, and JC own shares in GSK. JC and WRB are listed as the "Inventors" of patented malaria vaccines; however, neither individual holds a patent for a malaria vaccine. None of the other authors in this paper have declared conflicts of interest.

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Safety of the RTS,S/AS02A malaria vaccine in Mozambican children during a Phase IIb trial

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KEYWORDS
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Summary RTS,S/AS02A is a pre-erythrocytic vaccine candidate based on the *Plasmodium falciparum* circumsporozoite surface antigen and is currently the most advanced malaria vaccine candidate in development. A proof of concept phase IIb trial of the RTS,S/AS02A in Mozambican children aged 1—4 years determined a vaccine efficacy against risk of clinical malaria of 35.3%

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(95% CI 21.6—46.6; $p < 0.0001$) and against severe malaria of 48.6% (95% CI 12.3—71.0; $p = 0.02$). We evaluated the safety of the RTS,S/AS02A vaccine.

2022 children that received at least one vaccine dose of RTS,S/AS02A or control vaccines were included in the intention to treat safety analysis. Vaccine safety was evaluated using active and passive follow-up. Participants were observed for at least 1 h after each dose. Trained field workers visited children at home daily for the next 3 days to record solicited and unsolicited local and general symptoms. Investigators followed-up participants with severe adverse events until month 21.

Overall, we recorded 1712 unsolicited adverse events after vaccination, 53% in the intervention and 47% in the control group. Most unsolicited adverse events reported with RTS,S/AS02A were self-limited, and participants recovered without sequelae. Local reactogenicity increased with the number of doses. The proportion of children experiencing serious adverse events was lower in the RTS,S/AS02A recipients compared to the control group (*Engerix-B*™ or *Prevnar*™ and *Hiberix*™). Overall, these results indicate that the RTS,S/AS02A vaccine has a good safety profile and well tolerated when given in three doses to semi-immune children living in malaria-endemic areas.

Introduction

Malaria caused by *Plasmodium falciparum* is a tropical disease of major global health significance. Each year there are approximately 300—500 million acute malaria episodes [1] and an estimated 1—2 million deaths worldwide [2—4], mostly in children under age 5 years living in sub-Saharan Africa. While case management remains the cornerstone of control efforts, the need for prevention cannot be over emphasised, particularly in the African context. There is an urgent need to increase the use of effective control tools such as insecticide-treated nets (ITNs), and there is a wide consensus that a malaria vaccine would play a significant role in improved control.

The pediatric clinical development of the RTS,S/AS02A candidate malaria vaccine is being conducted under a partnership agreement with GlaxoSmithKline (GSK) and the PATH Malaria Vaccine Initiative (MVI). The vaccine antigen, RTS,S was formulated in the GSK proprietary Adjuvant System 2 (AS02A), containing an oil in water emulsion and the immunostimulants QS21 (a triterpene glycoside purified from the bark of Quillaja Saponaria) and 3D-MPL (3-deacylated monophosphoryl lipid A) [MPL]. This vaccine targets the pre-erythrocytic stage of the parasite and confers protection against infection by *P. falciparum* sporozoites delivered via laboratory-reared infected mosquitoes in malaria-naive adult volunteers and against natural exposure in semi-immune adults and children [5—10].

The development of this vaccine has included consecutive phase I and phase IIa/b studies in US [8,11], The Gambia [5,7,12] and Mozambique [9,10,13].

A proof of concept phase IIb randomised controlled trial was conducted in Mozambican children aged 1—4 years. At month 21, the study documented a reduction in the risk of clinical malaria of 35.3% (95% CI 21.6—46.6; $p < 0.0001$) and a 48.6% (95% CI 12.3—71.0; $p = 0.02$) reduction in severe malaria. The prevalence of *P. falciparum* asexual parasitaemia was 29% lower in the RTS,S/AS02A group (11.6%) than in the control group (16.2%; $p = 0.017$) 18 months after dose three [9,10]. In the context of this trial, we evaluated the safety of the RTS,S/AS02A vaccine.

This paper reports the safety results for all participants in this phase 2b study who received at least one dose of vaccine by intention to treat [ITT] analysis during the trial surveillance period (months 0—21) and represents the largest body of safety data on this vaccine reported thus far.

Materials and methods

Study site

This study was conducted at the Centro de Investigação em Saúde de Manhiça (CISM; Manhiça Health Research Centre) in Manhiça District, a rural area of Maputo Province, southern Mozambique, from April 2003 to May 2005. The characteristics of the area have been described in detail elsewhere [14,15]. The climate is subtropical with two distinct seasons: a warm and rainy season from November to April, and a generally cool and dry season during the rest of the year. Perennial malaria transmission with marked seasonality is mostly due to *P falciparum*. *Anopheles funestus* is the main vector, and the estimated entomological inoculation rate for 2002 was 38 infective bites per person per year [16].

Study design

This study is a phase IIb double-blind, randomised controlled trial to assess the efficacy, safety and immunogenicity of the candidate RTS,S/AS02A malaria vaccine. Details of the candidate malaria vaccine, the controls, and the trial profile for the double-blind (study months 0—8.5) and single-blind (study months 8.5—21) efficacy analysis have been presented elsewhere [9,10]. A total of 2022 healthy children aged 1—4 years were enrolled to receive either the candidate malaria vaccine or a comparison control vaccine.

The trial is registered with the ClinicalTrials.gov (identifier NCT00197041). The protocol (IND number: BB-IND 10514) was approved by the Mozambican National Ethics Review Committee, the Hospital Clínic of Barcelona Ethics Review Committee, and the PATH Human Subjects Protection Committee. The trial was conducted according to the

Table 1 Intensity grading of adverse events

Adverse event	Intensity grade	Parameter
Pain at injection site	0	Absent
	1	Minor reaction to touch
	2	Cries/protests on touch
	3	Cries when limb is moved/spontaneously painful
Swelling at injection site (Greatest diameter measured and scored at GSK)	0	None
	1	<5 mm
	2	5—20 mm
	3	>20 mm
Fever (axillary temperature measured and scored at GSK)	0	<37.5 °C
	1	37.5—38.0 °C
	2	>38.0—39.0 °C
	3	>39.0 °C
Irritability/fussiness	0	Behaviour as usual
	1	Crying more than usual with no effect on normal activity
	2	Crying more than usual with effect on normal activity
	3	Crying that cannot be comforted/prevents normal activity
Drowsiness	0	Behaviour as usual
	1	Drowsiness easily tolerated
	2	Drowsiness that interferes with normal activity
	3	Drowsiness that prevents normal activity
Loss of appetite	0	Normal
	1	Eating less than usual with no effect on normal activity
	2	Eating less than usual, interfering with normal activity
	3	Not eating at all

International Conference on Harmonisation of Good Clinical Practice Guidelines, and was monitored by GSK Biologicals. A local safety monitor and a data and safety monitoring board (DSMB) closely reviewed the conduct and safety data of the trial.

Parents or guardians of all participants gave written or thumb-printed informed consent prior to enrolment. A member of the community acted as an impartial witness and countersigned the consent form to guarantee an adequate understanding of the study procedures by all guardians. Eligibility screening included a brief medical history, a physical examination and blood sampling by finger-prick for haematology and biochemistry tests. Children were not screened for human immunodeficiency virus (HIV) infection. Hepatitis B surface Antigen (HBsAg) status was assessed at baseline, but was not an exclusion criteria to the trial. Other serological markers of hepatitis B status were not assessed.

Children were randomised in a 1:1 ratio to receive RTS,S/AS02A (0.25 mL dose) or the control vaccines. The RTS,S/AS02A vaccine was administered intramuscularly in the deltoid region of alternating arms starting with the left according to a 0, 1, and 2 months schedule. Children aged 24 months and older received three paediatric doses (0.5 ml) of hepatitis B vaccine (*Engerix*-B™, GSK Biologicals, Rixensart, Belgium). Children under 24 months received 2 paediatric doses of a 7-valent pneumoccocal conjugate vaccine (*Prevnar*™, Wyeth Lederle Vaccines, Madison, NJ, USA), administered at the first and third vaccinations and one dose of *Haemophilus influenzae type b* vaccine (*Hiberix*™, GSK Biologicals, Rixensart, Belgium) at the second vaccination.

Children were enrolled into two cohorts to measure the vaccine efficacy for clinical malaria disease and malaria infection. In cohort 1,1605 participants were followed-up using passive surveillance to detect clinical episodes of malaria. In cohort 2417 participants were followed-up using active surveillance to detect malaria infection through visits that started 14 days after dose three and were done every 2 weeks for 2.5 months and then monthly for an additional 2 months. In this cohort malaria parasitaemia was cleared 4 weeks before the start of the surveillance period with a combination of amodiaquine (10 mg/kg orally for 3 days) and sulfadoxine—pyrimethamine (one oral dose of sulfadoxine 25 mg/kg and pyrimethamine 1.25 mg/kg), the standard treatment at the time of the trial in the study area.

A total of 2022 children that received at least one vaccine dose were included in the ITT safety analysis. Of these, 1939 were included in the single-blind phase analysis of the trial.

Table 2 Severe malaria definitions (for reporting severe adverse events)

Severe malaria anaemia[a]	Asexual parasitaemia Hematocrit <15% No other more probable cause of illness	
Cerebral malaria[a]	Asexual parasitaemia	Assess coma score after correction of hypoglycemia and 60 min after control of fits. If fitting cannot be controlled within 30 min child is included
	Coma score ≤ 2 No other identifiable cause of loss of consciousness	
Severe malaria (other)	Asexual parasitaemia No other more probable cause of illness Does not meet criteria for severe malaria anaemia or cerebral malaria One of the following:	
	Multiple seizures	Two or more generalized convulsions within a 24-h period prior to admission
	Hyperparasitaemia[b]	5+ parasitaemia
	Prostration	Inability to sit unaided
	Hypoglycemia	<2.2 mmol/dl or <40 mg/dl
	Acidosis	Document supportive signs and/or laboratory readouts
	Circulatory collapse	Document supportive signs and/or laboratory readouts

[a] Severe malaria anaemia and cerebral malaria could be diagnosed in the same case.
[b] The criteria of hyperparasitaemia was deleted after month 8.5.

Study procedures

Vaccines were administered at the Manhiça and Ilha Josina health centres. Vaccine safety was evaluated using active and passive follow-up. Study participants were observed for at least 1 h after each vaccine dose by a paediatrician. Trained field workers visited the children in their homes daily for the following 3 days to record solicited and unsolicited local and general symptoms. All adverse events (AE) occurring within 1 month (30 days) of each vaccine dose were recorded irrespective of their severity or relationship to vaccination. All serious adverse events (SAE) occurring from the day of the administration of the first vaccine dose throughout the study duration were reported. Table 1 shows details of intensity grading for symptoms. All solicited local signs and symptoms were considered to be causally related to vaccination; general symptoms were assessed by the investigator to determine their relationship to the vaccination.

Solicited AEs included: local pain and swelling at the injection site, fever (defined as axillary temperature $\geq$37.5 °C), drowsiness, loss of appetite, and irritability or fussiness. Unsolicited AEs included all other general signs and symptoms detected through the passive case detection surveillance. SAEs were defined as any medical occurrence that resulted in death, was life-threatening, required inpatient hospitalisation or resulted in persistent or significant disability or incapacity. Investigators followed-up participants with SAEs until the event had resolved or until month 21. Deaths occurring at home were investigated by a review of all available medical records and by verbal autopsy, a previously described technique (Sacarlal J, in press INDEPTH monograph).

Safety monitoring of haematological parameters (haemoglobin, haematocrit, total white cell count (WCC), platelets) was done at screening and 1 month after dose three. Biochemical parameters [alanine aminotransferase (ALT), total bilirubin, creatinine] and antibodies against circumsporozoite protein (cohort 1) and HBsAg (cohort 2) were assessed 1, 6.5 and 19 months after dose three. The normal ranges applied were: hematocrit $\geq$ 25%, hemoglobin $\geq$70 g/L, WBC $5-17 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, ALT $\leq$60 IU/L, creatinine $\leq$ 45 μmol/L (<3 years) or $\leq$ 57 (>3 years) and total bilirubin $\leq$ 34 μmol/L.

Safety endpoints included the occurrence of solicited symptoms within the 4-day follow-up after each vaccination, the occurrence of unsolicited symptoms within the 30-day follow-up after vaccination and the occurrence of SAEs from the time of first vaccination until 21 months after dose three. For SAE reporting severe malaria was defined prospectively according to agreed case definitions (Table 2) and all cases meeting the case definition were reported as SAEs. The number of cases reported in the safety analysis is greater than that reported for the efficacy analysis, because in the latter more children were excluded by the analysis protocol.

