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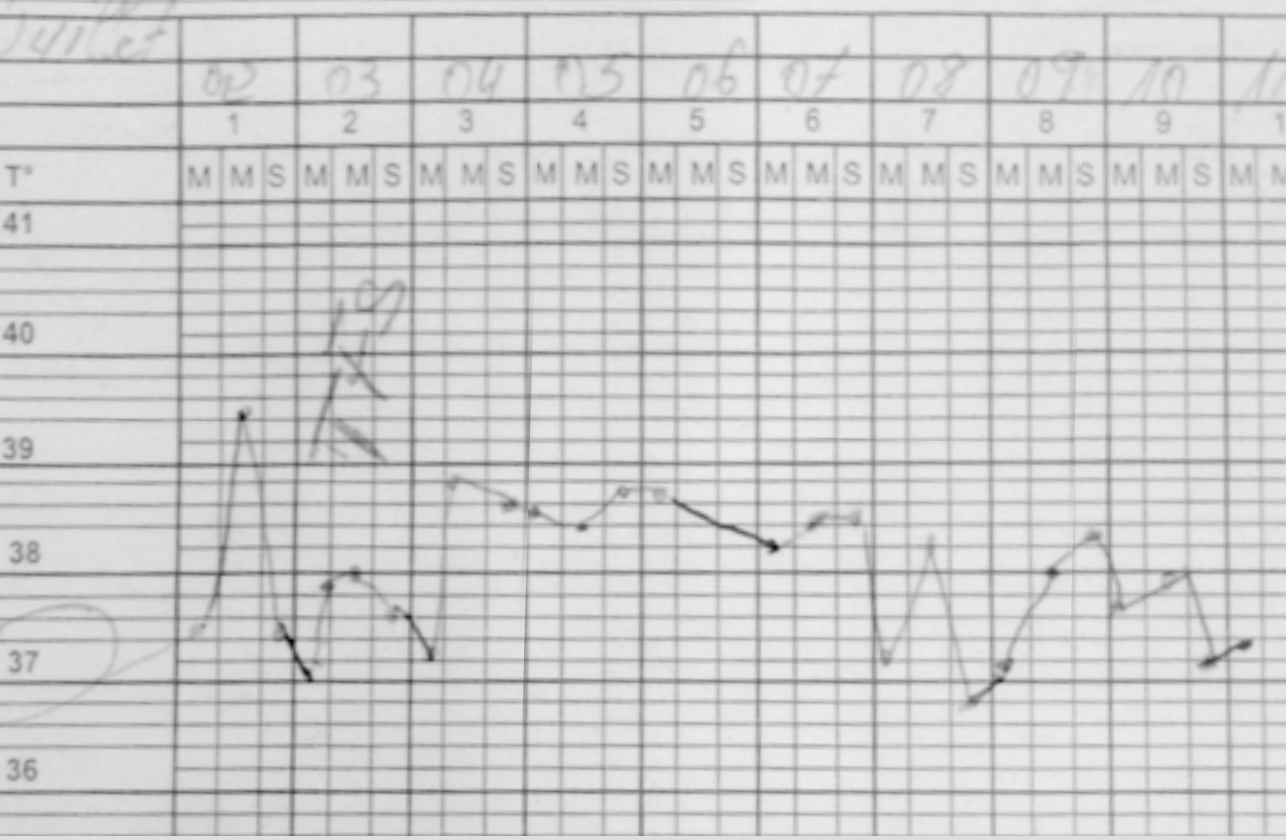

CAUSES OF FEVER IN GABON

Epidemiology and interventions

José Francisco Fernandes

CAUSES OF FEVER IN GABON – EPIDEMIOLOGY AND INTERVENTIONS

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Causes of fever in Gabon – Epidemiology and interventions

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CAUSES OF FEVER IN GABON – EPIDEMIOLOGY AND INTERVENTIONS

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TRABADJU KATA MATA!

Dr. João Adriano Fernandes

(*In memoriam*)

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GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

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BACKGROUND

Across ages, from ancient Greece, Hippocrates of Kos considered the father of medicine, already characterised fever (1). Mankind has always tried to interpret and explain this complex physiologic reaction of the human organism (2,3). Nowadays, from medical semiology perspective fever falls either into the category of symptom or of sign, depending on who characterizes it, but this clear-cut definition has not always been so obvious. Indeed, throughout the medical history, and across geographic regions and cultures, fever itself is taken as a disease *per se* (4). The term 'fever' is also used interchangeably for existing nosological entities or, the other way around, the name of a disease is used for the phenomenon – *e.g.* ague or swamp fever and malaria. The word 'fever' is also often associated to other qualifying nouns in order to describe some diseases or conditions such as African tick fever, aseptic fever, blackwater fever, boutonneuse fever, cat-scratch fever, dengue fever, drug fever, enteric fever, familial Mediterranean fever, Haverhill fever, hay fever, haemorrhagic fevers, Katayama fever, Lassa fever, mud fever, Oroya fever, paratyphoid fever, phlebotomus fever, Pontiac fever, pretibial fever, Q ("query") fever, rat-bite fever, relapsing fever, rheumatic fever, sandfly fever, scarlet fever, spotted fever, trench fever, typhoid fever, Valley fever, West Nile fever, yellow fever, and others; by thus embodying the main observational feature common to all of them. However, fever can characterize a large group of conditions, some of them without known etiology even when all technologies of modern medicine are applied (*i.e.* fever of unknown origin). In the vast field of infectious diseases, fever is standing on top of the list of reasons for medical attendances (5). Indeed, a myriad of infections cause fever as the main clinical manifestation, and frequently the only one diagnosed. Based on that, the increase of body temperature above normal ranges for age and physiology assessed by the thermometer is widely accepted to assess treatment effectiveness (6). Understanding mechanisms causing fever helps to formulate rational approaches to treatment and interventions against causative infections (3). However, those mechanisms are not always specific, clear, and straightforward. Thus, it is challenging to properly investigate fever, its etiologies, and their interplay. Nevertheless, it is an important undertaking as this might assist to define and implement better policies to more efficiently manage febrile diseases. In particular, febrile diseases that could be harmful and even lethal in negative scenarios.

To guide the reader across the chapter of this thesis, the coming sections of the introduction aim to provide a summary on fever, the link between both fever and infections, followed by the description two infectious diseases, one parasitic and one viral (*i.e. Plasmodium falciparum* malaria and Ebola virus disease). For malaria, besides candidate vaccines, alternative curative drugs to oppose the threat of antimalarial drug resistance are introduced. Whereas, for Ebola virus disease a vaccine development as a preventive tool to tackle outbreaks is described. And lastly, the research site in Lambaréné, where most of the presented work has been performed, is described.

FEVER

Fever can be defined as an increase in regulated body temperature due to the elevation of the thermoregulatory set point (7). In clinical practice, there is no universal consensus, but

commonly fever is defined as a body temperature above 38.0 degrees Celsius (°C). It is the chief complaint in paediatric medicine. Besides, fever is a physiological response and not a primary illness in itself (4). Likewise, fever is triggered by multiple organisms such as parasitic protozoans (*e.g. Plasmodium* spp.), the most common cause of fever in many endemic areas of sub-Saharan Africa (8); viruses, bacteria (9), and fungi (10,11), to some extent.

Pathogenesis of fever hinges on the re-setting towards a point higher than the normal hypothalamic set point, due to exogenous pyrogens (infectious agents, their breakdown products or toxins) acting on mononuclear cells which then release cytokines such as interleukin 1 (IL-1), IL-6, tumour necrosis factor-alpha (TNFα) along with prostaglandins (12). Conjugated actions of both cytokines and prostaglandins will then reset the hypothalamic set point for body thermoregulation (7).

In some cases, the mechanism of fever is more direct and rather physically than neurochemically mediated. In those cases, fever is due either to increase of heat production or to decreased heat loss seen for instance in heat stroke, neuroleptic malignant syndrome, malignant hyperthermia during anaesthesia (13).

In most conditions, low to moderate fever is beneficial in fighting infection (14), whereas high fevers may contribute to pathology and/or worsening the condition and the prognosis (2,6). Interestingly, some studies using antipyretic drugs to attenuate fever have shown an adverse effect on disease progression and mortality (*e.g.* in septic patients) (15,16). Overall, understanding of infections and their efficient management implies a deep knowledge of epidemiology, aetiology, and mecanisms of fever.

INFECTIONS

Medically, the term infection is understood as the growth of bacteria or other microorganisms in the body causing damage to the body (17). This mechanism implies an interaction between the pathogen and its (human) host. Often, host reactions are mechanisms of adaptation to protect itself against pathogens virulence, and replication (18). The primary goal of pathogens is to find the best strategy to multiply and by such, ensure/enable their transmission to the next host. The commonest strategies to achieve this are mucosal contact, invasion, immune evasion, or toxin production, whereas, at the host level, there is a deployment of both innate and adaptive defence systems leading to reduced replication and removal of the pathogen in most cases. Additionally, such acute or chronic reactions may lead to cross-protection or adverse effects, such as autoimmunity.

Despite the increasing body of knowledge accumulated in terms of prevention, hygiene, and sanitation but above all, the development of both antimicrobial drugs and vaccines, infectious diseases remain a major public health problem worldwide (19). Indeed, in Northern countries, after decades of relative controls, we see a substantial increase in so-called emerging and re-emerging infections (20). The reasons for this resurgence are especially due to the increase in international trades, travels, and climate changes. However, in resource-poor countries in South East Asia and Africa, for centuries, populations are still facing a huge burden in terms of morbidity and mortality of infectious diseases (21).

Table 1. Diversity of pathogens* constituting potential causative agents of febrile illness

Bacteria (22,23)	**Viruses** (22,23)	**Parasites** (22–24)	**Fungi** (22,25,26)
Acinetobacter baumannii	Arenaviruses	*Babesia* spp.	*Aspergillus* spp.
Bartonella spp.	Adenoviruses	*Entamoeba* spp.	*Blastomyces*
Bordetella pertussis	Astrovirus	*Plasmodium* spp.	*dermatitidis*
Borrelia spp.	Chikungunya virus	*Toxoplasma gondii*	*Candida* spp.
Brucella spp.	Coronavirus (NL63, 229E,	*Schistosoma* spp.	*Coccidioides immitis*
Campylobacter spp.	OC43 & HKU1)		*Cryptococcus*
Chlamydia trachomatis	Cytomegalovirus		*neoformans*
Clostridium spp.	Dengue virus 1-4		*Histoplasma capsulatum*
Corynebacterium spp.	Enterovirus		*Pneumocystis jirovecii*
Coxiella burnetii;	Epstein Barr virus		*Torulopsis glabrata*
Escherichia coli	Hepatitis A virus		
Haemophilus influenzae b	Hepatitis B virus		
Klebsiella spp.	Hepatitis C virus		
Legionella pneumophila	Hepatitis D virus		
Leptospira spp.	Hepatitis E virus		
Listeria monocytogenes	Herpes Simplex Virus 1 & 2		
Mycobacterium spp.	Human immunodeficiency		
Mycoplasma pneumonia	virus 1 & 2		
Neisseria meningitis	Human herpesvirus 6		
Nocardia asteroids	Human metapneumovirus		
Nontyphoidal Salmonella spp.	Respiratory syncytial virus		
Proteus spp.	A & B		
Providencia spp.	Human parainfluenza		
Pseudomonas aeruginosa	virus 1-4		
Rickettsia spp.	Influenza A & B		
Salmonella spp.	Measles morbillivirus		
Serratia spp.	Mumps virus		
Shigella spp.	Norovirus group 1 & 2		
Staphylococcus spp.	Parainfluenza viruses		
Streptococcus spp.	Parechovirus		
Ureaplasma spp.	RSV		
	Rhinovirus		
	Rotavirus		
	Sapovirus		
	Varicella-zoster virus		
	West Nile virus		
	Yellow fever virus		

(*): Listed are common pathogens; the list is not exhaustive.