Statistical methods

The ITT cohort for the safety endpoints analysis included all subjects vaccinated for whom data were available. Symptoms were investigated in terms of the percentage of participants reporting symptoms after each dose and in total. The intensity of symptoms and, for general and unsolicited symptoms, their relationship to vaccination were evaluated. The 95% confidence inter-

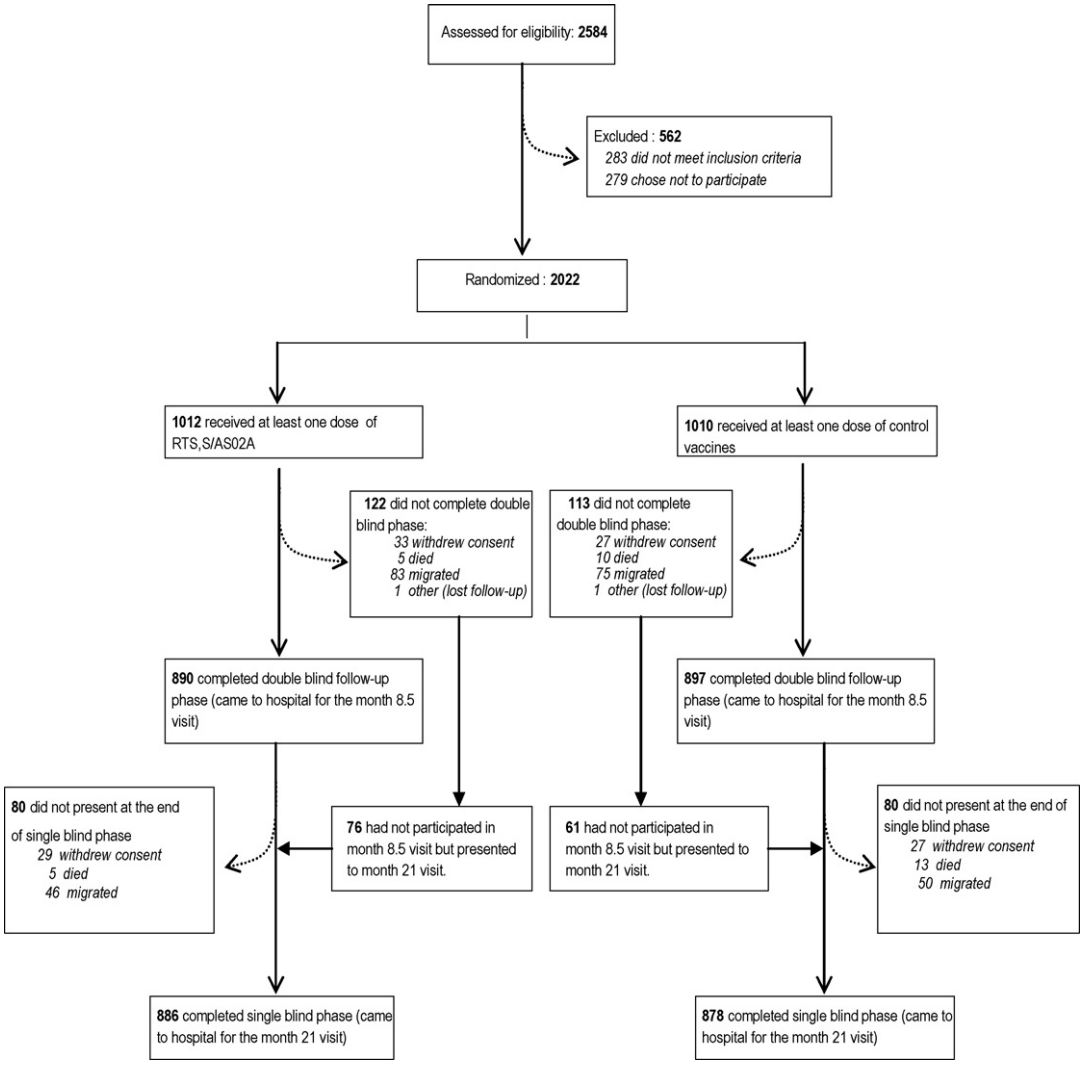

Fig. 1 Trial profile intention to treat cohort (ITT).

vals (CI) of the percentages were calculated using the exact method for binomial variables (StatXact PROCs for SAS, version 6; Cambridge, MA, USA). Unsolicited symptoms reported after vaccination and in the 30-day follow-up period were coded using the MedDRA dictionary [17]. Analyses were done with SAS version 8 (Cary, NC, USA).

Results

Two thousand and twenty-two children aged 1—4 years were recruited and randomised. Fig. 1 shows the trial profile for the ITT cohort. A total of 1764 subjects completed the single-blind phase up to month 21: 886 in the RTS,S/AS02A group and 878 in the control group.

Solicited adverse events (AEs)

Safety data was available for 1012 children who received a total of 2926 RTS,S/AS02A (0.25 mL) doses and for 1010 children who received a total of 2912 doses in the control group. Compliance for completion of symptom questionnaires was over 99%.

Overall, symptoms were reported more frequently following administration of RTS,S/AS02A than following either control vaccine regimen (Fig. 2). In children <24 months of age, at least one symptom was observed after 61.1% (95% CI: 57.3, 64.7) of RTS,S/AS02A doses, and in children ≥24

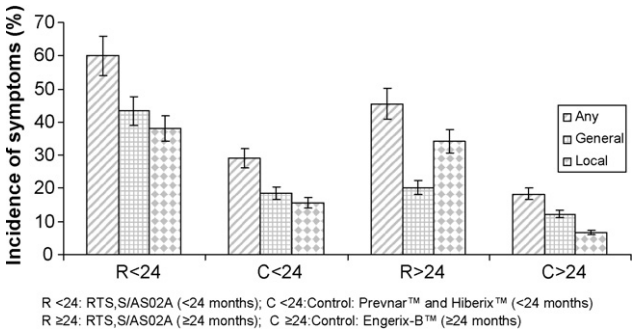

Fig. 2 Incidence and nature of symptoms (solicited and unsolicited) reported in the 4-day follow-up after vaccination.

Table 3 Local solicited symptoms reported after all doses

Group/cohort	$R < 24$ ($N = 701$)[a]				$C < 24$ ($N = 695$)[b]				$R \geq 24$ ($N = 2225$)[c]				$C \geq 24$ ($N = 2217$)[d]			
	n	%	95% CI		*n*	%	95% CI		*n*	%	95% CI		*n*	%	95% CI	
			LL	UL			LL	UL			LL	UL			LL	UL
Pain any	205	29.2	25.9	32.8	52	7.5	5.6	9.7	539	24.2	22.5	26.1	90	4.1	3.3	5.0
Grade 3	2	0.3	0.0	1.0	0	0.0	0.0	0.5	5	0.2	0.1	0.5	1	0.0	0.0	0.3
Swelling any	181	25.8	22.6	29.2	66	9.5	7.4	11.9	427	19.2	17.6	20.9	72	3.2	2.5	4.1
Grade 3	73	10.4	8.3	12.9	11	1.6	0.8	2.8	151	6.8	5.8	7.9	3	0.1	0.0	0.4

N: number of injected doses; %: number local solicited symptoms (*n*)/number of children in each group (*N*); 95% CI: exact 95% confidence Intervals, lower limit (LL), upper limit (UL). Grade 3 pain: cries when limb is moved/spontaneously painful. Grade 3 swelling: diameter of swelling >20 mm.

[a] RTS,S/AS02A (<24 months).

[b] Control: *Prevnar*™ and *Hiberix*™ (<24 months).

[c] RTS,S/AS02A (≥24 months).

[d] Control: *Engerix*-B™ (≥24 months).

months of age, this figure was 45.3% (95% CI: 43.2, 47.4). For recipients of the control vaccines, the corresponding figures were 30.1% (95% CI: 26.7, 33.6) in children <24 months (*Prevnar*™ and *Hiberix*™) and 18.6% (95% CI: 17.0, 20.3) in children ≥24 months (*Engerix*-B™).

Sequential doses of RTS,S/AS02A vaccine were associated with increasing local reactogenicity. Local symptoms were reported following 30.2%, 37.2% and 51.1% of 1, 2 and 3 doses, respectively, in children <24 months, and following 28.3%, 27.6% and 46.4% of doses 1, 2 and 3, respectively, in children ≥24 months.

Local reactogenicity was higher following administration of RTS,S/AS02A than of *Prevnar*™, *Hiberix*™ or *Engerix*-B™ (Table 3). For the RTS,S/AS02A recipients, pain was reported following 29.2% of doses in children <24 months, 24.2% of doses in children ≥24 months and swelling was reported following 25.8% and 19.2% of doses in the two age groups, respectively. Swelling > 20 mm occurred after 10.4% of doses in children <24 months and 6.8% of doses in children ≥24 months in the RTS,S/AS02A group. However among all participating children, only eight reports of grade 3 swelling persisted beyond the 4-day follow-up period; these most commonly followed the third dose and all resolved within 1 week. Of these, two occurred in children <24 months (one in RTS,S/AS02A after the second dose and another after the third dose of *Prevnar*™) and six in children ≥24 months (one occurred after the second dose and five after the third dose in RTS,S/AS02A group).

Pain was rarely severe; only eight cases of grade 3 pain (<0.3% of doses) in the intention to treat cohort were reported, and none persisted beyond the 4-day solicited period. Two cases occurred in children <24 months (all in RTS,S/AS02A after the second dose) and the other six in children ≥24 months (three after first dose and two after second dose of RTS,S/AS02A and one after second dose of *Engerix*-B™).

The incidence of all general solicited symptoms (drowsiness, irritability, loss of appetite and fever) was higher following doses of RTS,S/AS02A than doses of the control vaccines (Table 4). The incidence of general symptoms associated with the RTS,S/AS02A vaccine was higher in children <24 months than in those 24 months or older. However, the incidence of general reactogenicity of grade 3 severity was low, and no sequential dose effect was observed.

In the RTS,S/AS02A group, fever was the most common general solicited symptom, occurring in 27.4% of doses in children <24 months and in 10.3% of doses in those ≥24 months. Grade 3 severity fever was reported in 1.7% of children <24 months and in 0.4% of children ≥24 months. Although fevers grade 3, were judged by the medical staff to be predominantly unrelated to vaccination, the rates in the comparison groups were very low (33% in RTS,S/AS02A group and 16% in control group) (Table 4). All grade 3 fevers resolved within the 4-day follow-up period, except for two cases, that lasted until day 5.

Unsolicited adverse events (AEs)

Up to 30 days post-vaccination a total of 1712 unsolicited adverse events were observed; 910 (53.2%) in the RTS,S/AS02A group and 802 (46.8%) in the control group. Of these, 322 (155 in the RTS,S/AS02A and 167 in the control group) were considered to be grade 3 severity (Fig. 3).

In children <24 months, the most commonly reported AEs were upper respiratory tract infection (URTI), malaria

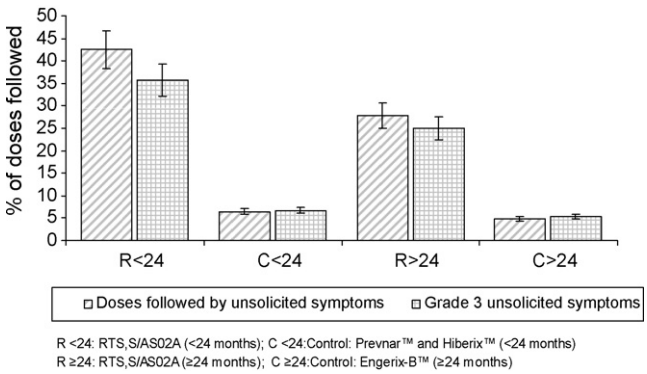

Fig. 3 Summary of doses followed by unsolicited symptoms.

Table 4 General solicited symptoms reported after all doses (intention to treat cohort)

Group/cohort		*R* < 24 (*N* = 701)[a]				*C* < 24 (*N* = 695)[b]				*R* ≥ 24 (*N* = 2225)[c]				C ≥ 24 (*N* = 2217)[d]			
		n	%	95% CI		*n*	%	95% CI		*n*	%	95% CI		*n*	%	95% CI	
				LL	UL			LL	UL			LL	UL			LL	UL
Drowziness	Any	52	7.4	5.6	9.6	23	3.3	2.1	4.9	60	2.7	2.1	3.5	32	1.4	1.0	2.0
	Grade 3	6	0.9	0.3	1.9	1	0.1	0.0	0.8	8	0.4	0.2	0.7	2	0.1	0.0	0.3
	Related	0	0.0	0.0	0.5	1	0.1	0.0	0.8	0	0.0	0.0	0.2	1	0.0	0.0	0.3
	Grade 3 related	0	0.0	0.0	0.5	0	0.0	0.0	0.5	0	0.0	0.0	0.2	0	0.0	0.0	0.2
Irritability	Any	92	13.1	10.7	15.9	16	2.3	1.3	3.7	66	3.0	2.3	3.8	26	1.2	0.8	1.7
	Grade 3	5	0.7	0.2	1.7	1	0.1	0.0	0.8	3	0.1	0.0	0.4	3	0.1	0.0	0.4
	Related	1	0.1	0.0	0.8	0	0.0	0.0	0.5	0	0.0	0.0	0.2	0	0.0	0.0	0.2
	Grade 3 related	0	0.0	0.0	0.5	0	0.0	0.0	0.5	0	0.0	0.0	0.2	0	0.0	0.0	0.2
Loss of appetite	Any	128	18.3	15.5	21.3	45	6.5	4.8	8.6	158	7.1	6.1	8.2	72	3.2	2.5	4.1
	Grade 3	7	1.0	0.4	2.0	2	0.3	0.0	1.0	6	0.3	0.1	0.6	3	0.1	0.0	0.4
	Related	2	0.3	0.0	1.0	1	0.1	0.0	0.8	1	0.0	0.0	0.3	0	0.0	0.0	0.2
	Grade 3 related	0	0.0	0.0	0.5	0	0.0	0.0	0.5	0	0.0	0.0	0.2	0	0.0	0.0	0.2
Fever	Any	192	27.4	24.1	30.9	41	5.9	4.3	7.9	230	10.3	9.1	11.7	139	6.3	5.3	7.4
	Grade 3	12	1.7	0.9	3.0	2	0.3	0.0	1.0	8	0.4	0.2	0.7	9	0.4	0.2	0.8
	Related	69	9.8	7.7	12.3	6	0.9	0.3	1.9	71	3.2	2.5	4.0	23	1.0	0.7	1.6
	Grade 3 related	4	0.6	0.2	1.5	0	0.0	0.0	0.5	1	0.0	0.0	0.3	1	0.0	0.0	0.3

N: number of injected doses; %: number of general solicited symptoms reported after all doses (*n*)/number of children in each group (*N*) 95% CI Exact 95% Confidence Intervals, lower limit (LL), upper limit (UL). Grade 3 drowsiness: drowsiness that prevents normal activity. Grade 3 irritability: crying that cannot be comforted/prevents normal activity. Grade 3 loss of appetite: not eating at all. Grade 3 fever: temperature ≥ 39.0 °C.

[a] RTS,S/AS02A (<24 months).

[b] Control: *Prevnar*™ and *Hiberix*™ (<24 months).

[c] RTS,S/AS02A (≥24 months).

[d] Control: *Engerix*-B™ (≥24 months).

and diarrhoea. These occurred with similar frequency in the RTS,S/AS02A and control group. Grade 3 events were most commonly malaria, URTI and pneumonia with frequencies of 3.0%, 1.3% and 0.9% in RTS,S/AS02A recipients and 4.5%, 0.9% and 1.4% in control vaccine recipients, respectively.

In children ≥24 months, the most commonly reported AEs were URTI, malaria and ascariasis. These occurred with similar frequency in the two groups. The most common grade 3 events were malaria, pneumonia and anaemia, with frequencies of 3.4%, 0.5% and 0.4% in RTS,S/AS02A recipients and 3.8%, 0.7% and 0.5% in *Engerix*-B™ recipients, respectively.

Using MedDRA definitions, there were no important differences in unsolicited symptoms or disease frequency between the RTS,S/AS02A and control vaccine recipients. Overall, eight unsolicited adverse events were judged by the investigators to be related to vaccination and these occurred in seven participants. Two were associated with administration of RTS,S/AS02A. The first was a case of fever (37.6 °C) for which no alternative cause was evident. The fever resolved within 1 day. The second was a tender swelling that was suspected clinically to be a sterile abscess that occurred after the second dose. It was managed conservatively and resolved without sequelae within 3 weeks. The participant subsequently received the third dose without a problem.

Serious adverse events (SAEs)

Over the total observation period 523 SAEs were reported in 212 subjects who received the RTS,S/AS02A vaccine and 634 SAEs in 289 subjects who received the control vaccines (Table 5). Overall, a higher number of participants experienced SAEs in the double-blind phase of the trial when compared to the single-blind phase. None of the SAEs was deemed to be related to vaccination.

In cohort 1, over the 21-month surveillance period, severe malaria requiring hospital admission was experienced by 3.7% [95%CI 2.5—5.1] of participants receiving RTS,S/AS02A in 35 reported cases. Among these, two cases of cerebral malaria, four cases of severe malaria anaemia and 29 cases of severe malaria (other) were reported (Table 6). In the control group, there were 54 cases reported by 50 children, 6.2% [95% CI 4.7—8.1] with at least one case of severe malaria. Three cases of cerebral malaria, nine cases of severe malaria anaemia and 43 cases of severe malaria (other) were reported in this group. In cohort 2, there were 12 cases of severe malaria that were equally distributed between the RTS,S/AS02 and control groups.

In total, nine children presented clinically with HIV infection or AIDS; four recipients of RTS,S/AS02A and five recipients of control vaccines. Of these, two children in the RTS,S/AS02A group and all those in the control group have

Table 5 Percentage of participants reporting SAEs classified by MedDRA primary system organ class and preferred term (intention to treat cohort) during follow-up

	RTS,S/AS02A ($N = 1012$)				Control ($N = 1010$)			
	n	%	95% CI		*n*	%	95% CI	
			LL	UL			LL	UL
At least one SAE over 21-month follow-up	212	21.0	18.5	23.6	289	28.6	25.8	31.5
At least one SAE requiring hospitalization over 21-month follow-up	122	12.1	10.1	14.2	142	14.1	12.0	16.4
Number of SAEs reported over 21 month follow-up	523				634			
At least one SAE in double-blind phase (months 0—8.5)	180	17.8	15.5	20.3	249	24.7	22.0	27.4
At least one SAE requiring hospitalization in double-blind phase (months 0—8.5)	100	9.9	8.1	11.9	112	11.1	9.2	13.2
	$N = 974$				$N = 965$			
At least one SAE in single-blind phase (months 8.5—21)	50	5.1	3.8	6.7	66	6.8	5.3	8.6
At least one SAE requiring hospitalization in single-blind phase (months 8.5—21)	46	4.7	3.5	6.3	64	6.6	5.1	8.4

At least one symptom = at least one symptom experienced (regardless of the MedDRA preferred term). *N* = number of subjects with at least one administered dose and included in ITT cohort. *n*/% = number/percentage of subjects reporting at least once the symptom. 95% CI = exact 95% confidence interval; LL: lower limit; UL: upper limit.

subsequently died. In the total study cohort, 23 subjects died; 10 in the RTS,S/AS02A group and 13 in the control group. No fatal SAE was considered to be related to vaccination.

Monitoring of haematological and biochemical parameters

The full blood cell count was monitored 1 month after dose three. A small proportion of participants were below the normal range for one or more parameters and these were balanced between RTS,S/AS02A and control vaccine recipients (Table 7). All abnormalities occurred in children who were considered healthy upon further clinical examination.

The biochemical parameters creatinine, ALT and total bilirubin were monitored at three time points post-vaccination corresponding to 1, 6.5 and 19 months after dose three. Few children had elevated creatinine levels, and these were equally distributed between recipients of RTS,S/AS02A and control vaccines (Table 8). All elevations of creatinine were mild, and occurred in children who had co-infections or were healthy upon clinical examination; no child was diagnosed with renal pathology. ALT values were elevated in a small proportion of children, and these were also balanced between RTS,S/AS02A and control vaccine recipients. In a small number of children the elevation was more than five times the upper limit of normality, these children had co-infections such as malaria that could explain high levels or were asymptomatic, and had no abnormal findings upon clinical examination.

HBsAg status was not an exclusion criteria for the trial, but was measured at baseline; 31 children in the RTS,S/AS02A group and 49 in the control group were positive. All children had normal ALT and bilirubin measurements at enrolment. One month post-vaccination, 10 of the 31 children in the RTS,S/AS02A group had mildly elevated ALT values compared to 11 of 49 children in the control group. All bilirubin measurements at this time point were normal and the children were asymptomatic. Although the mean values were similar between the groups, the proportions of children with a raised ALT was higher in the RTS,S/AS02A group compared with the controls. The proportion of children who were HBsAg positive at baseline and had raised ALT measurements at 6.5 and 19 months after the third dose of vaccine were similar between the two groups (Table 8). No abnormality of renal or hepatic function was judged to be related to vaccination.

Discussion

This trial included the largest number of volunteers vaccinated with RTS,S/AS02A, and therefore provides the best evidence to date on the safety and reactogenicity of this candidate vaccine when given in three doses according to a 0, 1, 2 months vaccination schedule to children living in a malaria endemic transmission area.

RTS,S/AS02A reactogenicity was similar to that recorded in previous vaccine trials in adults [5] and children in The Gambia [7] and Mozambique [13]. Local reactogenicity (pain and swelling) and general solicited symptoms (drowsiness, irritability, loss of appetite and fever) were reported more frequently in recipients of RTS,S/AS02A than in children receiving the control vaccine. Local reactogenicity increased with increasing dose levels, however, most local adverse events were mild to moderate in intensity, limited to the injection site, of short duration and terminated without sequelae.

Over the 21-month surveillance period, the proportion of children experiencing a SAE was lower in the RTS,S/AS02A recipients compared to the control group. This difference was largely accounted for by differences in the rate of

Table 6 Cases of severe malaria to month 21 in intention to treat cohort

Malaria severity	<24 months						≥24 months						Total					
	RTS,S/AS02A (*N* = 197)			Control (*N* = 195)			RTS,S/AS02A (*N* = 606)			Control (*N* = 607)			RTS,S/AS02A (*N* = 803)			Control (*N* = 802)		
	nc	ns	%	nc	ns	%	nc	ns	%	nc	ns	%	nc	ns	%	nc	ns	%
Cohort 1																		
Complete surveillance [0–21]																		
Cerebral malaria	2	2	1.1	3	3	1.5	0	0	0.0	0	0	0.0	2	2	0.3	3	3	0.4
Severe malaria anaemia	3	3	1.5	5	5	2.6	1	1	0.2	4	4	0.7	4	4	0.5	9	9	1.1
Severe malaria (other) requiring admission	15	11	5.6	19	19	9.7	14	13	2.2	24	21	3.5	29	24	3.0	43	40	5.0
	N = 51			*N* = 51			*N* = 158			*N* = 157			*N* = 209			*N* = 208		
	nc	ns	%	nc	ns	%	nc	ns	%	nc	ns	%	nc	ns	%	nc	ns	%
Cohort 2																		
Complete surveillance [0–21]																		
Cerebral malaria	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0
Severe malaria anaemia	1	1	2.0	2	2	3.9	0	0	0.0	0	0	0.0	1	1	0.5	2	2	1.0
Severe malaria (other) requiring admission	2	2	3.9	4	4	7.8	3	3	1.9	0	0	0.0	5	5	2.4	4	4	1.9

N: number of subjects in each group; nc: number of cases (cases can only appear in 1 category). ns: number of subjects (subjects reporting the same category more than once are only counted once); (%): proportion affected (100 × ns/*N*).

Table 7 Monitoring of haematological parameters (intention to treat)

	Study month	RTS,S/AS02A		Controls	
		% normal range	% below normal range	% normal range	% below normal range
Haemoglobin	Baseline	99.8	0.2	99.9	0.1
	1 month after dose 3	98.7	1.3	98.4	1.6
Haematocrit	Baseline	100.0	0.0	100.0	0.0
	1 month after dose 3	98.4	1.6	97.6	2.4
WBC[a]	Baseline	100.0	0.0	100.0	0.0
	1 month after dose 3	97.8	2.2	97.2	2.8
Platelets	Baseline	100.0	0.0	100.0	0.0
	1 month after dose 3	97.4	2.6	97.2	2.8

[a] White blood cell.