The negative interrelationship between pathogens and aggravating factors

Some pathological conditions promote or worsen infections with other pathogens. For instance: in malaria endemic-areas, falciparum malaria promotes a high number of invasive bacterial diseases, especially intestinal infections, in children (8). Also, the incidence, the severity, and the mortality of malaria are increased in adults with human immunodeficiency virus (HIV) infection and deteriorating immune status (27,28). This also applies to the classical opportunistic diseases (*e.g. Mycobacterium tuberculosis*, cytomegalovirus, *Pneumocystis jiroveci, Cryptococcus neoformans*), complicating as well as defining acquired immune deficiency syndrome (AIDS) (29).

Another deteriorating immune status condition, promoting the occurrence of infections (*e.g.* opportunistic, respiratory, measles, tuberculosis, skin infections, noma) is malnutrition (30) which is still largely present in sub-Saharan Africa, including malaria-endemic regions for several decades (31,32).

Malaria and Ebola virus diseases are the models of disease to be described here, with their strategies of control.

MALARIA

Malaria is a protozoan disease caused, in human beings, by five species of the genus *Plasmodium*, transmitted by *Anopheles* mosquitoes. *Plasmodium falciparum* and/or *Plasmodium vivax* cause the majority of malaria cases; however, the other species (*Plasmodium ovale curtisi*, *Plasmodium ovale wallikeri, and Plasmodium malariae*) to a lesser extent, cause malaria in humans (33). More recently in Southeast Asia, a zoonotic species *Plasmodium knowlesi* has been also found responsible for human cases (8).

Epidemiology

In 2018, an estimated 228 million cases, with 405 000 deaths of malaria occurred worldwide, with the World Health Organization (WHO) African Region counting 213 million cases (93%) alone, far ahead of both the WHO South-East Asia and Eastern Mediterranean Regions counting 3.4% and 2.1% of the proportion of cases of respectively.

Together, India plus 18 African countries hosted approximately 85% of all malaria cases. For instance: Nigeria (with 25%), the Democratic Republic of the Congo (12%), Uganda (5%), and Côte d'Ivoire, Mozambique (4%), and Niger (4%) have accumulated more than half of all cases in than year (34).

Clinical features and pathogenesis

Based on symptoms and signs and biological disorders seen in patients proven to harbour *Plasmodium* spp. in their blood, plasmodial infection episodes can be classified as either asymptomatic or as uncomplicated or severe disease.

After an incubation period of 1-6 weeks, the first symptoms, caused by the erythrocytic schizogony in the blood, appear. During this phase, *P. falciparum* invades red blood cells,

degrades haemoglobin to haemozoin and releases glycosylphosphatidylinositol (GPI) and other potential toxins. The synthesis and release of tumour necrosis factor and cytokines including interleukin 1, 6 and 12 (IL-1, IL-6 and IL-12), interferon-gamma and soluble factors like nitric oxide intermediates and reactive oxygen intermediates, leading to the 'clinical expression' of fever, chills, headache, body aches, dizziness, vertigo, altered behavior, weakness, nausea, vomiting, diarrhoea, jaundice, hepatosplenomegaly often seen in uncomplicated malaria (35,36).

If untreated, acute malaria can progress from its uncomplicated form to severe illness, and even be fatal; particularly, in malaria-naive patients (i.e. children living in endemic areas or travellers from non-endemic areas).

In 2000, WHO reconsidered the definition of severe malaria as acute *falciparum* malaria with signs of severity and/or evidence of vital organ dysfunction (37). These features may include both clinical and laboratory disorders such as hyperpyrexia (body temperature above 40˚C), hypotension, jaundice, generalized convulsions, impaired consciousness, coma, metabolic acidosis, acute respiratory distress syndrome, pulmonary oedema, acute kidney failure, and disseminated intravascular coagulation, hyperparasitaemia; where the parasite count is higher than 500,000 per microliter of blood, hypoglycemia, severe anaemia; where the haemoglobin level is under 5 grams per decilitre (Figure 1) (8,38).

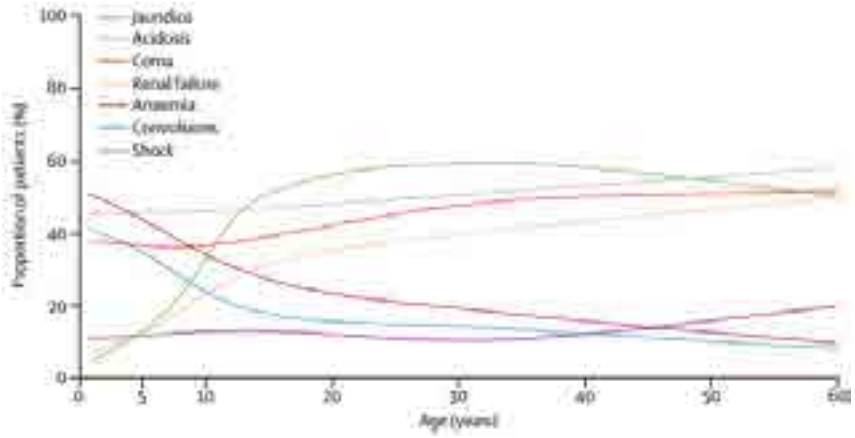


Figure 1. Manifestations of severe falciparum malaria by age
(adapted from White et al., 2014 (8)).

Laboratory diagnostics

Malaria diagnostic tools are summarised in Table 2 below adapted from Cheaveau and colleagues (39).

Differential diagnosis

Clinical diagnosis on its own is notoriously inaccurate in the diagnosis of malaria, which is a great mimic of several clinical presentations/diseases. Malaria diagnosis cannot be retained without evidence of malaria parasite either directly (*e.g.* thick/thin blood smears) or indirectly (*e.g.* RDT or PCR). However in areas of high and stable malaria transmission intensity where there is a high population parasite rate positivity of malaria diagnostic tools can be non-specific, thus not enough to exclude other causes associated.

Table 3 presents a non-exhaustive list of diseases/conditions to be distinguished from malaria whenever key clinical features are seen in seriously ill patients with or without a positive malaria test.

Table 2. Established malaria diagnostic methods

Type/Method of Diagnostics		**Detection target**
Quantitative	Microscopy	Parasite morphology
	Quantitative polymerase chain reaction (qPCR)	18S rRNA, cox1, cytb, TARE-2
	Quantitative nucleic acid sequence-based amplification (QT NASBA)	18S rDNA
Non-quantitative	Rapid diagnostic tests (RDT)	HRP-2, pLDH, 'pan-specific' Aldolase
	Quantitative buffy coat (acridine orange stain)	Parasite DNA/RNA
	Regular PCR (nested, One-step)	18S rDNA, dhfr-ts, 28S rDNA, mitochondrial DNA
	Loop-mediated isothermal amplification (LAMP)	18S rRNA, mitochondrial DNA
	Nucleic acid sequence-based amplification (NASBA)	18S rRNA

Cox1: cyclooxygenase-1; **Cytb:** cytochrome b; **TARE-2:** telomere-associated repetitive element 2; **pLDH:** parasite lactate dehydrogenase; **HRP2:** histidine-rich protein 2; **dhfr-ts:** Dihydrofolate reductase-thymidylate synthase.

Table 3. Main differential diagnosis of malaria

Key signs	Diseases/Conditions
Fever	Common bacterial/viral infections Typhoid Rickettsia infections Arboviral illnesses Influenza Brucellosis Respiratory Tract infections Urinary tract infections Visceral leishmaniasis Trypanosomiasis Relapsing fevers
Coma (Cerebral malaria)	Meningitis Encephalitis Enteric fevers Trypanosomiasis Brain abscess Other etiologies of non-traumatic coma
Anemia	Iron/folate deficiency anemia Haemolytic anemia due to haemoglobinopathies (sickle cell anemia)
Renal failure	Massive intravascular haemolysis Side effect of traditional herbal medicines Leptospirosis Hypertension Snake envenoming
Jaundice and hepatomegaly	Viral hepatitis A/B/E/CMV/EBV Leptospirosis Yellow fever Biliary disease Drug-induced liver reaction/disease

CMV: cytomegalovirus; EBV: Epstein-Barr virus.

Treatment and prevention of malaria

Curative treatment

The recommended treatment of uncomplicated *P.falciparum* malaria in children and adults (not during the first trimester of pregnancy) consists of one of the following artemisinin-based

combination therapy (ACTs): (*i*) Artemether + Lumefantrine; (*ii*) Artesunate + Amodiaquine; (*iii*) Artesunate + Mefloquine; (*iv*) Dihydroartemisinin + piperaquine, and (*v*) Artesunate + sulfadoxine-pyrimethamine. As per the regimens summarized in Table 4 (40).

Reducing the transmissibility of treated P.falciparum infections in areas of low-intensity transmission

In low-transmission areas, it is recommended to give a single dose of 0.25mg/kg body weight primaquine alongside ACT to patients with *P. falciparum* malaria to reduce transmission. However, this should not be given to particular populations such as infants younger than 6 months old, pregnant women, and women breastfeeding infants younger than 6 months old). Furthermore, there is no need to test for glucose-6-phosphate dehydrogenase (G6PD) deficiency (40).

Treatment of uncomplicated P.falciparum malaria in special population

Table 6 is summarizing the recommended treatment for uncomplicated *P. falciparum* infection in five categories of the population considered sensitive and deserving special attention (40).

Table 4. Treatment regimen of the different ACTs based on patient body weight

Body weight (kg)	**Artemether + Lumefantrine** [Dose (mg) given daily for 3 days]	**Body weight** (kg)	**Artesunate + Amodiaquine** [Dose (mg) given daily for 3 days]
5 to <15	20+120	4.5 to < 9	25+67.5
15 to <25	40+240	9 to <18	50+135
25 to 35	60+360	18 to <36	100+270
≥35	80+480	≥36	200+540
Body weight (kg)	**Artesunate + Mefloquine** [Dose (mg) given daily for 3 days]	**Body weight** (kg)	**DHA + Piperaquine *** [Dose (mg) given daily for 3 days]
5 to < 9	25+55	5 to < 8	20+160
9 to <18	50+100	8 to <11	30+240
18 to <30	100+220	11 to <17	40+320
≥30	200+440	17 to <25	60+480
Body weight (kg)	**Artesunate + SP** [Dose (mg) given daily for 3 days]	25 to <36	80+640
5 to <10	25+250/12.5	36 to <60	120+960
10 to <25	50+500/25	60 < 80	160+1280
25 to <50	100+1000/50	>80	200+1600
≥50	200+1500/75		

<: smaller than; **≥:** greater than or equal to ; **(*):** revised dose recommendation for DHA + Piperaquine in young children (<25kg): a minimum of 2.5 g/kg (body weight) per day of DHA and 20mg/kg per day of piperaquine daily for 3 days; **DHA:** Dihydroartemisinin; **SP:** Sulfadoxine-Pyrimethamine.

Table 5. Dosing of Primaquine based on patient body weight

Body weight (Kg)	**Single dose of Primaquine** (mg base)
10* to <25	3.75
25 to <50	7.5
50 to 100	15

*: dosing of young children weighing <10kg is limited.