Table 8 Monitoring of biochemical parameters (intention to treat)

	Study month	RTS,S/AS02A		Controls	
		% normal range	% above normal range	% normal range	% above normal range
ALT[a]	Baseline	100.0	0.0	100.0	0.0
	1 month after dose 3	96.1	3.9	95.5	4.5
	6.5 months after dose 3	96.6	3.4	97.3	2.7
	21 month after dose 3	97.1	2.9	96.8	3.2
Bilirubin	Baseline	100.0	0.0	100.0	0.0
	1 month after dose 3	100.0	0.0	99.8	0.2
	6.5 months after dose 3	99.8	0.2	99.6	0.4
	21 month after dose 3	99.9	0.1	99.8	0.2
Creatinine	Baseline	100.0	0.0	100.0	0.0
	1 month after dose 3	99.3	0.7	99.8	0.2
	6.5 months after dose 3	98.3	1.7	99.6	0.4
	21 month after dose 3	99.1	0.9	98.5	1.5

[a] Alanine aminotransferase.

malaria between the two groups. There was a higher number of SAEs requiring hospital admission in the double-blind phase (months 0 to 8.5) than in the single-blind phase (months 8.5 to 21). This difference is largely due to a change in the diagnostic criteria defining severe malaria for SAE reporting made at the end of the double-blind phase. Experience gained during the study showed that hyperparasitemia was frequently well-tolerated and could be managed on an outpatient basis. Therefore, this criteria for severe malaria was removed from the clinical case definition during the single-blind phase.

The pattern of inpatient morbidity observed in this trial is lower than of background population and consistent with that observed in other studies in the region [18,19].

No significant difference between vaccine groups was observed in the proportion of participants who had laboratory values outside the normal ranges for the measured parameters. We have previously documented the high level of circulating hepatitis A in children from this area that may explain the elevated liver enzymes observed [13]. There were very few children in whom a raised ALT was associated with a raised bilirubin value and all were mild and sub-clinical.

In conclusion, these data confirm that the RTS,S/AS02A vaccine has a good safety profile and is well tolerated when given to semi-immune children living in a malaria-endemic area.

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Contributors: Each of the authors participated in the design, implementation, analysis and interpretation of the study. Jahit Sacarlal, Pedro Alonso and John J. Aponte were involved in all phases of the study. Amanda Leach led the clinical team at GSK Biologicals. John J. Aponte and Marc Lievens led the data analysis. Jahit Sacarlal, Pedro Aide, Quique Bassat, Caterina Guinovart and Eusebio Macete were responsible for field and hospital activities as well as safety surveillance. Jessica Milman and Caterina Guinovart were the programme managers of the trial. Inacio Mandomando and Mateu Espasa coordinated all laboratory work at the CISM. Sabine Corachan was the central study coordinator and Opokua Ofori-Anyinam was the clinical development manager at GSK Biologicals. Marie-Claude Dubois was the malaria vaccine project manager at GSK Biologicals. Joelle Thonnard was responsible for safety monitoring at GSK Biologicals. Joe Cohen and Ripley Ballou head malaria Vaccine Research and Development at GSK Biologicals. Jahit Sacarlal led the manuscript preparation with inputs from all other investigators.

Conflict of interest statement: MVI supports the development and testing of a number of malaria vaccines that can be seen as competitors. Amanda Leach, Opokua Ofori-Anyinam, Joelle Thonnard, Sabine Corachan, Marie-Claude Dubois, Marc Lievens, W Ripley Ballou and Joe Cohen are employees of GlaxoSmithKline Biologicals. Amanda Leach, Opokua Ofori-Anyinam, W Ripley Ballou and Joe Cohen own shares in GlaxoSmithKline. Both Joe Cohen and W Ripley Ballou are listed as the 'Inventors' of patented malaria vaccines. However neither individual holds a patent for a malaria vaccine. None of the other authors in this paper have declared a conflict of interest.

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ARTICLE 5: Sacarlal J, Aide P, Aponte JJ, Renom M, Leach A, Mandomando I, et al; Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children, **JID 2009**– accepted in press

MAJOR ARTICLE

Long-Term Safety and Efficacy of the RTS,S/AS02A Malaria Vaccine in Mozambican Children

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Background. We previously reported that the RTS,S/AS02A vaccine had an acceptable safety profile, was immunogenic, and demonstrated efficacy against *Plasmodium falciparum* malaria disease for 21 months.

Methods. We conducted a randomized, controlled, phase 2b trial of RTS,S/AS02A in 2022 Mozambican children aged 1–4 years. We now report safety results for all randomized subjects and vaccine efficacy (VE) findings for children in the Manhiça area over the 45-month surveillance period.

Results. During the surveillance period, the $VE_{(2.5–45)}$ (VE over months 2.5–45 of surveillance) against a first or only episode of clinical malaria disease was 30.5% (95% confidence interval [CI], 18.9%–40.4%; $P < .001$), and the $VE_{(2.5–45)}$ against all episodes was 25.6% (95% CI, 11.9%–37.1%; $P < .001$). When the same period was considered, the $VE_{(2.5–45)}$ for subjects protected against severe malaria was 38.3% (95% CI, 3.4%–61.3%; $P = .045$). At study month 45, the prevalence of *P. falciparum* was 34% lower in the RTS,S/AS02A group than in the control group (66 [12.2%] of 541 patients vs 101 [18.5%] of 547 patients) ($P = .004$).

Conclusion. These results show evidence that RTS,S/AS02A maintained protection during the 45-month surveillance period, and they highlight the feasibility of developing an effective vaccine against malaria. In combination with other malaria-control measures, such a vaccine could greatly contribute to reducing the intolerable global burden of this disease.

Trial registration. ClinicalTrials.gov identifier NCT00197041 and NCT00323622.

During the 20th century, economic and social development, together with antimalarial campaigns, have resulted in the eradication of malaria from large swathes of the planet, thereby reducing the percentage of the world's areas that are malaria prone from 50% to 27%. Nonetheless, given expected population growth, it is projected that, by 2010, one-half of the world's population—nearly 3.5 billion people—will be living in areas where malaria is transmitted [1]. Today, Africa continues to absorb the brunt of the disease, with approximately 350–550 million clinical episodes and 700,00 to 1.6 million deaths occurring annually, mostly among children <5 years of age [1, 2].

The past decade has witnessed a renewed effort to study and control malaria. New tools are becoming available, and the development of a vaccine is considered to be a key component of future improved control activities. RTS,S (GlaxoSmithKline Biologicals [GSK]), a recombinant circumsporozoite product that is formulated with the AS02A Adjuvant System and that con-

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Potential conflicts of interest: PATH Malaria Vaccine Initiative (MVI) supports the development and testing of a number of malaria vaccines that can be seen as competitors. A.L., M.L., J.V., J.T., M.-C.D., M.A.D., W.R.B., and J.C. are current or previous employees of GlaxoSmithKline Biologicals (GSK). A.L., W.R.B., M.-C.D., and J.C. own shares in GSK. Both J.C. and W.R.B. are listed as the "inventors" of patented malaria vaccines. However, neither individual holds a patent for a malaria vaccine. All other authors: no conflicts.

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Engerix-B and Hiberix are trademarks of the GlaxoSmithKline group of companies. Prevenar is a trademark of Wyeth-Lederle.

(See the article by Kester et al, on pages XXX–XX, and the editorial commentary by Bremen and Plowe, on pages XXX–XX.)

[a] Employed by GlaxoSmithKline Biologicals at the time of the study.

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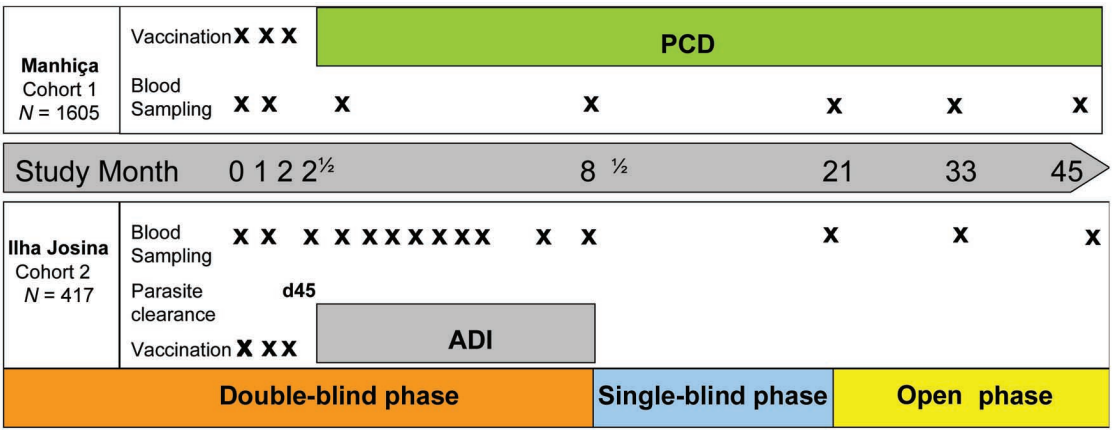

Figure 1. Study design. ADI, active detection of information; d45, day 45; double-blind phase, study months 2.5–8; open phase, study months 21–45; PCD, passive case detection; single-blind phase, study months 8–21.

tains an oil-in-water emulsion and the immunostimulants QS21 (a triterpene glycoside purified from the bark of *Quillaja saponaria*) and 3D-MPL (3-deacylated monophosphoryl lipid A [MPL]), is currently the most clinically advanced malaria vaccine candidate in the world. RTS,S/AS02A specifically targets the preerythrocytic stage of *Plasmodium falciparum* and has been shown to confer protection against experimental *P. falciparum* infection, delivered via laboratory-reared infected mosquitoes, in immunized malaria-naive volunteers and against natural infection in semi-immune adults [3–7].

Consecutive phase 1 trials in children aged 6–11 years and 1–5 years in The Gambia showed that the vaccine was safe, well tolerated, and immunogenic [3, 6, 8]. Short-term protection against infection (71% [95% confidence interval {CI}, 46%–85%] during the first 9 weeks of follow-up) was demonstrated in immunized adult men in The Gambia in 1998 [3]. Subsequently, a pediatric vaccine dose was selected and studied in a phase 1 trial of Mozambican children aged 1–4 years, in whom it was found to be safe, well tolerated, and immunogenic [9].

In 2004, we reported the first proof-of-concept study involving African children aged 1–4 years who were living in a *P. falciparum*–endemic area in Mozambique. During the first 6-months of follow-up in this double-blind, randomized, controlled trial, immunization with RTS,S/AS02A was associated with vaccine efficacy (VE) of 29.9% (95% CI, 4.8%–11.0%; $P = .004$) against clinical malaria, 45% (95% CI, 31.4%–45.9%; $P < .001$) against infection, and 57.7% (95% CI, 16.2%–80.6%; $P = .019$) against severe malaria [10].

An extended follow-up showed that, at 21 months after the first dose, the risks of clinical malaria and severe malaria were reduced by 35.3% (95% CI, 21.6%–46.6%; $P < .001$) and 48.6% (95% CI, 12.3%–71.0%; $P = .02$), respectively, in the RTS,S/AS02A group [11].

We recently completed a phase 1/2b clinical trial in infants living in a malaria-endemic area of Mozambique. Administration of RTS,S/AS02A, staggered with EPI vaccines, showed that RTS,S/AS02A had a good safety profile, was well tolerated and immunogenic, and was associated with a VE against new infection of 65.9% (95% CI, 42.6%–79.8%; $P < .001$) [12].

Future deployment of any vaccine will depend on the level of VE and the duration of protection, both of which are critical elements of any target product profile. The present study reports the long-term safety and efficacy noted during 45 months of follow-up of Mozambican children who were 1–4 years of age at the time that they received a first dose of either RTS,S/AS02A or control vaccines.