Table 6. Summary of recommended treatment for each special population

Population	**Recommended intervention**
First trimester of pregnancy	Quinine+clindamycin for 7 days
Infants less than 5kg body weight	ACT at the same mg/kg body weight target dose as for children weighing 5kgs
Patients co-infected with HIV	Avoid Artesunate+SP (if cotrimoxazole is ongoing) Avoid Artesunate+Amodiaquine (if efavirenz/zidovudine ongoing)
Non-immune travellers	ACT
Uncomplicated Hyperparasitaemia	ACT and should be closely monitored, due to their increased risk of treatment failure, severe malaria, and death

ACT: artemisinin-based combination therapy; **CTX:** cotrimoxazole; **EFV:** efavirenz; **AZT:** zidovudine; **mg/kg:** milligram of medication per kilogram of the body weight; **HIV:** human immunodeficiency viruses.

Treatment of uncomplicated malaria caused by *P. vivax, P. ovale, P. malariae* or *P. knowlesi*

When the malaria species is not known with certainty, treatment is given as for uncomplicated *P. falciparum* malaria. Therefore, it is recommended to use (*i*) ACT or Chloroquine in areas with chloroquine-susceptible infections ; (*ii*) ACT (except pregnant in their first trimester) in areas with chloroquine-resistant; and (*iii*) quinine in pregnant women (1st trimester) with *P. vivax* (40).

Preventing relapse in *P. vivax* or *P. ovale* malaria

To prevent relapses following malaria episodes caused by either *P. vivax* or *P. ovale*, it is suitable to screen for G6PD deficiency, first. Then, the patients with deficient would be given 14 days course (0.25 – 0.5 mg/kg body weight daily) of primaquine, whereas, those without the deficiency would take primaquine base at 0.75mg/kg body weight once a week for eight weeks under close medical supervision (40).

Preventing relapse in pregnant or lactating women

To prevent further relapses in pregnant women or breastfeeding, better consider weekly chemoprophylaxis with chloroquine until delivery and breastfeeding terminated, then, primaquine, if no G6PD deficiency (40).

Until now, no resistance against ACTs in sub-Saharan Africa has been observed, nonetheless, one study showed a declining response of *P. falciparum* to dihydroartemisinin-piperaquine (DHA-PPQ), and artemether-lumefantrine (AL) in Kenya (41). Though, some treatment failure with ACT is seen and may occur, often because of incorrect approaches (*i.e.* use of monotherapy and/or incomplete dosing).

Nonetheless, previous examples are warning: in 1957 chloroquine (CQ) was the first antimalarial drug to which resistance has been documented worldwide (first emerging in the Thai-Cambodian border and then spreading gradually and reaching Africa in 1978) (42). Then resistance to sulfadoxine-pyrimethamine (SP) spreading from Thailand, other South-East Asian countries, reached Africa in the late 1990s (43). Afterward, atovaquone was introduced in 1996, and resistance was described in the same year (44). Based on this unfortunate development learned from malaria drug history, it is therefore understandable that the priority is to continuously develop new antimalarial compounds to keep pace with the parasites' ability to develop resistance.

Preventive approaches

The core preventive strategies are based on two approaches, namely vector control and protection of high-risk groups.

Vector control

Insecticide-treated mosquito nets (ITNs)

Long-lasting insecticidal nets (LLINS) are the main tools for populations living in endemic areas. They are designed to be effective for three years for people at risk of malaria. In 2015, 43% people at risk were still not protected (45).

Indoor Residual Spraying of insecticides (IRS)

IRS is a powerful way to rapidly reduce malaria transmission, involving spraying insecticides on indoor walls and ceilings where mosquitoes might lie down. IRS is effective for 3-6 months on average. In order to confer significant community protection a minimum of 80% of homes should be sprayed in a targeted area (46).

Supplementary methods:

In some settings and circumstances, vector control methods are on one hand, larval source management which includes modifying, manipulating, or applying biological or chemical agents and findable. And on the other hand, personal protection measures to reduce contact between mosquitoes and humans by using one or several of the following: window screens; insecticide-treated blankets; hammocks; window curtains; repellents; and protective clothing (47).

The protection of high-risk groups

Among the most sensitive populations, three categories of people (*i.e.* pregnant women, infants, and children under five years) have their protection based on the use of preventive therapies

(either in monotherapy or combination) adapted to the resistance profile to the antimalarials depending on geographic regions of the world.

Intermittent preventive treatment of malaria in pregnancy (IPTp)

As part of antenatal care in malaria-endemic African regions, intermittent preventive treatment with sulfadoxine-pyrimethamine (SP) should be provided to all women in their first or second pregnancy (SP-IPTp). Starting in the second trimester and doses should be given at least one month apart with at least a total of three doses (48).

Intermittent preventive treatment in infants (IPTi)

In areas of moderate-to-high malaria transmission in Africa where SP is not resistant yet, provide intermittent preventive treatment with SP to infants under 12 months of age (SP-IPTi) at 10 weeks, 14 weeks, and nine months (49).

Seasonal malaria chemoprophylaxis (SMC)

In some regions in sub-Saharan Africa where the malaria transmission is highly seasonal, SMC should be provided with monthly Amodiaquine+SP for all children under 6 years during each transmission season (50).

Vaccines

Several malaria candidate vaccines are in the pipeline (51). However, to date only the RTS, S/AS01 vaccine (Mosquirix®) proved efficacious in reducing malaria, and *P. falciparum* severe malaria after completion of a four-doses regime, in young African children, vaccinated in seven African countries during the experimental phase III. To evaluate Mosquirix' potential role in decreasing child mortality and its safety whilst being integrated into the common vaccine program, WHO's advisory bodies for malaria and immunization have recommended a pilot program, introducing the Mosquirix® in Ghana, Kenya, and Malawi from 2019 onwards (52).

EBOLA VIRUS DISEASE

Ebola virus disease (EVD) is a viral infection caused by a member of the *Filoviridae* family, which causes severe, sometimes haemorrhagic febrile disease in humans and non-human primates (53). The best known and most prevalent strain, the Ebola Zaire virus (ZEBOV) strain caused 27 of the 37 outbreaks recorded by the WHO since 1976 (see Table 7) when EVD was first identified during the course of two simultaneous African outbreaks, one in Nzara, South Sudan, and the other in Yambuku, DR Congo, near the Ebola River, after which the disease was named.

Transmission

The natural Ebola virus reservoirs are thought to be fruit bats of the *Pteropodidae* family (54).

Ebola virus is transmitted to humans through either blood, meat, or secretions and fluids of infected animals (*e.g.* fruit bats, antelopes, porcupines, monkeys, gorillas, or chimpanzees) (55).

Human-to-human transmission occurs via either direct contact with body fluids (*i.e.* blood, semen, faeces, vomit) of an Ebola-infected individual or cadaver; or indirect contact with previously contaminated objects. These mechanisms put families and health-care workers at high risk of contamination (56), and make burial ceremonies (dead people remaining infectious as long as their body contains the virus), in rural/traditional African communities /regions a hazard to health (55).

Pathogenesis & clinical symptoms

According to animal studies, EBOV viremia starts from 2 to 4 days after inoculation. A decreased endothelial cell function, compounded by activation of cytokine tumour necrosis factor-α, which is known to induce a long-lasting decrease in endothelial cell barrier function (57).

After an incubation period from 2 to 21 days (where infected people cannot also spread the disease), symptoms of EVD can be sudden and include: fever; fatigue; myalgia; headache, and sore throat. Those symptoms may be followed by vomiting; diarrhoea; rash; symptoms of impaired renal and liver function; bleeding (*e.g.* oozing from the gums, or blood in the stools) (55).

Laboratory Diagnosis

Confirmatory diagnosis of Ebola virus infection is made out of two preferred specimens (extreme biohazard risk): whole blood, collected in ethylenediaminetetraacetic acid (EDTA) from live patients exhibiting symptoms; or (when blood collection is not possible) saliva, stored in universal transport medium collected (as done from deceased patients).

The laboratory diagnostic methods to be used are: (*i*) virus isolation by cell culture (58); (*ii*) reverse transcriptase polymerase chain reaction (RT-PCR) (58); (*iii*) assay antigen-capture detection tests (58); (*iv*) serum neutralization test (58); (*v*) electron microscopy; (*vi*) antibody-capture enzyme-linked immunosorbent assay (ELISA) (58), with reverse transcription (RT-PCR) being the gold standard.

Outbreak control and responses

According to WHO, both surveillance for Ebola virus disease and the development of preparedness plans in certain countries at risk are the key preventive approaches against Ebola outbreaks:

In the case of an outbreak detected, the WHO responds by supporting community engagement; promoting disease detection and contact tracing; deploying vaccination; ensuring proper case management, equipping laboratory services, and delivering training and assistance for safer burial practices, whilst reducing the risk of possible sexual transmission: Ebola survivors are strongly advised to either abstain from sex or to use correctly and consistently condoms until two negative tests of their semen (59).

Finally, it is worth noting that there are now interventions (*i.e.* on one hand, curative agents such as the triple monoclonal antibody ZMapp, the antiviral agent remdesivir, the single monoclonal antibody MAb114, or the triple monoclonal antibody REGN-EB3. Both MAB114 and REGN-EB3 were superior to ZMapp in reducing mortality from EVD. Indeed, at 28 days,

death had occurred in 61 of 174 patients (35.1%) in the MAB114 group, *versus* 84 of 169 (49.7%) in the ZMapp group (p=0.007) and 52 of 155 (33.5%) in the REGN-EB3 group as compared with 79 of 154 (51.3%) in the ZMapp subgroup (p=0.002) (60). On the other hand vaccines (61,62) have been successfully to stop the outbreak before the ongoing one in Eastern DRC and those interventions are available in the field. However, the deployment of the therapeutic arsenal cannot be done effectively in the conflict zone (*e.g.* the province of North Kivu) (63). Such geographic area trapped by violence and military conflicts the ongoing outbreak is mouldering for almost two years, second biggest in history, became hard to manage the movement of health workers being restricted and their lives at risk (63).

Towards a new and efficient vaccine

Historically, several small, sporadic and well-located outbreaks occurred during the last 2 decades in the Central Africa region (see Table 7); mainly in the Democratic Republic of Congo, formerly Zaire (64–66), in Gabon (*i.e.* in Mayibout 1996, Booué 1996, and Mékambo 2001) (67), and also in the Republic of Congo (53). It is only since the largest and unprecedented Ebola outbreak in 2014-2016 in Western Africa (in Guinea, Liberia and Sierra Leone) that the World Health Organization has declared the outbreak of Ebola virus disease in West Africa a public health emergency of international concern and has called for a strong and coordinated international response (68). This global awareness helps mobilize unusual resources and to establish a protocol for Ebola vaccine evaluations. During this process, a scientific consortium, the VEBCON Consortium (VSV-EBola CONsortium) designed and conducted a parallel dose-escalation phase 1 trial of the recombinant VSV-ZEBOV (rVSV-ZEBOV) candidate vaccine in Germany, Kenya, and Gabon while a double-blind phase 1 randomized controlled trial was done in Switzerland in adult populations. Later, another phase 3 trial was launched in Guinea (69). However, Gabon was the only country where both adults and pediatric populations (cohorts aged 6-17 years old) received the candidate vaccine according to a study protocol, before its implementation on the field of an outbreak in Guinea, within the frame of an open-label, cluster-randomized ring vaccination trial of suspected cases in Basse-Guinée (Guinea) (70).

VACCINES

A vaccine is a non-pathogenic or attenuated antigen that mimics a particular pathogen in order to elicit an immune response.

Vaccination has allowed the eradication or the reduction of incidence and mortality of various infectious diseases, and it is considered as one of the major advances in biomedical sciences (71).

The terms *vaccine* and *vaccination* are derived from the Latin root words *Variolae vaccinae* (smallpox of the cow), the term devised by Edward Jenner, in 1978, to designate cowpox (72).