METHODS

Study site. The study was conducted at the Centro de Investigação em Saúde de Manhiça (CISM; Manhiça Health Research Centre) in Manhiça District, a rural area of Maputo Province, southern Mozambique, from April 2003 through May 2007. The characteristics of the area and the dates of malaria transmission have been described in detail elsewhere [13, 14]. Malaria transmission, mostly due to *P. falciparum,* is perennial, with marked seasonality. *Anopheles funestus* is the main vector, and the estimated entomologic inoculation rate for 2002 was 38 infective bites per person per year [10]. Combination therapy with amodiaquine and sulfadoxine pyrimethamine was the first-line treatment used for uncomplicated malaria during the first 2 years of the study, and it was replaced by the combination of sulfadoxine pyrimethamine plus artesunate in 2006. All antimalarial drugs were readily available at health care facilities in Mozambique throughout the study. Each participant received an ITN during the study. Throughout the duration of the trial, IRS was promoted in the study area by the Mozam-

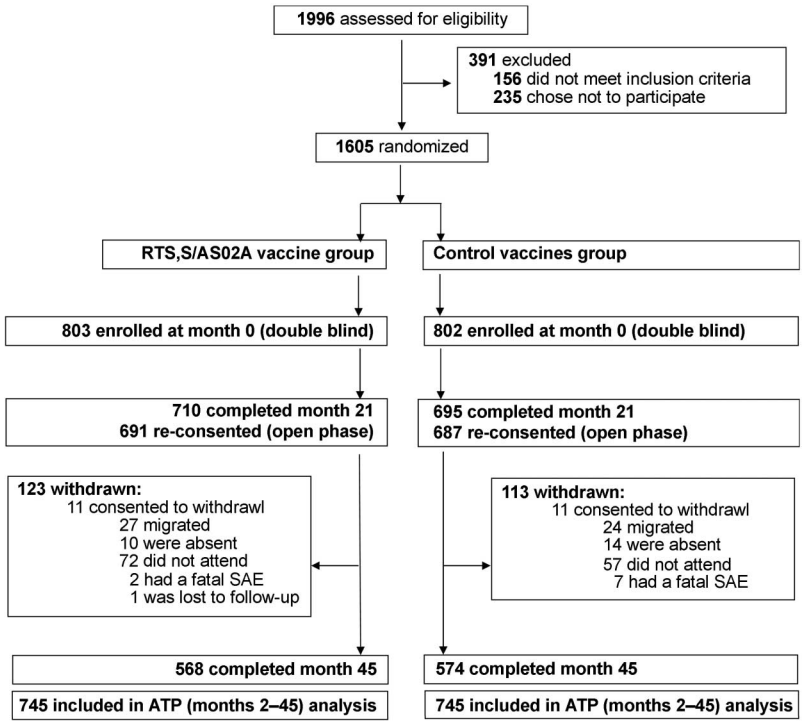

Figure 2. Trial profile for cohort 1. ATP, according-to-protocol analysis; SAE, serious adverse event.

bique Ministry of Health. Adjacent to the CISM is the Manhiça Health Centre, a 110-bed referral health care facility. The district health network consists of an additional 8 peripheral health care posts and another rural hospital.

Study design. This study is a phase 2b, randomized controlled trial to assess the efficacy, safety, and immunogenicity of 3 doses of the candidate RTS,S/AS02A malaria vaccine administered to children aged 1–4 years. The present study includes different follow-up periods (figure 1). The initial double-blind phase included study months 0–8.5. At that time point, and according to protocol, the investigators were unblinded, and a first analysis of safety and efficacy was performed and reported [10]. Study participants and case ascertainment mechanisms remained blinded, and follow-up was sustained, in accordance with protocol, in the single-blind phase occurring from study months 8.5 to 21 [11]. A subsequent new protocol was developed to expand follow-up of the safety and efficacy of the study cohorts from study months 21 to 45. The present study includes safety and efficacy data for the entire study period from month 0 to month 45.

A total of 2022 healthy children aged 1–4 years were enrolled to receive either the candidate malaria vaccine or a comparison control vaccine. The parents or guardians of all participants provided written or thumb-printed informed consent before study enrollment. A member of the community acted as an impartial witness and countersigned the consent form to guarantee an adequate understanding of the study procedures by all guardians. Eligibility screening included a brief medical history, a physical examination, and blood sampling by finger stick for hematologic and biochemical tests. Children did not undergo screening tests for human immunodeficiency virus (HIV) infection. Hepatitis B surface antigen (HBsAg) status and anti-HBsAg antibody levels were assessed at baseline but were not criteria for exclusion from the trial. Other serologic markers of hepatitis B status were not assessed.

Children were randomized 1:1 to receive RTS,S/AS02A (in a 0.25-mL dose) or the control vaccines. The RTS,S/AS02A candidate vaccine was administered intramuscularly in the deltoid region of alternating arms, starting with the left arm, according to a 0-, 1-, and 2-month schedule. Children in the control group who were $\geqslant$24 months of age received 3 pediatric doses (0.5 mL) of hepatitis B vaccine (Engerix-B; GSK). Children <24 months of age received 2 pediatric doses of 7-valent pneumoccocal conjugate vaccine (Prevenar; Wyeth Lederle Vaccines), which was administered at the first and third vaccinations, and 1 dose of *Haemophilus influenzae* type B vaccine (Hiberix; GSK Biologicals), which was administered at the second vaccination.

Children were enrolled in 2 cohorts to measure the VE against clinical malaria disease and malaria infection. In cohort 1 (from the Manhiça area), 1605 participants were monitored using passive surveillance, to detect clinical episodes of malaria, and safety surveillance, until month 45. In cohort 2 (from the Ilha Josina village), 417 participants were monitored using ac-

Table 1. Percentage of Participants Reporting Serious Adverse Events (SAEs), as Classified by the *Medical Dictionary for Regulatory Activities (MedDRA)* [19] Primary System Organ Class and Preferred Term, over 45 Months of Follow-up (Intention-to-Treat [ITT] Analysis of Months 0–45)

	Control vaccine recipients with SAEs (n = 1010)		RTS,S/AS02A vaccine recipients with SAEs (n = 1012)	
Finding	No.[a]	%[b] (95% CI)	No.[a]	%[b] (95% CI)
Subjects with ⩾1 SAE[c]				
Reported	326	32.3 (29.4–35.3)	235	23.2 (20.7–25.9)
Reported and requiring hospitalization	199	19.7 (17.3–22.3)	159	15.7 (13.5–18.1)
SAEs reported and classified by *MedDRA* preferred term[d]				
Among all subjects	770		639	
Among subjects requiring hospitalization[d]	525		454	
Death				
Due to all causes	22	2.2 (1.4–3.3)	12	1.2 (0.6–1.2)
Excluding those due to trauma	18	1.8 (1.0–2.8)	11	1.1 (0.5–1.9)
Malaria related	5	0.5 (0.1–1.1)	1	0.1 (0.0–0.5)

NOTE. CI, confidence interval.

[a] No. of subjects who had ⩾1 dose administered, were included in an ITT cohort, and reported the symptom at least once.

[b] Percentage of subjects who reported the symptom at least once.

[c] At least one symptom experienced (regardless of the *MedDRA* preferred term).

[d] Symptoms reported by a subject after administration of a given dose and classified by the same preferred term are counted once.

tive surveillance, to detect malaria infection through visits that started 14 days after administration of dose 3, continued every 2 weeks for 2.5 months, and then continued monthly for an additional 2 years. At the end of the single-blind phase, new informed consent was obtained to continue follow-up for 2 more years. Surveillance for this cohort was continued through a health care facility–based passive case-detection system, to monitor safety.

The protocol (IND no. BB-IND 10514) was approved by the Mozambican National Ethics Review Committee, the Hospital Clínic of Barcelona (University of Barcelona) Ethics Review Committee, and the PATH Human Subjects Protection Committee. The trial was conducted in accordance with International Conference on Harmonisation of Good Clinical Practice guidelines and was monitored by GSK. A local safety monitor and a data and safety monitoring board closely reviewed the conduct and safety data of the trial.

Study procedures. Vaccines were administered at the Manhiça and Ilha Josina health care centers. Vaccine safety was evaluated using active and passive follow-up [15].

A serious adverse event (SAE) was defined as any medical event that resulted in death, was life-threatening, required inpatient hospitalization, or resulted in persistent or significant disability or incapacity. Investigators monitored participants with SAEs until the event had resolved or until month 45 of surveillance. Deaths that occurred at home were investigated by a review of all available medical records and through a verbal autopsy.

Statistical methods. Safety analysis was based on intention-to-treat (ITT) analysis of study participants included in both cohorts 1 and 2 during months 0–45. Analyses of VE against clinical malaria were based on cohort 1 study participants who were compliant with study procedures (ie, the according-to-protocol [ATP] cohort for analysis) from month 2.5 to month 45 during the study period

A child with a clinical episode was defined as a child who presented to a health care facility with an axillary temperature of ⩾37.5°C and *P. falciparum* asexual parasitemia of ⩾2500 parasites/μL (as per primary case definition). A child requiring admission to the hospital for malaria was defined as a child with *P. falciparum* asexual parasitemia for whom malaria was judged to be the sole cause of illness or a substantial contributing factor. All cases of severe malaria were defined by the presence of asexual *P. falciparum* parasitemia in a severely ill child, with there being no other more-probable cause of illness. Severe malaria was defined by the presence of any the following conditions: severe malaria anemia (packed-cell volume, <15%), cerebral malaria (Blantyre coma score, <2), and/or severe disease of other body systems (eg, multiple seizures [⩾2 generalized convulsions in the previous 24 h], prostration [defined as an inability to sit unaided], hypoglycemia [<2.2 mmol/L], clinically suspected acidosis, or circulatory collapse) [16]. All hospital admissions were independently reviewed by 2 groups of clinicians, to determine whether malaria was the cause of the admissions and whether the cause fulfilled the definition of severe malaria. Discrepancies were resolved by consensus.

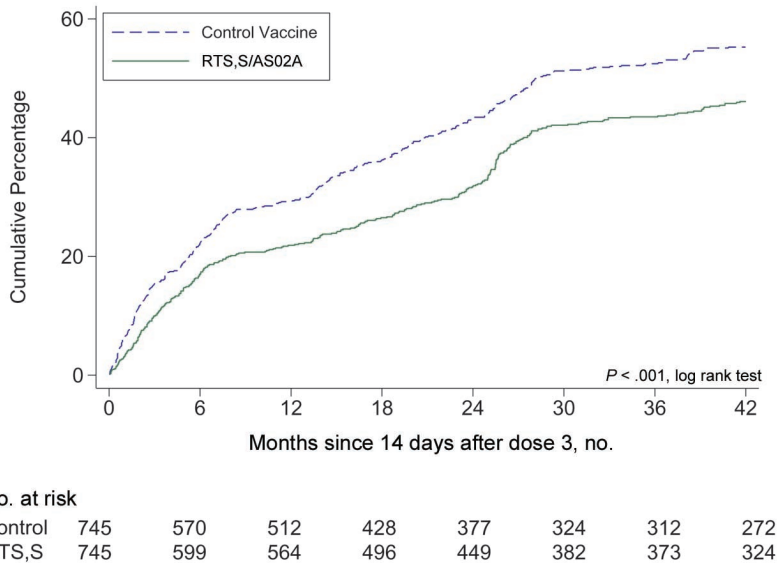

Figure 3. Kaplan-Meier curves for the cumulative proportion of children with ⩾1 episode of clinical malaria

For the efficacy analyses, except for analyses of hospital admissions, the time at risk was calculated with absences from the study area and antimalarial drug use both considered. For analysis of multiple episodes of clinical malaria, a subject was not considered to be susceptible for 28 days after the previous episode. After receiving malaria treatment, a child was not considered to be at risk for an arbitrary period of 28 days after receiving sulfadoxine pyrimethamine, 7 days after chloroquine alone, 7 days after quinine alone, 7 days after amodiaquine, and 20 days after artemether plus lumefantrine.

For the time to a first or only episode of clinical malaria, VE was assessed using Cox regression models and was defined as: (1 − hazard ratio) × 100. The VE was adjusted for predefined covariates of age, bed net use, geographic area (administrative divisions), and distance from a health care center. Cox regression assumes proportional hazards throughout follow-up. This assumption was checked graphically by plotting per group the log of the cumulative hazard against the log of time, as well as by using a test based on the Schoenfeld residuals and time-dependent Cox regression models.

For multiple episodes of clinical malaria and hospital admission, the group effect was assessed using Poisson regression models with normal random intercepts, including the time at risk as an offset variable.

Differences in the proportions of children with ⩾1 episode of severe malaria disease, as well as the differences in the prevalence of asexual *P. falciparum* at each cross-sectional survey, were compared using Fisher's exact test. For severe malaria, VE was calculated as 1 − risk ratio, with the exact 95% confidence interval determined using StatXact PROCs for SAS software (version 6).

The humoral immune response against *P. falciparum* was assessed as described elsewhere by determining titers of antibody to the circumsporozoite protein. Seropositivity was defined as anti–circumsporozoite protein titers of ⩾0.5 EU/mL. Analyses were performed using SAS software (version 8; SAS) and STATA software (version 9.0; Stata).