As per July 2020, 153 vaccines (under 246 presentations) have got WHO prequalification (73).

There are different types of vaccines depending on the strategies used to elicit an immune response in the vaccinated person, as presented in the table below.

Table 7. Chronological list of Ebola cases per country caused by Zaire strain with their fatality rates

Year	Country	Cases	Deaths	Case fatality
2018-2020	DR Congo	ongoing		
2018	DR Congo	54	33	61%
2017	DR Congo	8	4	50%
2015	Italy	1	0	0%
2014	Spain	1	0	0%
2014	UK	1	0	0%
2014	USA	4	1	25%
2014	Senegal	1	0	0%
2014	Mali	8	6	75%
2014	Nigeria	20	8	40%
2014-2016	Sierra Leone	14124*	3956*	28%
2014-2016	Liberia	10675*	4809*	45%
2014-2016	Guinea	3811*	2543*	67%
2014	DR Congo	NA	NA	NA
2008	DR Congo	32	14	44%
2007	DR Congo	264	187	71%
2005	Congo	12	10	83%
2003 (Nov-Dec)	Congo	35	29	83%
2003 (Jan-Apr)	Congo	143	128	90%
2001-2002	Congo	59	44	75%
2001-2002	Gabon	65	53	82%
1996	South Africa (ex-Gabon)	1	1	100%
1996 (Jul-Dec)	Gabon	60	45	75%
1996 (Jan-Apr)	Gabon	31	21	68%
1995	DR Congo	315	254	81%
1994	Gabon	52	31	60%
1977	DR Congo	1	1	100%
1976	DR Congo	318	280	88%

* Include suspect, probable, and confirmed Ebola virus diseases cases.
DR Congo: The Democratic Republic of the Congo; **NA**: not available;
UK: United Kingdom; **USA:** United States of America; **ex-Gabon:** exported case from Gabon.
Data source: WHO Ebola virus diseases fact sheet (55).

Two vaccines will be studied in this work the vaccine against malaria and Ebola respectively.

The malaria vaccine

For malaria researchers, developing a malaria vaccine has been a goal for a long time, hence, attempts of vaccine development in humans started four decades ago (44), as a complementary asset in this war against malaria, following animal models, earlier, in the 1940s (75). Several malaria vaccine candidates exist targeting one of the specific steps of the malaria life cycle (*e.g.* pre-erythrocytic, blood-stage, transmission-blocking), all are intended either to prevent infection or to prevent clinical illness and death (76). Among all those candidate vaccines

Table 8. Vaccine types, targeted diseases and methods of preparation
(adapted from Kocourkova et al., 2017(74))

VACCINE TYPE		Examples of TARGETED DISEASE
First-generation	Inactivated	Polio, Hepatitis A, Japanese encephalitis
		Influenza, Rabies, Encephalitis
		Whooping cough
	Live attenuated	Measles, Mumps, Rubella, Rotavirus, Varicella, Herpes Zoster
		Yellow fever, Influenza
		Tuberculosis
Second generation	Polysaccharide or protein-based Subunit	Pneumococcus
		Meningococcus
		Pertussis
		Typhus
	Conjugate subunit	*H. influenzae b* infection
	Recombinant subunit	Hepatitis B
		Human papillomavirus (HPV)
	Toxoid	Diphtheria
		Tetanus
Third generation / Experimental*	DNA vaccine	Leishmaniasis
	RNA vaccine	COVID-19 (several candidates)
	Recombinant vector	HIV, Ebola virus disease
	T-cell receptor peptide vaccines	valley fever, stomatitis, and atopic dermatitis

*: In this category of vaccines, there is a range of innovative vaccines still under development and/or already used; **BCG:** Bacillus Calmette-Guérin; **HBsAg:** Hepatitis B surface antigen; **COVID-19:CoronaVIrus Disease 2019**; **DNA:** deoxyribonucleic acid; **RNA:** ribonucleic acid; **HIV:** human immunodeficiency virus.

(Figure 2), the most advanced in the pipeline is a pre-erythrocytic vaccine, the RTS, S vaccine, which is a fruit of a long and multilateral scientific collaboration since 1987 (77), is made of the tandem repeat region of the circumsporozoite protein, expressing the central repeat ('R') fused to the C-terminal region known to contain T cell epitopes ('T') fused in turn to the hepatitis B surface antigen ('S'), yielding a yeast-expressed protein RTS. Co-expressed with the unfused S protein resulted in 'RTS, S' (76). This vaccine (RTS,S/AS01 or Mosquirix®) began

pilot implementation in 2019, in Ghana, Kenya, and Malawi, three African malaria-endemic countries (77).

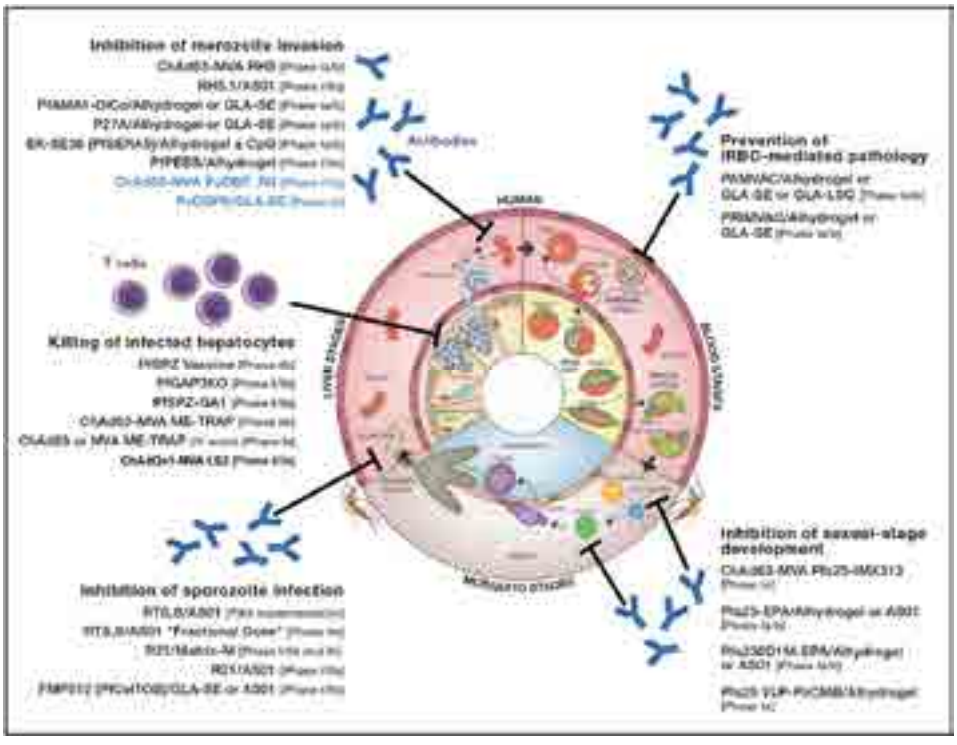
Ebola vaccine candidates

Table 9 below provides an overview of the Ebola virus disease candidate vaccines pipeline (79).

RESEARCH SETTING AND CONTEXT

Gabon is a country of the Gulf of Guinea that straddles the equator, sparsely populated with an average of less than 5 people per Km^2 (81) for a total population of 1,725,000 people (2015) (82) in a land area of 267,667 km^2. An estimate of 69-80% of its territory is covered by dense equatorial rainforest (81). Yearly, there are two rainy seasons (from the middle of March to middle of June, and then middle of September to the middle of December) separated by two dry seasons (from the middle of December to the middle of March and from the middle of June to the middle of September) (83).

Historically, three outbreaks of Ebola hemorrhagic fever have been recognized in Gabon (84). In 2007 and 2010, Gabon recorded simultaneous outbreaks of Chikungunya and Dengue (85). In Gabon, cases of dengue haemorrhagic fever (DHF) caused by up to three different



Vaccines for *P. vivax* are coloured blue. Figure from Simon J Draper et al., 2018 (78)

Figure 2. Overview of malaria vaccine candidates in clinical development.

Table 9. Example of Ebola virus disease candidate vaccines pipeline*
(adapted from Karen A. Martins et al., 2016 (80))

VACCINE CLASS	VACCINE NAME	CLINICAL TRIAL STATUS
VECTORED LIVE	rVSV-ZEBOV	Phase 3
	HPIV3-Ebov Z GP	Phase 1
VECTORED NON-REPLICATING	ChAd3.EBOZ/ChAd3.EBO	Phase 2
	Ad26.ZEBOV	Phase 3
	Ad5-EBOV	Phase 2
	VRC-EBOADV018-00-VP	Phase 1
	MVA-BN-Filo	Phase 3
	MVA-EbolaZ(VRC-EBOMVA079-00-VP)	Phase 1
DNA	VRC-EBODNA023-00-VP, VRCEBODNA012-00-VP, and VRCMARDNA025-00-VP	Phase 1
	INO-4201, INO-4202, and INO-4212	Phase 1
SUBUNIT/PROTEIN	EBOVGP$_{1,2}$ with Matrix-M	Phase 1

***:** pipeline as per 2016. The list is maybe not exhaustive by now and meant to be informative about the huge diversity of candidates within the pipeline; **rVSV-ZEBOV:** Recombinant vesicular stomatitis virus vector expressing the Zaire Ebola virus glycoprotein; **HPIV3-EbovZ GP:** Chimeric human parainfluenza virus type 3 bearing the Ebola virus glycoprotein; **ChAd3-EBO-Z:** chimpanzee adenovirus 3 vector expressing the Zaire Ebola virus glycoprotein; **Ad26.ZEBOV:** human adenovirus serotype 26 (Ad26) expressing the Ebola virus Mayinga variant glycoprotein ; **Ad5-EBOV:** human adenovirus serotype 5 vector (Ad5) with the glycoprotein gene from ZEBOV; **MVA-BN-Filo:** vaccinia Ankara expressing Zaire Ebola virus glycoprotein and other filovirus antigens; **VRC-EBOADV018-00-VP:** recombinant product composed of two replication-deficient recombinant adenovirus serotype 5 (rAd5) vectors encoding for glycoprotein (GP), one from the Zaire strain and one from the Sudan-Gulu strain of Ebola; **VRC-EBOMVA079-00-VP (MVA-EbolaZ):** recombinant Modified Vaccinia Virus Ankara Ebola Vaccine, **MARV GP$_{1,2}$:** Marburg virus glycoprotein subunit 1 & subunit 2; **EBOVGP$_{1,2}$:** Ebola virus glycoprotein subunit 1 & subunit 2 ; **SUDV GP$_{1,2}$:** virus glycoprotein subunit 1 & subunit 2 ; **EBOV NP:** Ebola virus Nucleoprotein ; **VRC-EBOADC069-00-VP:** composed of two recombinant cAd3 vectors that express Ebola wild-type glycoprotein from Zaire and Sudan strains ; **VRCEBODNA012-00-VP:** an ebolavirus DNA vaccine that included three plasmids expressing EBOV GP$_{1,2}$, SUDV GP$_{1,2}$, and EBOV NP ; **VRCMARDNA025-00-VP:** a DNA vaccine for Marburg virus disease, which expressed full-length MARV GP$_{1,2}$; **INO-4201:** Inovio DNA vaccine expressing the EBOV GP$_{1,2}$ from pre-2013 EBOV variants; **INO-4202:** Inovio DNA vaccine expressing the EBOV Makona GP$_{1,2}$; **INO-4212:** is a one-to-one mixture of INO-4201 and INO-4202.

DENV serotypes have been reported, and dengue seroprevalence has been found between 5% and 20% (86,87).

Lambaréné, a semi-urban town of about 35,000 inhabitants surrounded by villages (88) – situated approximately 250 km southeast of the capital Libreville – is one of the melting pots where various populations and ethnic groups cohabit together. Infectious diseases continue

to be of major importance in the area and contribute significantly to overall morbidity and mortality (89). *P. falciparum* was known to be highly endemic with an estimated 50 infectious bites/person/year in Lambaréné, two decades ago (90,91). More recently, it has been found that the malaria burden remained mostly unchanged or even increased in some rural provinces of Gabon (92). Besides malaria, in Lambaréné, there is also a high diversity of potential pathogens and high resistance rates within the spectrum of the encountered bacteria, *e.g.* methicillin-resistant *S. aureus*, extended-spectrum beta-lactamase (ESBL) producing *K. pneumoniae* and ESBL-producing Enterobacteriaceae. In total 5.8% of all *S. aureus* isolates were methicillin-resistant. The proportion of ESBL producing Enterobacteriaceae was 15.4% and of all *K. pneumoniae* were ESBL-producer (93). Viral infections are also frequent: a study demonstrated seroprevalence of dengue among toddlers approximately 30 months old of age in semirural Lambaréné between 2007 and 2010. Ig G-antibodies against dengue was found in 12.3% and IgG antibodies against chikungunya in 0.6% of infants tested (94).

Life expectancy at birth for males and females is 65 years and 67 years, respectively with a probability of dying at an age under five years of 45/1000 live births in 2018 (82). The general population of Lambaréné is relatively young with more than 50% of the population under 25 years of age, as shown by the age pyramid of the population living there (Figure 3).

Despite many studies of febrile illnesses, there was still a lack of exhaustivity regarding the causes of fever in febrile hospitalized children in Gabon and Central Africa. Therefore, we decided to conduct the first study of this kind in Lambaréné to describe the current spectrum of pathogens along with the distribution of infections, co-infections, and co-morbidities hospitalized febrile children living in that area.

The CERMEL, established in 1981 as the medical research unit of the Albert Schweitzer Hospital, has a strong research collaboration for longer than 20 years with the Institute of

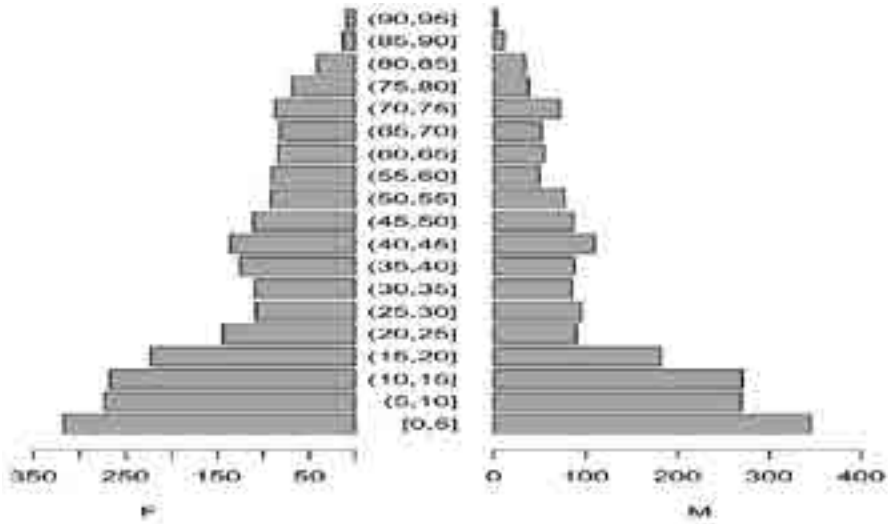


Figure 3. Age Pyramid of Lambaréné (data source: CERMEL 2011).

Tropical Medicine of the University of Tübingen. The research center has extensive experience conducting epidemiological studies (malaria, geohelminths, schistosomiasis, and arboviruses), basic research (immunology and genetics), and clinical studies (phase 1-3 vaccine and drug trials). All facilities on site are equipped to conduct clinical trials following ICH-GCP guidelines and the Declaration of Helsinki, including pediatric phase 1 to phase 3 trials on chemotherapy, prevention, and vaccines.

SCOPE AND AIMS OF THIS THESIS

The main objective of this thesis is to improve our understanding of the challenges surrounding the topic of febrile infections. This research aimed to assess the causes of fever. It also describes the results of global strategies meant to tackle fever-related health with both novel drug and vaccine candidates.

Pooling data together, this work supports a better understanding of the complexities in establishing the causes of fever, epidemiology of infectious agents, interactions among them, preventive and curative strategies of some of the deadliest diseases (*i.e.* malaria and Ebola) in human populations living in Central African regions and Gabon.

The present academic work is aimed at adding a piece of valuable information helping to guide health decision-makers, local stakeholders, scientists, and health care professionals through the complexity of fever across the spatiotemporal barrier of knowledge. This thesis is structured into three thematic parts:

Part one

In the first part, I conducted a systematic, prospective, cross-sectional hospital-based study in hospitalized children with fever at the Albert Schweitzer hospital of Lambaréné, Gabon (**Chapter 2**). I also described the spectrum of pathogens, distribution of infection, co-infections, and comorbidities in those children.

Part two

In the second part, I performed a meta-analysis of clinical trials evaluating fosmidomycin, a novel antimalarial drug, mainly Gabonese children to inform the decision making concerning further development of this potentially valuable supplementary tool (**Chapter 3**). I also conducted a systematic review to assess data of phases I to III in adults (including malaria-naïve adults), children, and infants from 11 sites in seven countries across malaria-endemic settings in Sub-Saharan Africa (**Chapter 4**).

Part three

In the third and last part, I described the results of the investigation of the safety and immunogenicity of the recombinant Vesicular Stomatitis Virus- vectored vaccine expressing the Ebola surface glycoprotein (rVSVΔ-ZEBOV) vaccine in both adults and children in Lambaréné, Gabon (**Chapter 5**).

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CAUSES OF FEVER IN GABONESE PEDIATRIC POPULATION

CAUSES OF FEVER IN GABONESE CHILDREN: A CROSS-SECTIONAL HOSPITAL-BASED STUDY

2

ABSTRACT

The causes of infections in pediatric populations differ between age groups and settings, particularly in the tropics. Such differences in epidemiology may lead to misdiagnosis and ineffective empirical treatment. Here, we investigated the current spectrum of pathogens causing febrile diseases leading to pediatric hospitalization in Lambaréné, Gabon. From August 2015 to March 2016, we conducted a prospective, cross-sectional, hospital-based study in a provincial hospital. Patients were children≤15 years with fever≥38 °C and required hospitalization. A total of 600 febrile patients were enrolled. Malaria was the main diagnosis found in 52% (311/600) patients. Blood cultures revealed septicemia in 3% (17/593), among them four cases of typhoid fever. The other causes of fever were heterogeneously distributed between both bacteria and viruses. Severe infections identified by Lambaréné Organ Dysfunction Score (LODS) were also most often caused by malaria, but children with danger signs did not have more coinfections than others. In 6% (35/600) of patients, no pathogen was isolated. In Gabon, malaria is still the major cause of fever in children, followed by a bacterial and viral disease. Guidelines for both diagnosis and management should be tailored to the spectrum of pathogens and resources available locally.

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INTRODUCTION

Causes of fever in African pediatric populations are more diverse than previously thought. A landmark study conducted in Tanzania showed that due to a change in epidemiology, a broad spectrum of pathogens replaced *P. falciparum* malaria as the most common cause of disease in children in this area (1). However, a few years later, *P. falciparum* malaria, is still seen to be the main cause of febrile illnesses in Ghana, West Africa (2). When unaware, these differences in epidemiology might lead to misdiagnosis as well as inefficient treatment by the medical personnel. The process of medical diagnosis includes the joint interpretation of symptoms, clinical signs and laboratory findings. Careful selection and prioritization of a diagnostic setup are informed by *a priori* knowledge of the seasonal, local and worldwide frequency and distribution of a given disease (3,4).

Our study describes the distribution of infections, co-infections, and co-morbidities in children hospitalized for febrile illnesses at the Albert Schweitzer Hospital (HAS) in Lambaréné, Gabon, as an example for a hospital in a semiurban Central African region. In addition, we present the current spectrum of pathogens causing severe disease identified by Lambaréné Organ Dysfunction Score (LODS) in these children.

RESULTS

Study patients

A total of 600 febrile patients≤15 years were enrolled in our study. Of these, 280 (47%) were females; 69% (415/600) patients were < 5 years, and median (IQR) age was 29 [12–68] months (Table 1). Seven percent (40/549, NA = 51) had at least one known chronic medical condition prior to admission, among the main ones: 4% (23/600) patients had homozygous sickle cell disease; 1% (6/600) were HIV positive. Vaccination coverage of the expanded program on immunization (EPI) vaccines was above 80% for scheduled doses of BCG, poliomyelitis and pentavalent (diphtheria, pertussis, tetanus, hepatitis B and *Haemophilus influenzae* type b) vaccines, and 54% and 55% for measles and yellow fever vaccines, respectively (Supplementary Fig. S1).

General condition

On admission, 4% (23/593, NA = 7) patients were prostrated. Malnutrition – defined by a weight-for-age Z-score < -2SD was found in 22% (132/600) patients. Table 2a depicts differences of clinical parameters that werefound statistically significant, in relation to major clinical signs.

Anemia was more pronounced in severely malnourished patients – having a weight-for-age Z-Score< -3SD, with a hemoglobin concentration of 8.3 g/dL *versus* 9.2 g/dL (p= 0.02) (Table 2b).

Laboratory values and imaging

Laboratory values were mostly as expected for the respective clinical condition. Table 2b depicts differences of hematological parameters and liver function tests in relation to major clinical signs.

Table 1. Characteristics of 600 febrile children enrolled in the study. (*) hemoglobin level <5 g/dL or hematocrit <15%. (ǂ) weight for age Z-score <-2 standard deviations mean.
SD: *standard deviation*. **IQR**: *interquartile range*.

Variables	Value
Temperature (°C) Median (IQR)	39 (38.4–39.7)
Weight (kg) Median (IQR)	12 (8.5–18.2)
Age (Months) Median (IQR)	29 (12–68)
Male	320 (53.3%)
Female	280 (46.7%)
Age <12 Months	148 (24.6%)
Age 12–36 Months	182 (30.3%)
Age 36–60 Months	85 (14.2%)
Age >60 Months	185 (30.9%)
Severe anemia*	41 (6.8%)
Sickle cell anemia	23 (4.2%)
Normal weight for age	468 (78%)
Abnormalǂ weight for age (< 2 SD mean)	132 (22%)
Duration of hospitalization (days) Mean (SD)	5 (3)

Chest radiography was done in children with respiratory signs and/or leucocytes ≥20,000/mm3 as early as possible after admission (Fig. 1).

Parasitology

Overall, 52% (311/600) patients were diagnosed with *Plasmodium falciparum* (*P.f.*) malaria, ten of them as mixed infections: seven *P. malariae* (*P.m*), three *P. ovale curtisi* (*P.o.c.*), and one *P. ovale wallikeri* (*P.o.w.*). One patient was infected with *P.f.*, *P.m.*, and *P.o.c.* at the same time.

PCR-screening for *Babesia* spp. was negative in all patients.

Urine analysis revealed one *Schistosoma haematobium* infection.