RESULTS

For the safety analysis (surveillance months 0–45), a total of 2022 children aged 1–4 years were recruited and randomized to the RTS,S/AS02A group and the control group (1605 children for cohort 1 and 417 children for cohort 2). A total of 1465 subjects (72.5%; 1142 subjects in cohort 1 and 323 subjects in cohort 2) completed the follow-up to study month 45. For the efficacy analyses (months 2.5–45), including only those participants in cohort 1, a total of 1490 (73.7%) of 2022 children completed the follow-up (figure 2).

Over the 45-month surveillance period analyzed for the intent-to-treat cohort, 639 SAEs classified according to the preferred term in the *Medical Dictionary for Regulatory Activities* [19] were noted in 235 subjects who received the RTS,S/AS02A vaccine, and 770 SAEs were noted in 326 subjects who received the control vaccines (table 1). The pattern of the causes of SAEs observed in this trial is similar to the morbidity background of the area. The most important diseases are malaria, anemia, gastroenteritis, and pneumonia. During this period, 62 cases of severe malaria were experienced by 4.6% (95% CI, 3.4%–6.1%) of the study participants who received RTS,S/AS02A. In the control group, there were 83 cases of severe malaria among 7.0% (95% CI, 5.5%–8.8%) of the study participants. Blood

Table 2. Vaccine Efficacy (as Determined by According-to-Protocol Analysis of Follow-up Months 2.5–45) in Cohort 1, by Outcome

Fever	Control vaccine group (n = 745)			RTS,S/AS02A vaccine group (n = 745)			Vaccine efficacy, % (95% CI)	P
	Events, no.	PYAR, no.	Event rate	Events, no.	PYAR, no.	Event rate		
First or only episode								
And parasitemia								
>0 parasite/mL	393	1380.1	0.29	342	1571.3	0.22	27.2 (15.6–27.1)	<.001
>2500 parasites/mL	370	1440.1	0.26	307	1637.4	0.19	30.5 (18.9–40.4)	<.001
>15,000 parasites/mL	334	1520.3	0.22	282	1699.4	0.17	28.2 (15.7–38.9)	<.001
Or history of fever and parasitemia >0 parasites/mL	490	1137.0	0.43	421	1378.8	0.31	31.9 (22.2–40.4)	<.001
Several episodes and parasitemia >2500 parasites/mL	774	2142.8	0.36	658	2194.3	0.30	25.6 (11.9–37.1)	<.001

NOTE. The 1605 participants in cohort 1 were monitored using passive surveillance, to detect clinical episodes of malaria, and safety surveillance. Vaccine efficacy estimates were adjusted by age at baseline, bed net use at baseline, distance from health care facility, and geographic region. CI, confidence interval; PYAR, person-years at risk.

transfusions were performed for 58 subjects (2.7% of patients in the group receiving study vaccine and 3.1% of patients in the control group). There were 34 deaths, with 12 (1.2% [95% CI, 0.6%–2.1%]) occurring in the RTS,S/AS02A group and 22 (2.2% [95% CI, 1.4%–3.35%]) occurring in the control group (P = .087). Six of these deaths were judged to be associated with malaria: 1 occurred in the RTS,S/AS02A group, and 5 occurred in control group. No SAE or death was considered to be associated with vaccination.

VE. In the VE analyses (VE analysis for the according-to-protocol cohort for months 2.5–45 [$ATP_{\{2.5–45\}}$]), 677 children had first or only clinical episodes that met the primary case definition. Of these, 307 were in the RTS,S/AS02A group and 370 were in the control group, yielding a crude VE estimate of 25.6% (95% CI, 13.4%–36.0%; P < .001) and an adjusted $VE_{(2.5–45)}$ of 30.5% (95% CI, 18.9%–40.4%; P = < .001) (figure 3). The VE estimates obtained using several case definitions based on different parasite-density cutoff levels are shown in table 2. The adjusted $VE_{(2.5–45)}$ of surveillance, including all clinical episodes, was 25.6% (95% CI, 11.9%–37.1%; P < .001).

In the RTS,S/AS02A group (n = 745), there were 29 children who had ⩾1 episode of severe malaria, compared with 47 children in the control group (n = 745) (VE, 38.3% [95% CI, 3.4%–61.3%]; P = .045). The number of hospital admissions due to all causes was also lower for the RTS,S/AS02A group than for the control group (175 vs 194 admissions), and the VE was 22.2% (95% CI, −3.8% to 41.7%; P = .088). The VE against malaria resulting in hospitalization was 23.0% (95% CI, −1.7% to 41.9%; P = .078).

Analysis of VE over different follow-up periods showed a VE of 16.8% over months 21–33 (95% CI, −2.5% to 32.4%; P = .084) and a VE of 11.8% over months 33–45 (95% CI, −20.1% to 35.2%; P = .426). There is a trend toward lower estimates of efficacy over the latter follow-up periods, but the proportionality of the hazard assumption did not find evidence of waning efficacy either by graphical inspection of the plot of log [−log(survival time)] with log of the survival time, the time-dependent Cox models, or the test based on the Schoenfeld residuals (P = .210).

Anti–circumsporozoite protein response and parasitemia. Anti–circumsporozoite protein antibody levels were still ~30-fold higher than prevaccination levels in cohort 1 at month 45 in the RTS,S/AS02A group, with a geometric mean titer (GMT) of 8.9 (range, 7.8–10.1), whereas most of the children in the control group had a GMT of 0.3 (range, 0.3–0.3). At least 96% of subjects in the RTS,S/AS02A group were seropositive for anti-circumsporozoite protein antibodies at month 45.

The prevalence of asexual-stage parasites was lower in the RTS,S/AS02A group than in the control group, in the yearly cross-sectional surveys that were performed (figure 4). At study month 33, the prevalence of *P. falciparum* asexual parasitemia was 22% lower in the RTS,S/AS02A group (93 [15.8%] of 590 patients) than in the control group (121 [20.3%] of 596 patients; P = .049). At study month 45, prevalence was 34% lower in the RTS,S group (66 [12.2%] of 541 patients) than in the control group (101 [18.5%] of 547 patients) (P = .004). Among children bearing asexual-stage *P. falciparum* parasites during the cross-sectional surveys, parasite densities were similar in the 2 groups and at both surveys conducted at months 33 and 45 (geometric mean density, 1878 vs 1621 [P = .467] and 594 vs 1057 [P = .065], respectively) (figure 4).

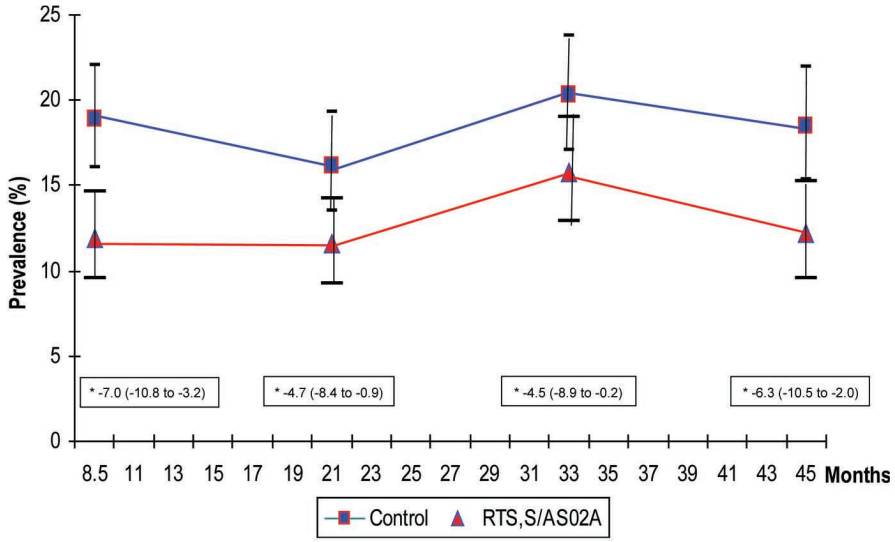

Figure 4. Prevalence (95% confidence interval) of *Plasmodium falciparum* asexual parasitemia at the different cross-sectional surveys. *Difference in the affected proportion of the RTS,S/AS02A group and the control group.

DISCUSSION

The present study reports what is, to our knowledge, the first long-term follow-up of a pediatric malaria vaccine trial in Africa. Over a 45-month period, the candidate vaccine had an acceptable safety profile, with significantly less SAEs and a trend toward a reduced mortality rate among individuals in the RTS,S/AS02A group.

Previous reports confirmed efficacy during an initial 6-month follow-up as well as sustained protection up to 21 months of follow-up. Analysis up to 45 months allows us to exclude the theoretical risk that partial protection with this vaccine could have impaired acquisition of natural immunity and that subsequent loss of vaccine-induced protection could be followed by a rebound in the risk of clinical malaria among previously protected children.

It is challenging to assess the duration of protection against a communicable disease when repeated infections and clinical episodes are required to slowly build up naturally acquired immunity, and when the risk of malaria consequently is age dependent. Indeed, over the past 2 years, the incidence of clinical malaria in the control group decreased from 0.37 episodes/person-years at risk (during follow-up from months 21 to 33) to 0.15 episodes/person-years at risk (during follow-up from months 33 to 45). Analysis of efficacy broken down into 12-month periods yields estimates that show a tendency toward decreasing efficacy from 30% to 11%, with overlapping confidence intervals that are wider at the end indicating less precision on the estimate at the end of the study. The statistical method used to evaluate the proportionality of the hazard assumption showed no evidence of waning efficacy, but because the study was not designed to have sufficient power to evaluate this, it could reflect a lack of power to detect it.

On the other hand, the prevalence of parasites at the end of the 45-month follow-up was significantly lower in the vaccine group than in the control group. Given that the prevalence of *P. falciparum* asexual parasitemia must reflect the recent risk of infection, we interpret this finding as a strong indication that significant efficacy remains at the end of the 45-month follow-up.

VE against clinical malaria and against severe malaria over the entire follow-up was 30.5% (95% CI, 18.9%–40.4%) and 38.3% (95% CI, 3.4%–61.3%), respectively. VE against all clinical episodes was 25.6% (95% CI, 11.9%–37.1%; $P < .001$). In other words, immunization with RTS,S/AS02A reduced the burden of malaria during this period by approximately one-quarter.

These exciting results confirm the potential of developing malaria vaccines that may influence relevant end points of clinical and public health and that may consequently reduce the unacceptable burden of malaria in African children. Together with recently reported data showing a favorable safety profile and a proof-of-concept efficacy of 65% in reducing the risk of new infections when vaccine is administered to young infants at 10, 14, and 18 weeks of age, these results strengthen the rationale for advancing toward a phase 3 trial aiming to register RTS,S/AS as the first malaria vaccine.

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Evidencias en Pediatría

Editorial

Últimos avances en el desarrollo de una vacuna de la malaria

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EDITORIAL

Últimos avances en el desarrollo de una vacuna de la malaria

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La malaria es un problema de salud global que produce una gran carga de enfermedad en todos los países en vías de desarrollo. Se estima que unos 3.000 millones de personas, casi la mitad de la población mundial, vive en áreas donde hay transmisión de malaria[1] Esta enfermedad infecciosa afecta todos los años entre 300 y 500 millones de personas y causa entre 1 y 3 millones de muertes en el mundo[2-4].

El continente más afectado es África, y es la región Subsahariana la que sufre el mayor peso de la enfermedad. Se estima que en África alrededor de 220 millones de casos de malaria ocurren anualmente y el 30% de todos los ingresos hospitalario son debidos a esta enfermedad. Se calcula que entre 700.000 y 1.6 millones de personas mueren debido a las consecuencias directas de la enfermedad, siendo la mayoría de ellos niños menores de cinco años y mujeres embarazadas[5].

La enfermedad es causada por el parásito *Plasmodium* que, a través da picadura infectada de la hembra del mosquito del género *Anopheles*, es capaz de entrar en el torrente sanguíneo de las personas sanas. Los cuatro tipos de parásitos que infectan al hombre son *Plasmodium vivax*, *ovale*, *malariae* y *falciparum*. Este último se encuentra en África y Asia y es responsable del 90% de las muertes; sin embargo, la especie más prevalente es el *Plasmodium vivax*[5].