Bacteriology

Blood cultures were taken from 593/600 patients, with 3% (17/593) positives; 4/17 positives were diagnosed with typhoid fever. Pathogens identified in blood cultures are given in Table 3(a). Analyses of pus from two patients requiring abscess drainage revealed *S. aureus* in one and *K. pneumoniae* in the other. Bacteria from urine and stool samples are depicted in Table 3(b,c), respectively. Cerebro-spinal fluid (CSF) cultures were performed in two participants; both were negative after 48 hours of culture. 504 (NA =96) EDTA blood samples subjected to PCR-screening for *Brucella* spp., *Leptospira* spp., *Bartonella* spp., *Borrelia* spp., *Coxiella burnetii* and *Rickettsia* spp. were found negative. We also screened for *Chlamydiae*, *Haemophilus influenzae*, and *Mycoplasma pneumoniae* in pharyngeal swabs by PCR. Only *H. influenzae* was found in 16% (31/191) patients (Fig. 1).

Table 2. Differences in biomedical parameters among study patients in relation to major clinical signs. HEENT: head, eyes, ears, nose, and throat; **Values in bold**: statistically significant (the actual p-values are provided in the Supplementary Table S8) (*) Transaminases: are reflecting ALT values except when specified by a "ǂ" it is rather AST: Aspartate aminotransferase. **HEENT:** head, eyes, ears, nose, and throat. **Values in bold**: statistically significant (the actual p-values are provided in the Supplementary Table S9).

(2a) Differences in clinical parameters among study patients in relation to major clinical signs							
Clinical features		**All participants**	**Status**	**Age (Months)**	**Body temperature (°C)**	**Number of stools**	**Respiratory rate (per minute)**
		N (%)		**Value**	**Value**	**Value**	**Value**
General signs	Restless	182 (30.6)	Absent	—	—	—	**44**
			Present	—	—	—	**49**
	Wasted	63 (10.6)	Absent	—	—	**1**	—
			Present	—	—	**0.7**	—
HEENT	Rhinorrhea	159 (27)	Absent	—	—	—	**44**
			Present	—	—	—	**50**
Gastrointestinal	Vomiting	326 (54.3)	Absent	—	—	**0.6**	—
			Present	—	—	**1.3**	—
	Diarrhea	188 (32)	Absent	—	—	**0**	—
			Present	—	—	**3**	—
	Bloody stool	69 (12)	Absent	—	—	**0.6**	—
			Present	—	—	**3**	—
	Abdominal pain	109 (18.4)	Absent	—	—	—	**47**
			Present	—	—	—	**41**
Neurological	Prostrated	23 (4)	Absent	—	39	—	—
			Present	—	38.6	—	—
	Unconscious	66 (11)	Absent	47		—	—
			Present	36		—	—
Respiratory	Frequent sneezing	6 (1)	Absent	—	39	—	—
			Present	—	38.3	—	—
	Cough	270 (46)	Absent	—	—	—	**42**
			Present	—	—	—	**50**
	Bronchial breath sounds	105 (18.4)	Absent	—	—	—	**44**
			Present	—	—	—	**53**
	Flaring	63 (11)	Absent	—	—	—	**44**
			Present	—	—	—	**58**
	Sore throat	5 (0.8)	Absent	—	—	—	**45**
			Present	—	—	—	**61**
Urinary	Pain in passing urine	14 (2.4)	Absent	—	—	—	**46**
			Present	—	—	—	**36**
	Increased frequency of urination	11 (1.9)	Absent	**47**	—	—	—
			Present	**20**	—	—	—

(2b) Differences in biochemical parameters among study patients in relation to major clinical signs								
Clinical features		**All participants**	**Status**	**Hemoglobin (g/dL)**	**Hematocrit (%)**	**Leukocytes (x10⁹/L)**	**Thrombocytes (x10⁹/L)**	**Transaminases* (IU/L)**
		N (%)		**Value**	**Value**	**Value**	**Value**	**Value**
General signs	Restless	182 (30.6)	Absent	9.1	26.2	11.9	**216**	33.7
			Present	9.1	26.7	13.2	**280**	33.4
	Lethargic	499 (83.9)	Absent	**9.9**	**28.9**	12.6	**297**	—
			Present	**8.9**	**25.9**	12.2	**224**	—
	Wasted	63 (10.6)	Absent	**9.2**	**26.6**	12.2	239	33
			Present	**8.3**	**24**	13.0	212	37.7
HEENT	Rhinorrhea	159 (27)	Absent	9.2	26.2	11.8	**221**	34.8
			Present	8.4	27	13	**275**	30.9
Gastro-intestinal	Vomiting	326 (54.3)	Absent	**8.8**	**25.6**	12.9	252	34.4
			Present	**9.3**	**27.1**	11.7	223	32.6
	Diarrhea	188 (32)	Absent	**8.8**	**25.6**	12.3	**210**	33.9
			Present	**9.6**	**27.9**	12.1	**292**	31.3
	Abdominal pain	109 (18.4)	Absent	9.1	26.4	12.3	239	33.4ǂ
			Present	9.1	26.1	11.9	218	32.6ǂ
	Hepatomegaly	199 (35)	Absent	**9.5**	**27.8**	11.6	**268**	**55.3ǂ**
			Present	**8.1**	**23.5**	13.4	**175**	**67.6ǂ**

Continued

Table 2. (continued)

(2b) Differences in biochemical parameters among study patients in relation to major clinical signs								
Clinical features		**All participants**	**Status**	**Hemoglobin (g/dL)**	**Hematocrit (%)**	**Leukocytes (x10⁹/L)**	**Thrombocytes (x10⁹/L)**	**Transaminases* (IU/L)**
		N (%)		**Value**	**Value**	**Value**	**Value**	**Value**
Neurological	Convulsion	80 (13.5)	Absent	9.1	26.6	13.3	**244**	32.1
			Present	8.6	24.6	11.9	**186**	40.6
	Prostrated	23 (4)	Absent	9.1	26.4	12.2	237	33.6
			Present	8.6	24.8	13.5	211	32.6
	Unconscious	66 (11)	Absent	9.1	26.6	12.2	**244**	32.9
			Present	8.5	24.4	12.8	**175**	38.9
Respiratory	Frequent sneezing	6 (1)	Absent	9.1	**26.4**	12.2	**234**	**33.8**
			Present	10.9	**35**	12.4	**447**	**22.3**
	Cough	270 (46)	Absent	9	26.1	**11.4**	**201**	**54.9**
			Present	9.2	26.8	**13.08**	**278**	**65.4**
	Crackles	66 (11.5)	Absent	9	26.2	**11.7**	**227**	32.7
			Present	9.6	27.9	**14.7**	**306**	37.7
	Bronchial breath sounds	105 (18.4)	Absent	9	26.4	**11.4**	**221**	33.8
			Present	7.6	26.7	**14.3**	**300**	16
	Flaring	63 (11)	Absent	9.1	**26.7**	**11.7**	234	32
			Present	8.8	**24.4**	**15.2**	246	51.2
	Sore throat	5 (0.8)	Absent	**9**	**26.4**	12.1	234	33.8
			Present	**10.7**	**34.2**	15.4	**447**	23
Urinary	Pain in passing urine	14 (2.4)	Absent	9.1	26.4	**12.2**	**239**	—
			Present	9.4	25.7	**8.3**	**137**	—
	Increased frequency of urination	11 (1.9)	Absent	9.1	26.5	12.1	**241**	**32.9**
			Present	8.7	24.5	10.3	**112**	**21**
Lymphatic	Splenomegaly	253 (44.2)	Absent	**9.9**	**28.8**	12.1	**296**	**53.6**[‡]
			Present	**7.9**	**23.1**	12.4	**161**	**66.9**[‡]

Virology

Hepatitis B serology (HBsAg determination) was performed in seven patients presenting with an at least five-fold increase in alanine aminotransferase (ALT ≥225 UI/L); all were HBsAg negative.

A subset of 89/108 samples that tested negative for all pathogens represented in the initial standard panel (Supplementary Table S1) were screened, by PCR, for cytomegalovirus (CMV), Epstein Barr virus (EBV), human herpesvirus 6 (HHV6). Nineteen samples were not tested for technical reasons. Those three targeted herpesviruses were positive in 25% (22/89), 30% (27/89) and 33% (29/89) patients, respectively (Fig. 1). Co-infections of EBV and HHV6 were present in 13 patients. One child was co-infected by three viruses (Table 4(a)). Screening for dengue viruses 1-4 (DENV 1-4), West Nile virus (WNV), yellow fever virus (YFV) and chikungunya virus (CHIKV) was negative (Fig. 1).

Based on clinical suspicion, eight patients were tested for human immunodeficiency virus type 1 and 2 (HIV1&2), and two were found positive for HIV1, 4 patients were already known to be HIV positive on admission. A total of 191 nasopharyngeal specimens underwent PCR analysis for viruses (Supplementary Table S1), Table 4(b) presents the distribution of the 25 (13%) pathogens identified.

Stool samples of 62 patients underwent PCR, and 21 (34%) were positive for gastrointestinal viruses (Table 4(c)).

Table 3. Distribution of bacteria by class and site of sampling. (a): bacteria in blood culture. (b): list of bacteria found in urine. (c): bacteria in stool culture. *: one parasite (Schistosoma haematobium) found in urine.

BACTERIA					
(a)	**BLOOD**	**(b)**	**URINE**	**(c)**	**STOOL**
PATHOGENS	**N (%)**	**PATHOGENS**	**N (%)**	**PATHOGENS**	**N (%)**
Gram-negative		**Gram-negative**		**Gram-negative**	
Enteropathogenic E. coli (EPEC)	1 (0.2)	*Acinetobacter baumannii*	1 (0.3)	*Group D Salmonella*	1 (1.1)
Escherichia coli Type III	1 (0.2)	*Enterobacter cloacae*	2 (0.7)	*Klebsiella pneumoniae*	1 (1.1)
Group D Salmonella	1 (0.2)	*Escherichia coli*	26 (9.1)	*Salmonella enterica durham*	1 (1.1)
K. pneumoniae ESBL	1 (0.2)	*Escherichia coli ESBL*	2 (0.7)	*Shigella ssp.*	3 (3.3)
S. enterica enteritidis	2 (0.3)	*Klebsiella oxytoca*	1 (0.3)	**Negative**	85 (93.4)
S. enterica typhimurium	2 (0.3)	*Klebsiella pneumoniae*	7 (2.4)	**Overall**	91 (100)
Escherichia coli Type III	1 (0.2)	*Klebsiella pneumoniae ESBL*	4 (1.4)		
Shigella ssp.	1 (0.2)	*Kluyvera spp.*	1 (0.3)		
Gram-positive		*Proteus mirabilis*	2 (0.7)		
Group C Streptococcus	2 (0.3)	*Proteus penneri*	1 (0.3)		
Micrococcus luteus	1 (0.2)	*Proteus vulgaris*	1 (0.3)		
Staphylococcus aureus	3 (0.5)	*Ralstonia pickettii*	1 (0.3)		
Staphylococcus saprophyticus	1 (0.2)	*Salmonella enterica enteritidis*	1 (0.3)		
Streptococcus pneumoniae	1 (0.2)	**Gram-positive**			
Negative	576 (97.1)	*Enterococcus faecalis*	1 (0.3)		
Overall	593 (100)	*Group D Streptococcus*	1 (0.3)		
		Staphylococcus aureus	2 (0.7)		
		Contamination	107 (37.4)		
		Negative	124 (43.4)		
		Others*	1 (0.3)		
		Overall	286 (100)		

Table 4. Distribution of viruses by class and site of sampling. (a): viruses in blood. (b): list of viruses found in pharyngeal swabs. (c): viruses found in stool.

VIRUSES					
(a)	**BLOOD**	**(b)**	**THROAT**	**(c)**	**STOOL**
PATHOGENS	**N (%)**	**PATHOGENS**	**N (%)**	**PATHOGENS**	**N (%)**
Cytomegalovirus (CMV)	22 (24.7)	Adenovirus	7 (3.7)	Adenovirus	8 (12.9)
Epstein-Barr virus (EBV)	27 (30.3)	Coronavirus OC43	3 (1.6)	Astrovirus	3 (4.8)
Human herpesvirus6 (HHV6)	29 (32.6)	Coronavirus 229E	2 (1.0)	Norovirus	2 (3.2)
Negative	11 (12.4)	Enterovirus	3 (1.6)	Rotavirus	7 (11.3)
Overall	89 (100)	Influenza A	3 (1.6)	Sapovirus	1 (1.6)
Mixed infections	**N**	Parainfluenza type 2	1 (0.5)	**Negative**	41 (66.1)
HHV6 + EBV	13	Parainfluenza type 3	1 (0.5)	**Overall**	62 (100)
HHV6 + CMV	8	Rhinovirus	3 (1.6)	**Mixed infections**	**N**
HHV6 + CMV + EBV	1	Respiratory Syncytial Virus	4 (2.1)	Astrovirus + Rotavirus	1
		Negative	166 (86.9)	Adenovirus + Astrovirus + Rotavirus	1
		Overall	191 (100)		
		Mixed infections	**N**		
		Coronavirus OC43 + Coronavirus 229E	1		
		Parainfluenza type 2 + Rhinovirus	1		

Imaging

Thirty-five chest radiographs were performed. Two showed no abnormalities, three could not be interpreted due to technical constraints, and 30 showed pathological findings. Among these,

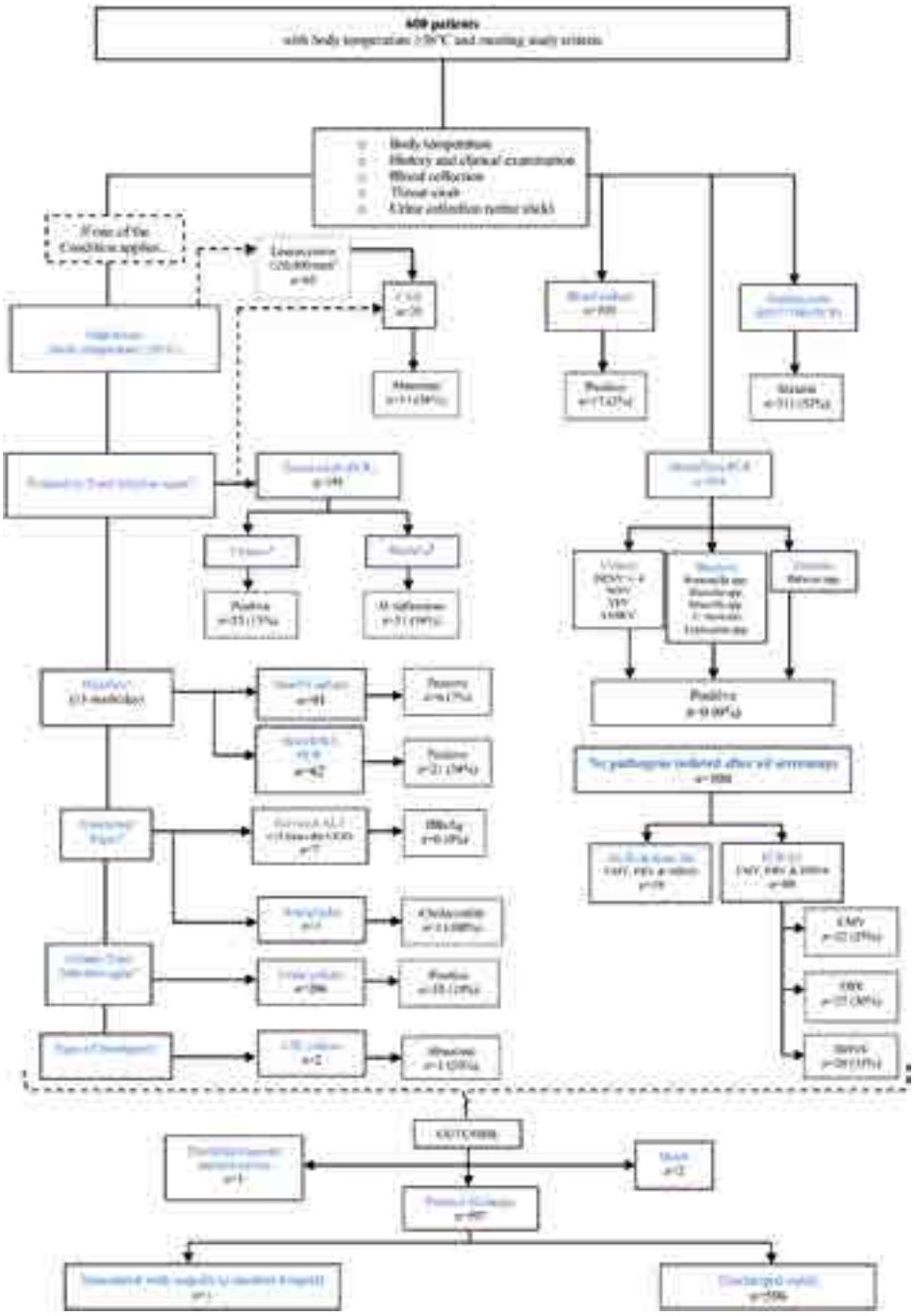

Figure 1. Flow diagram of signs and symptoms, laboratory findings, and diagnoses in all patients throughout the study. *: Influenza A & B, Rhinovirus, Enterovirus, Parainfluenza 1-4, Coronavirus (NL63, 229E, OC43 & HKU1), Human metapneumovirus, Respiratory syncytial virus A & B and Adenovirus **§:** Chlamydia pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae.

33% (10/30) showed radiologic features of pneumonia with one case also presenting with pleural effusion, 3% (1/30) revealed miliary tuberculosis. A majority of 63% (19/30), although showing abnormal features, did not meet the criteria of pneumonia (5).

One single abdominal sonography was performed and revealed acute calculous cholecystitis treated by surgery (Fig. 1).

More frequent infections and their characteristics

In malarial patients, the body temperature was higher compared to non-parasitemic patients (39.2 °C *versus* 38.8 °C; p<0.001). Consequently, malaria was positively associated with fever grade 3 (39.4 °C – 40 °C; adjusted odds ratio for both sex and age (AOR) 3.2 [1.9–5.6]) and negatively associated with fever grade 1 (<38.6 °C; AOR 0.59 [0.4–0.8]) (Supplementary Table S2). Malaria was equally distributed in all age groups. The evolution of the main clinical and biological parameters among study patients in relation to both infections malaria and *H. influenza* are described in Supplementary Table S3.

Laboratory results showed that anemia was not associated with *H. influenzae* infection, whilst the alanine aminotransferase was lower than in uninfected patients by factor 2 (18.9 IU/L *versus* 41.8 IU/L; p < 0.001) (Supplementary Table S3).

The main pathogen causing urinary tract infections, irrespective of sex and age, was *E. coli* in 52% (28/54) patients.

Figure 2 shows the frequency of the main conditions/diagnoses and their co-occurrences; with malaria and malnutrition being the second-most frequent association, seen in 12% (71/600) patients. Systemic infection (bacteria and viruses isolated from blood) occurred together with malaria in 2% (13/600) patients.

All diagnoses and their proportions are listed in Supplementary Fig. S2. In 6% (35/600) of patients, no pathogen was found.

Children with danger signs

Twelve percent (72/600) children met the criteria of emergency (high risk of death) based on the adapted LODS. Females were 49% (35/72), the median (IQR) age was 19 [10.8–51] months, the nutritional status was normal (weight-for-age Z-score ≥ -2.0) in 79% (57/72) patients. Among those 72 patients, only one child had homozygous sickle cell disease and another one was HIV-positive. On admission, 29% (21/72) patients had a body temperature equal or greater than 39.5 °C, and the most common associated clinical signs were vomiting and convulsions in 53% (38/72) and 42% (30/71, NA= 1), respectively. Severe anemia – hemoglobin <5 g/dL and/or hematocrit <15% – was found in 12% (7/60, NA =12) patients. Regarding the main diagnoses; severe malaria was found in 47% (34/67, NA=5) and lower respiratory tract infections (LRTI) in 26% (19/72) patients; the two cases of meningitis and one case of encephalitis were among them. In two patients the cause of fever remained undetermined.

Figure 3 shows the frequency of the main diagnoses, including all co-occurrences amongst them, diagnoses coded with MedDRA's preferred terms are listed in Supplementary Fig. S3.

The two cases of death were part of this group of children presenting with danger signs.

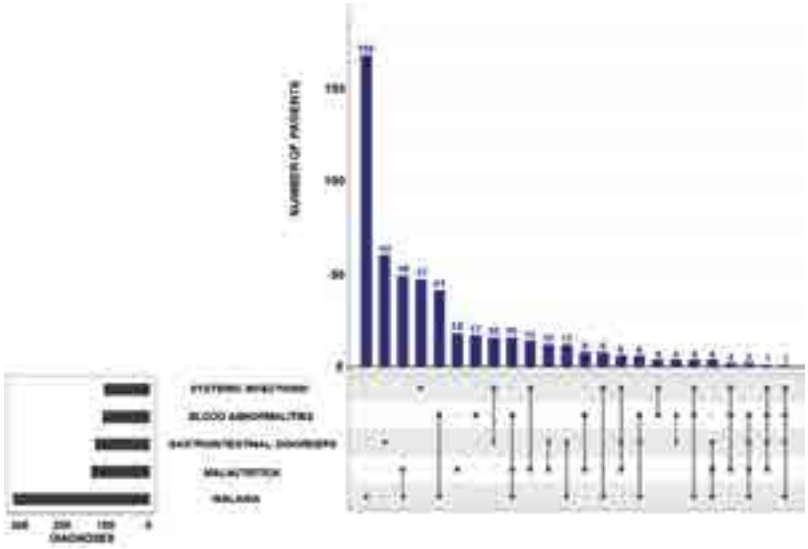

Figure 2. Distribution, frequencies and overlapping of main conditions/diagnoses (according to MedDRA preferred terms) in all 600 patients. *Systematic infections other than malaria.

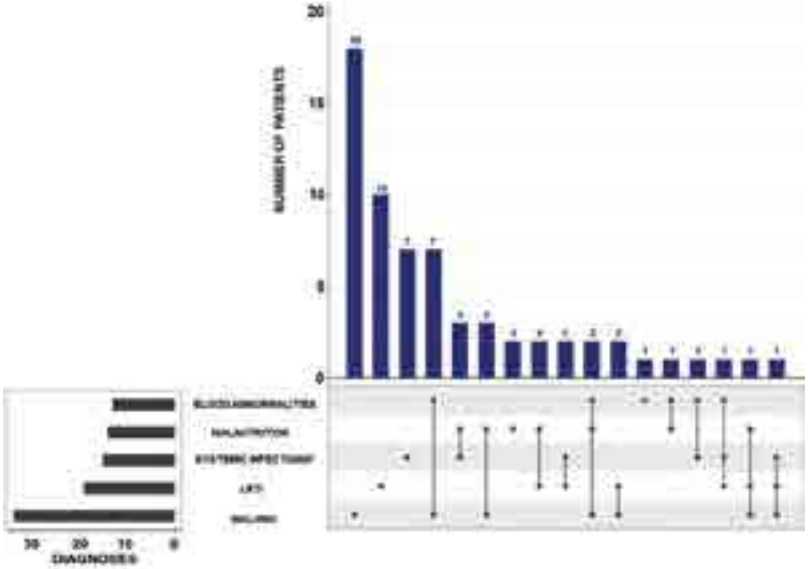

Figure 3. Distribution, frequencies, and overlapping of main conditions/diagnoses (according to MedDRA preferred terms) in patients with danger signs. *Systemic infections other than malaria. LRTI: Lower Respiratory Tract Infections.