Durante las primeras décadas del siglo XX las áreas con malaria endémica se redujeron en un 30%. Los primeros avances se registraron en América del Norte y la mayor parte de Europa Occidental, regiones en las que el rápido desarrollo socioeconómico permitió destinar más recursos a combatir la transmisión de la infección. Durante las décadas de 1950 y 1960 se logró reducir aun más la transmisión de esta enfermedad parasitaria en grandes áreas de la India y la antigua Unión Soviética mediante el empleo del insecticida dicloro-difeniltricloroetano (DDT) y las campañas antivectoriales realizadas como parte de un programa mundial para el control de la malaria. En España la malaria fue erradicada en los años 60.

Una vez conseguida la erradicación de la malaria en los países industrializados, el programa mundial para el control de esta enfermedad fracasó debido a la disminución del interés junto con la insuficiencia de recursos económicos destinados para su control, aunado al limitado interés por el desarrollo de nuevas tecnologías.

Empezaron a aparecer las primeras cepas de parásitos resistentes a los medicamentos antimaláricos mas usados, como la cloroquina, así como a los insecticidas utilizados habitualmente. Esta situación, a su vez, se vio agravada por algunos factores determinantes entre los que cabe destacar: el escaso interés por parte de la industria farmacéutica en el desarrollo de nuevos fármacos; los cambios en el medio ambiente; la superpoblación; la insuficiente e inadecuada distribución e implementación de medidas de control; el desmoronamiento de los programas nacionales de control de la enfermedad; el aumento del turismo y de los movimientos migratorios, con el consiguiente movimiento de poblaciones no inmunes a zonas endémicas[6].

Como consecuencia, los casos de malaria han aumentado en todo el mundo y esta enfermedad se ha convertido actualmente en uno de los problemas de salud más importantes en vastas zonas de África subsahariana, Asia, América Latina y Oceanía.

Actualmente disponemos de diferentes herramientas para controlar la enfermedad, entre las que destacan las siguientes: el rápido diagnóstico e inicio del tratamiento de los casos con un antimalárico eficaz y apropiado, considerando su disponibilidad, validez y costo-efectividad; la disminución del contacto entre hombre y vector (fundamentalmente con mosquiteras impregnadas de insecticida); el tratamiento preventivo intermitente en niños y mujeres embarazadas; y el sistema de vigilancia y control vectorial integrado mediante fumigación intradomiciliaria o con larvacidas, así como una efectiva participación comunitaria e intersectorial.

En muchos países la mayoría de estos mecanismos de control no han sido implementados por diversas razones económicas, sociales y políticas. Entre ellas es importante resaltar unos servicios sanitarios básicos muy deficientes.

Una vacuna efectiva, segura y de bajo coste que proteja los niños de zona endémica, sumada a las demás estrategias de control ya existentes, sería un elemento clave en el control de la enfermedad.

El desarrollo de la vacuna de la malaria empezó en el siglo XX y, a pesar de los avances biomédicos y los estudios realizados en la época, no se fue capaz de conseguir una vacuna preventiva. A principios de los años 70 del siglo pasado se demostró que los esporozoítos irradiados con rayos ultravioletas de *P. falciparum* y *P. vivax* inoculados en voluntarios sanos conferían inmunidad completa en 90% de ellos frente a la picadura de mosquitos infectados [7,8]. La introducción de las técnicas de biología molecular en el estudio de la malaria, junto con la posibilidad del cultivo in vitro de *P. falciparum* y la mayor mortalidad por la infección causada por esta especie, han hecho que se convierta en el principal objetivo de estudio. Sin embargo, también se han realizado avances en la obtención de una vacuna frente a la especie *P. vivax* que tiene distribución geográfica mucho mayor que la de *P. falciparum*. La dificultad del cultivo in vitro es uno de los obstáculos técnicos en el desarrollo de una vacuna frente a este tipo de parasito.

Los parásitos del género *Plasmodium* tienen un ciclo vital complejo y atraviesan diferentes estadios, cada uno de los cuales presentan múltiples antígenos que pueden ser inmunógenicos. Las investigaciones en el desarrollo de una vacuna se centran en tres vías diferentes:

• vacunas contra el estadio pre-eritrocítico, que protegen contra los esporozoitos (forma infectiva inyectada por el mosquito) o impiden la invasión de los hepatocitos [9-13];

• vacunas eritrocíticas o contra el estadio sanguíneo, que inhiben la multiplicación del parásito en los hematíes, previniendo la enfermedad grave durante la infección sanguínea[14-16];

• vacunas del estadio sexual del parásito, que tratan de prevenir el desarrollo de formas sexuales una vez ingeridas por el mosquito, rompiendo así el ciclo biológico del parásito[17].

El desarrollo de una vacuna esta siendo mucho más complicado de lo que parecía hace 30 años. Esto es debido a varios factores entre los que cabe destacar: el complejo desarrollo y la alta variabilidad antigénica del parásito (que le confieren unos extraordinarios mecanismos de evasión inmune), la falta de marcadores inmunológicos que se correlacionen con la protección frente a malaria y la falta de modelos animales.

El Plasmodium presenta multitud de antígenos que varían a lo largo de los diferentes estadios de su ciclo vital y contra los cuales son requeridas respuestas inmunes secuenciales encadenadas. Así, un anticuerpo desarrollado contra la fase inicial de la infección no protegerá contra las fases posteriores.

Además, muchas proteínas parasitarias exhiben muchas formas diferentes, y un mismo clon parasitario puede llegar a disponer de hasta cincuenta copias diferentes del gen que codifica una proteína esencial para su acción, expresando una versión diferente de la proteína en cada oleada sucesiva de parásitos en sangre. Esta variabilidad es crítica para la supervivencia del parásito y desfavorable para el individuo infectado.

La única manera de conocer la eficacia de una vacuna es realizando ensayos clínicos de las vacunas candidatas en zonas endémicas de malaria. Estos estudios son siempre logísticamente muy complejos. Los costes totales necesarios para desarrollar una vacuna son extremadamente elevados; el proceso que va desde el desarrollo de una vacuna hasta que la inversión puede ser devuelta gracias a la comercialización posterior a su registro puede llevar hasta 30 años. Esta es la una de las razones por la que las empresas farmacéuticas demuestran poco interés en el desarrollo de una vacuna para esta enfermedad.

A pesar de todo ello, los últimos años se ha producido un progreso significativo en el desarrollo de una vacuna que ha demostrado una protección significativa y duradera en niños africanos tras ensayos clínicos.

Muchas han sido las formulaciones utilizadas en la búsqueda de una buena vacuna candidata y, hasta ahora, sólo la RTS,S ha demostrado inducción de respuesta protectora en humanos. Existe consenso en cuanto a que los adyuvantes como AS02 son imprescindibles para promover el desarrollo de títulos elevados de anticuerpos.

La vacuna RTS,S/AS02A, desarrollada por GSK (GlaxoSmithKline Biologicals), está constituida por la parte C-terminal de la proteína CS (aminoácidos 207 a 395) fusionada con antígeno de superficie de hepatitis B expresado en forma de virus-like. El adyuvante es conocido como AS02 y consta de dos inmunoestimulantes: MPL y QS21 en una emulsión de agua y aceite.

Esta vacuna fue inicialmente probada en adultos voluntarios no inmunes demostrándose una protección del 41%[18]. En otros estudios realizados en Gambia se observo un 71% de protección durante la primeras 9 semanas y un 34% de reducción de la infección durante un periodo de 15 semanas, con una reducción de la eficacia, hasta desaparecer[19]. En ambos estudios se informó que la vacuna era segura e inmunogénica.

Posteriormente, en Mozambique, se realizó un ensayo clínico aleatorizado, doble ciego, controlado en 2.022 niños de 1 a 4 años para evaluar la eficacia, inmunogenicidad y reactogenicidad de la vacuna. El objetivo primario de este estudio era evaluar la eficacia en los casos clínicos de malaria por *P. falciparum* a los 6, 18 y 45 meses de seguimiento[9,10]. Se incluyeron dos

cohortes con distintos tipos de control. La cohorte 1 tenía como finalidad principal valorar la eficacia de la vacuna frente a los episodios de malaria (considerando episodio una temperatura axilar de ≥37.5 ºC y > 2.500 parásitos asexuados por microlitro de sangre). En la cohorte 2 el objetivo más importante era determinar la eficacia de la vacuna frente a nuevas infecciones. Por eso, a estos niños se les administró una dosis de antimaláricos antes de la última dosis de la vacuna candidata para poder evaluar el tiempo hasta la primera infección. Los participantes fueron aleatorizados para recibir la vacuna RTS,S/ASO2A o las vacunas de control (vacuna neumococo heptavalente alternada con la vacuna de *Hemofilus influenza* o la vacuna Hepatitis B). Los resultados mostraron a los 6 meses de la vacunación una eficacia frente al primer episodio de malaria clínica del 29.9% (IC95%: 11-44.8; p=0.004), una eficacia frente a la primera infección del 45% (IC95%: 31.4-55.9; p<0.0001) y frente a la malaria grave del 57.7% (IC95%: 16.2-80.6; p=0.019)[9]. Recientemente ha sido comprobado que la eficacia se mantiene hasta los 45 meses de seguimiento; con unos valores de 30.5% (95% CI 18.9–40.4; p=<0.0001) frente a la primera infección y de 38.3% (95% CI 3.4 - 61.3; p=0.045) para la malaria grave.

Con esta misma vacuna se hay realizado ensayos para evaluar la seguridad, inmunogenicidad y eficacia, además de la no inferioridad de respuesta inmune, cuando se administra junto con las vacunas del PAV (programa ampliado de vacunación) en niños mozambiqueños menores de 6 meses. Para ello se administro la vacuna a estudio intercalada con las vacunas del PAV (difteria, tétanos, tosferina y Hemophillus influenza tipo b). Los resultados de este estudio demostraran una eficacia de 65.9% (95% CI 42.6 – 79.8; p<0.0001)[20]. Este artículo es analizado en el presente número de Evidencias en Pediatría[21].

Actualmente, múltiples vacunas candidatas se hallan en diferentes estadios de desarrollo, la mayor parte de ellas aún en fase preclínica. Más de la mitad de las aproximadamente 90 vacunas candidatas que están siendo desarrolladas actualmente se basan en únicamente tres antígenos, clonados hace más de dos décadas: la proteína del circumsporozoíto (CSP), la proteína de superficie del merozoíto (MSP) y el antígeno apical de membrana 1 (AMA-1).

Tras la revisión histórica, y gracias a los avances significativos logrados en los últimos años, podemos concluir que hay buenas razones para ser optimistas. A pesar de las dificultades científicas a las que todavía nos enfrentamos, creemos que el registro de una primera generación de vacuna contra la malaria se encuentra en un horizonte cercano. Necesitaremos más investigaciones para desarrollar una vacuna definitiva que evite muchas muertes en la población más vulnerable: los niños. El registro de esta primera vacuna candidata no es el destino final, sino una estación intermedia, en el esfuerzo global para el desarrollo de las mejores herramientas de control y eventualmente la erradicación de la malaria.

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VII. SUMARY OF RESULTS

ARTICLE 1: A 10 year study of the cause of death in children under 15 years in Manhiça, Mozambique. BMC 2009

From January 1997 to December 2006, 568499 person year at risk (pyrs) and 10037 deaths were recorded in the Manhiça DSS. 3730 deaths with 246658 pyrs were recorded in children under 15 years old. Verbal autopsy interviews were conducted for 3002 (80.4%) of these deaths. Forty seven percent of the interviews were conducted within a period of 6 months and 83.9% within 1 year from the time of death.

According to respondents, 54% of deaths occurred outside a health facility. However, medical and other assisted care during the terminal illness was sought by 81.9% of those who died. Most of the paediatric deaths occurred in children aged 1– 4 years (41.3%), followed by infants aged 29 days – 1 year (30.6%) Communicable diseases were responsible for most deaths (73.6%), non-communicable diseases accounted for 9.5%, and injuries for 3.9%. Among communicable disease, the most frequent diagnosis was infectious and parasitic diseases (60.0%), followed by perinatal disease (17.4%).

Malaria was the leading cause, accounted for 21.8% of total diagnosis given physicians and for 3.2 deaths/1000 pyrs. Acute lower respiratory infection (ALRI) including pneumonia, was the second leading cause of death with 9.8% of total diagnosis and 1.5 deaths/1000 pyrs, followed by HIV/AIDS with 8.3% and 1.3 deaths/1000 pyrs. Diarrheal

diseases with 8.0% and 1.2 deaths/1000 pyrs and malnutrition with 6.4% and 0.96 deaths/1000 pyrs were other important conditions. In the age group between 29 days to 1 year, malaria (12.5 deaths/1000 pyrs), ARLI (10.6 deaths/1000 pyrs) and HIV/AIDS (6.5 deaths/1000 pyrs) accounted for 37% of infant mortality. Malaria was very common in children 1 to 4 years of age, with 34.4% of the total diagnoses and a mortality rate of 6.1 deaths/1000 pyrs. Malaria and pneumonia largely predominated among causes of death, accounting for 30% of the mortality during the study. The first period (1997 to 2001), was marked by an increase in death rates attributed to malaria, pneumonia, diarrhoeal disease and HIV/AIDS, but not malnutrition. Deaths attributed to malaria almost triplicate between 1997 and 2001, increasing from 2.2 deaths/1000 pyrs to 7.7 deaths/1000 pyrs, respectively, and then declined substantially between 2001 and 2006 to 2.8 deaths/1000 pyrs. During the second period all main mortality causes, declined in the study area (16).

ARTICLE 2: Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children randomised controlled trial. Lancet 2004

In 2004, we reported the first proof of concept study in 2022 African children aged 1 to 4 years living in a *P. falciparum* endemic area of Mozambique. This was a double-blind, individually-randomized, placebo-controlled trial.

The primary objective was to evaluate the safety, efficacy and immunogenicity of the paediatric dose of the RTS,S/AS02A malaria candidate vaccine. The study included two cohorts of children living in two separate areas which underwent different follow-up schemes. Participants were randomly allocated three doses of either RTS,S/AS02A candidate malaria vaccine or control vaccines. The primary endpoint, determined in cohort 1 (n=1605), was time to first clinical episode of *P falciparum* malaria (axillary temperature 37.5ºC and P falciparum asexual parasitaemia >2500 per/L) over a 6-month surveillance period.

Efficacy for prevention of new infections was determined in cohort 2 (n=417). 115 children in cohort 1 and 50 in cohort 2 did not receive all three doses and were excluded from the perprotocol analysis. Vaccine efficacy for the first clinical episodes was 29.9% (95% CI 11.0–44.8; p=0.004). At the end of the 6-month observation period, prevalence of *P falciparum* infection was 37% lower in the RTS,S/AS02A group compared with the control group (11.9% vs 18.9%; p=0.0003). Vaccine efficacy for severe malaria was 57.7% (95% CI 16.2–80.6; p=0.019). In cohort 2, vaccine efficacy for extending time to first infection was 45.0% (95% CI 31.4–55.9; p<0.0001)(87)

ARTICLE 3: Duration of protection with RTS,S/AS02A malaria vaccine in prevention of Plasmodium falciparum disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. – Lancet 2005

In this article we report VE in a single blind phase up to month 21 (VE 8.5–21). The primary endpoint was time to first or only clinical episode of *P.falciparum* malaria (axillary temperature 37·5ºC and *P falciparum* asexual parasitaemia 2500 per/L) detected through a passive case detection system. We also determined VE for other case definitions and for episodes of severe malaria.

During the single-blind phase, $VE_{(8.5–21)}$ was 28.9% (95% CI 8.4–44.8; p=0.008). At month 21, prevalence of *P. falciparum* infection was 29% lower in the RTS,S/AS02A group than in the control (p=0.017). Considering the entire study period, $VE_{(2.5–21)}$ was 35.3% (95% CI 21.6–46.6; p_0.0001) for clinical episodes and $VE_{(2.5–21)}$ for severe malaria was 48.6% (95% CI 12.3–71.0; p=0.02). (86).

ARTICLE 4: Safety of the RTS,S/AS02A malaria vaccine in Mozambican children during a Phase IIb trial.- Vaccine 2008

Safety data was available for 1012 children who received a total of 2926 RTS,S/AS02A (0.25mL) doses and for 1010 children who received a total of 2912 doses in the control group. Overall, symptoms were reported more frequently following administration of RTS,S/AS02A than following

either control vaccine regimen. In children < 24 months of age, at least one symptom was observed after 61.1% (95% CI: 57.3-64.7) of RTS,S/AS02A doses, and in children ≥24 months of age, this figure was 45.3% (95% CI: 43.2-47.4). For recipients of the control vaccines, the corresponding figures were 30.1% (95% CI: 26.7-33.6) in children <24 months (Prevnar™ and Hiberix™) and 18.6% (95% CI: 17.0-20.3) in children ≥24 months (Engerix-B™).

Up to 30 days post-vaccination a total of 1712 unsolicited adverse events were observed; 910 (53.2%) in the RTS,S/AS02A group and 802 (46.8%) in the control group. Of these, 322 (155 in the RTS,S/AS02A and 167 in the control group) were considered to be grade 3 severity. Over the total observation period 523 SAEs were reported in 212 subjects who received the RTS,S/AS02A vaccine and 634 SAEs in 289 subjects who received the control vaccines. None of the SAEs was deemed to be related to vaccination.(88)

ARTICLE 5: Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children – JID 2009 in press

For the safety analysis (0-45), 2022 children aged 1-4 years were recruited and randomised to the RTS,S/AS02A group and control group (1605 children for Cohort 1 and 417 children for Cohort 2). A total of 1465 (72.5%) subjects completed the follow up to study month 45: 1142 in the Cohort 1 and 323 in the Cohort 2. For the efficacy analyses (2.5-45),

including only participants in Cohort 1, 1490 (73.7%) children completed the follow-up. Over the 45-month surveillance period analysed on the intent-to-treat cohort (ITT(0-45)), 639 SAEs classified by MedDRA preferred term level were reported in 235 subjects who received the RTS,S/AS02A vaccine and 770 SAEs in 326 subjects who received the control vaccines. In the vaccine efficacy analyses (ATP(2.5-45)), 677 children had first or only clinical episodes meeting the primary case definition. Of these, 307 were in the RTS,S/AS02A group and 370 in the control group, yielding a crude vaccine efficacy estimate of 25.6% (95% CI 13.4–36.0; p=0.0001) and an adjusted VE(2.5–45) of 30.5% (95% CI 18.9–40.4; p=<0.0001). The VE estimates using several case definitions based on different parasite density cut-offs. The adjusted VE(2.5–45) including all clinical episodes was 25.6% (95% CI 11.9 – 37.1; p=0.0006).

ARTICLE 6: Last advances in the development of a malaria vaccine. (Últimos avances en el desarrollo de una vacuna de la malaria). Evid Pediatr. 2008

Malaria is a global health problem and a major cause of morbidity and mortality in many countries in all developing countries. The recent estimates that around 3 billion people lived in areas at risk of malaria transmission. Each year there are approximately 300-500 million acute malaria episodes and an estimated 700.000 and 1.6 millions of people die

as a direct consequence of an illness, most of them being children less than 5 years old and pregnant women mostly in Sub-Saharan Africa(18). Currently, there are different tools available to control the disease, as for instance: the rapid diagnosis and initiation of the treatment with an appropriate and effective antimalarial, considering its availability, validity and cost-effectiveness; the reduction of the contact between the person and the vector (basically with impregnated mosquito nets); the intermittent preventive treatment in children and pregnant women; the surveillance system and vector control integrated thanks to housing fumigation or the use of larvicides; as well as an effective and cross sector community participation. A vaccine, effective, safe and with a low cost, that could protect the children from the endemic zones, added to the already existing control tools, could be a key element to control the disease.

However, during the last years a significant progress has been made to develop a vaccine that has demonstrated a significant and lasting protection in African children after several clinical trials.

Many have been the formulations used in the search for a good vaccine candidate and, until now, only the RTS,S has demonstrated an induction of a protector response in humans. There is a consensus about the fact that the adjuvants as the AS02 are essential to promote the development of large titles of anti bodies. After a historical review and thanks to the significant developments achieved during the last years, we

can conclude that there are good reasons for being optimistic. In spite of the scientific difficulties that we are still facing, we believe that the register of a first generation vaccine against malaria may be on the near horizon. We will need more research to develop a definitive vaccine that could prevent many deaths among the most vulnerable population: the children. The registration of a first vaccine candidate is not the final destiny but an intermediate station in the global effort for the development of the best control tools and eventually, the eradication of malaria (100).

VIII. CONCLUSIONS

ARTICLE 1: A 10 year study of the cause of death in children under 15 years in Manhiça, Mozambique. BMC 2009

i. About 54% of all deaths took place outside a health facility in Manhiça study area.

ii. 73.6% of deaths were attributed to communicable diseases. Non-communicable diseases accounted for 9.5% of the defined causes of death, and injuries accounted for 3.9% of causes of deaths.

iii. Malaria was the single largest cause of death in Manhiça study area in children less than 15 years accounting for 21.8% of cases.

iv. Pneumonia with 9.8% was the second leading cause of death, followed by HIV/AIDS (8.3%) and diarrhoeal diseases with 8%.

v. Deaths attributed to malaria almost triplicate between 1997 and 2001, increasing from 2.2 deaths/1000 pyrs to 7.7 deaths/1000 pyrs, respectively, and then declined substantially between 2001 and 2006 to 2.8 deaths/1000 pyrs. This may be due to several reasons such as use of more effective antimalarial drugs for treatment of non severe malaria cases.

vi. This study highlight the big challenge that lies ahead in the fight against preventable infectious diseases in developing countries.

ARTICLE 2: Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children randomized controlled trial. Lancet 2004

vii. The RTS,S/AS02A vaccine was safe, well tolerated, and immunogenic.

viii. During double blind phase (2,5- 8,5m), vaccine efficacy for the first clinical episodes was 29.9% (95% CI 11.0–44.8; p=0.004)

ix. Vaccine efficacy for severe malaria was 57.7% (95% CI 16.2–80.6; p=0.019).

x. In cohort 2, vaccine efficacy for extending time to first infection was 45.0% (31.4–55.9; p<0.0001).

xi. At the end of the 6-month observation period, prevalence of *P falciparum* infection was 37% lower in the RTS,S/AS02A group compared with the control group (11.9% vs 18.9%; p=0·0003).

xii. Our results indicate the feasibility of development of an effective vaccine against malaria.

ARTICLE 3: Duration of protection with RTS,S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. Lancet. 2005

xiii. During the single-blind phase (8,5–21m), vaccine efficacy for the first clinical episode was 28.9% (95% CI 8.4–44.8; p=0.008).

xiv. Considering the entire study period (2,5–21m), vaccine efficacy was 35.3% (95% CI 21.6–46.6; p0.0001) and severe malaria was 48.6% (95% CI 12.3–71.0; p=0.02).

xv. These results show that RTS,S/AS02A confers partial protection in African children aged 1–4 years living in rural endemic areas against a range of clinical disease caused by *P falciparum* for at least 18 months

ARTICLE 4: Safety of the RTS,S/AS02A malaria vaccine in Mozambican children during a Phase IIb trial. Vaccine 2008.

xvi. Results indicate that the RTS,S/AS02A vaccine a good safety profile and well tolerated when given in three doses to semi-immune children living in malaria-endemic areas.

xvii. The proportion of children experience SAEs was lower in the RTS,S/AS02A recipients compared to the control group (Engerix-BTM or PrevnarTM and HiberixTM. None of the SAEs was deemed to be related to vaccination.

xviii. No imbalances for Biochemical and Hematological variables at month 21

xix. The pattern of inpatient morbidity observed in this trial is lower than of background population

ARTICLE 5: Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children – JID accepted in press, 2009

xx. Good safety profile has been confirmed: the RTS,S group had less SAE and lower mortality

xxi. Immunization with RTS,S/AS02A did not interfere with acquisition of naturally induces of immunity, and so, not rebound in the risk of malaria was documented.

xxii. Vaccine efficacy against clinical malaria and against severe malaria over the entire follow-up period (2.5–45) was 30.5% (95% CI 18.9–40.4) and 38.3% (95% CI 3.4- 61.3), respectively.

xxiii. Anti-CS antibodies levels decrease with time but were still approximately 30-fold higher compared to pre-vaccination levels in cohort 1 at Month 45 in the RTS,S/AS02A group while the control group.

xxiv. Given that the prevalence of *P. falciparum* asexual parasitaemia must reflect recent risk of infection, we interpret that difference in parasite prevalence between two group, this as a strong indication that significant efficacy remains at the end of the 45-month follow-up.

xxv. These exciting results confirm the potential of developing malaria vaccines that may impact clinical and public health relevant endpoints, and that may consequently reduce the unacceptable burden of malaria in African children.

xxvi. These results strengthen the rationale for advancing toward a phase III trial aiming to register RTS,S/AS as the first malaria vaccine.

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