Outcome

A total of 596 of 600 included patients, were discharged as cured after a mean of five days of hospitalization. Two patients died while in hospital; one of meningitis presenting clinically with meningismus and deep coma, one of pneumonia (clinically suspected pulmonary tuberculosis complicated by bacterial pneumonia). One child, diagnosed with *Staphylococcus aureus* bacteremia, was referred to another hospital after the development of Guillain-Barré syndrome. Another child was taken from the hospital, by his parents, against medical advice (Fig. 1).

Overall, in our study population, neither pre-existing conditions, such as malnutrition or sickle cell anemia nor concomitance of multiple diagnoses were associated with a negative outcome.

DISCUSSION

In Lambaréné and surrounding villages, malaria remained the leading cause of hospitalization for fever in children, an observation that is in accordance with findings from Ghana (2) and Burkina Faso (6). This contrasts with a study from Tanzania where malaria was found only in 10.5% of the patients (1,7). Consequently, in Lambaréné, infections were most often caused by parasites (62%) (Plasmodium species and one Schistosoma haematobium infection), followed by bacteria (21%) and viruses (18%). Malaria cases were significantly associated with fever grade 3 (body temperature between 39.4 °C and 40 °C), lethargy, unconsciousness, and convulsions as described in neighboring Congo (8), and with anemia, malnutrition, and thrombocytopenia. Malnutrition can be an important factor related to malaria as described in hospitalized Mozambican children (9) and is also pre-disposing to bacteremia (10). Interestingly, we observed an association of malaria and malnutrition despite the generally low prevalence of malnutrition in Gabon. Anemia is particularly important in malaria-endemic areas because it is the most frequent complication in severe malaria (11,12).

Respiratory tract infections were the second-most frequent cause of fever in Lambaréné putting young children at risk of respiratory distress, which may be life-threatening (13), especially where pediatric resuscitation and advanced means of respiratory support are not available. Furthermore, one of the two fatalities that occurred in our study was caused by respiratory failure. Lower respiratory tract infections (LRTI) were the main cause of febrile illness in children, as found in similar studies (14). These results stress the need for fast and correct malaria diagnosis and radiology in resource-limited areas.

The other identified sources of fever were a heterogeneous group of infections related to pathogens found in blood, urine, stool, and nasopharyngeal specimen.

The relatively large number of CMV by PCR in blood of immune-competent children were most likely not all acute cases even though primary infection with CMV occurs during the first year of life (15). A certain proportion of the PCR-positives might be explained by delayed sample preparation and consequently, the release of leukocyte-derived CMV DNA (16). EBV is rather expected to be seen in young adults than in young children (17). We identified HHV6 in blood by PCR without any exanthema found in these patients possibly due to previous or acute infection without visible skin lesions.

Neither dengue nor chikungunya virus was diagnosed in our study, even though both pathogens were regularly reported in the Lambaréné area (18). This might be explained by the predominantly sylvatic life cycle of both viruses in the region, where they occur in seasonal small outbreaks (19).

The proportion of typhoid fever was low (0.7%) compared to a similar study in Tanzania (3.7%) (1). We acknowledge regional differences in epidemiology and think that continuous improvements in hygiene and sanitation in African communities are further lowering the prevalence of typhoid fever. The same might be the case for bacterial and viral meningitis, which was almost absent (0.2%). This low proportion of meningitis further underlines that not only geographically, Gabon is not part of the West-African meningitis belt (20). The incorporation of the H. influenzae type b vaccine into the Gabonese EPI ten years ago might have contributed to the decline of meningitis cases as seen in Ivory Coast (21). The 31/191 cases of H. influenza found by PCR from pharyngeal swabs were most likely colonization rather than true infections.

Respiratory viruses isolated from nasopharyngeal throat swabs were low in numbers but in accordance qualitatively with what is found in the same age population in other African settings (22).

E. coli was the most frequently isolated pathogen of urinary tract infections, as commonly seen in pediatric populations (23). In stool samples, viruses were identified less frequently than in Tanzania1. In addition, we observed lower rates of invasive bacteria, typhoid and non-typhoid Salmonella species in our cohort than in Kenyan children(24). Those differences may be related to different pathogen dynamics, ecology and level of hygiene between the areas. Co-morbidities were common. In our population, the measles vaccination rate was 54%, well below the WHO (25) recommended vaccination rate of 95%, in order to prevent measles epidemics and ultimately to eradicate the disease. However, the situation for the other EPI vaccines was better with a vaccination coverage of above 80% for all vaccines that are scheduled to be given within the first 14 weeks of life and not like measles and yellow fever vaccine at 9 months of age.

Therefore, measles need to be considered as one possible differential diagnosis in a proportion of cases even in patients with only unspecific signs and symptoms, e.g. lacking characteristic skin eruptions. Pre-existing conditions and co-morbidities, such as sickle cell disease, known HIV-infection and malnutrition had no strong negative impact on the outcome of the febrile diseases, although our methodology might not have been ideal to identify discrete signs.

Malnutrition was present in one-fifth of the patients but did not seem related to immune disorders known to increase the sensitivity of malnourished patients to infections (26), conversely to what is described from other populations (27,28).

The cause of fever remained undetermined in 35 patients (6%), similar to the rate of 3.2% reported in Tanzania1. Since no control group was recruited the population attributable fractions cannot be estimated. Particularly diseases attributed to herpesviridae and low-grade P. falciparum parasitemias may be affected and could increase the proportion of undiagnosed cases, as seen in Ghana2.

Based on our results, we recommend that pediatric health care providers should take more care when prescribing antibiotic treatment and if they do, they should base this on the local

epidemiology and susceptibility profiles of the bacterial pathogens. Further studies should be designed to assess the relevance of presumptive antibiotic treatment.

METHODS

Study design and setting

We report, according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (Table S4) (29), a systematic, prospective, cross-sectional study in hospitalized children with a fever at the HAS in Lambaréné, Gabon. HAS is one of the two main hospitals of the Moyen-Ogooué province located in the center of Gabon.

Participants

Children aged ≤15 years, hospitalized at HAS, from August 2015 to March 2016, were included in the study. Apart from consent to participate, the only inclusion criterion was fever (rectal or axillary temperature ≥38 °C). No exclusion criteria were applied.

Variables

A full diagnostic toolkit to support the identification of infectious agents causing febrile illness was established (Supplementary Table S1).

Clinical case definitions

Definitions of keywords for characterization of symptoms/signs, medical conditions, and diagnoses were established for harmonization (Supplementary Table S5). To assess severity, we adapted the Lambaréné Organ Dysfunction Score (LODS) focusing on three key items (prostration, coma and chest wall in-drawing) (30). Prostration was defined by the presence of at least one of the four signs: inability to breast-feed/eat, sit, stand, or walk, depending on child age; whereas coma was defined as Blantyre Coma Score (BCS) ≤2. Symptoms and diagnoses were coded according to the Preferred Terms of the Medical Dictionary for Regulatory Activities (MedDRA) version 19.1 of September 2016 from verbatim descriptions.

Data sources and bias assessment.

Study physicians completed the questionnaire, including sociodemographic and clinical information as presented in Supplementary Table S6.

Based on symptoms and signs, children were assigned to a specific syndrome (Supplementary Table S1) and samples were taken accordingly (Fig. 1). Sample collection was based on two principles: first, to find the immediate diagnosis decisive for treatment based on investigations (*i.e.* rapid diagnostic kits and laboratory-based investigations), routinely available in Lambaréné; second, to allow for diagnosis by advanced techniques carried out in Lambaréné and at the partner institutions presented in Supplementary Table S7.

We tried to minimize the selection biases (*i.e.* admission, volunteer, and non-response bias) common in cross-sectional studies by recruiting almost all febrile hospitalized children in the study period.

Sample size considerations

This observational study aimed at detecting non-dominant fever-causing pathogens controlling for seasonal variations in disease frequency. The pediatric ward had 1,933 admissions in 2014, of which 69% had fever as the lead symptom. To cover at least one rainy and one dry season and to detect uncommon pathogens, we decided to recruit 600 children over a period of eight months, requiring the inclusion of at least 50% of eligible children; yielding a 95% probability to detect uncommon pathogens (prevalence ≤0.05%).

Data management and statistical methods.

Data were collected by filling a paper questionnaire that was manually entered into an electronic database - Research Electronic Data Capture (REDCap) Software - Version 5.7.2, 2015 (31).

Missing data were handled by list-wise deletion (complete-case analysis). Missing values are reported as "not available".

Analyses were done with R software V3.5.1 (2018) (www.r-project.org). Continuous exposure data were described and compared according to their distribution. Prevalence ratios and odds ratios were used to show associations among dichotomous variables. Stratification was used to show effect differences amongst a third variable. Corresponding multivariate models were established to account for confounding and interaction.

Results of the bivariable and multivariable analysis were reported as crude and adjusted odds ratio at 95% confidence intervals (95% CI), and statistical significance was defined as a two-sided p-value <0.05.

All methods were performed in accordance with relevant guidelines and regulations.

ETHICS DECLARATIONS

After being validated by both the Scientific Review Board and the Institutional Ethics Committee of Centre de Recherches Médicales de Lambaréné (CERMEL), our study protocol was submitted to the Gabon National Ethics Committee on 06 February 2015 and obtained an approval (Number 006/2015/SG/P) on 28 February 2015.

Written informed consent was obtained from all parents/legal guardians prior to enrolment. Children aged eight years and above were enrolled only when they agreed and provided their assent form; except for those unconscious, in which case only the informed consent provided by a parent or guardian was considered sufficient. The study team decided upon the medical condition of the patient and determined whether he/she was able to sign the assent.

Two copies of the informed consent form, as well as the assent form, had to be signed. One was kept by the study personnel for documentation, while the second copy was given to the patient's parents/guardians.

DATA AVAILABILITY

All data supporting the findings of this study are available from the corresponding authors upon request.

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AUTHOR CONTRIBUTIONS

Patient recruitment, enrollment, and sample collection (J.F.F., M.D. and C.K.). Performed experiments (J.F.F., A.L., F.S., A.A., M.D.A., A.B.A., D.E., A.A.A., I.E., B.He. and B.Ho.), planning/ designing of experiments (J.F.F., J.H., B.Ho., J.M., M.P.G. and B.M., analysis (J.F.F. and B.M.), writing – original draft preparation (J.F.F.) and writing – review & editing (J.F.F., J.H., M.D., A.L., F.S., A.A., M.D.A., C.K., A.B.A., M.E., D.E., A.A.A., S.T.A., B.L., I.E., B.He., B.Ho., J.M., P.G.K., M.P.G. and B.M.).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-58204-2.

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Publisher's note

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ANTIMALARIA DRUGS AND MALARIA VACCINES

11

FOSMIDOMYCIN AS AN ANTIMALARIAL DRUG: A META-ANALYSIS OF CLINICAL TRIALS

3

ABSTRACT

With first indications of resistance against artemisinin compounds, the development of novel alternative antimalarials remains an urgent need. One candidate is fosmidomycin (Fos), a phosphonic acid derivative. This PRISMA guideline-adhering and PROSPERO- registered systematic review and meta-analysis provides an overview of the state-of-the-art of the clinical development of Fos as an antimalarial. Pooling six clinical trials of Fos against uncomplicated malaria in African children yielded an overall day 28 cure rate of 85% (95% CI: 71–98%); a parasite clearance time of 39 h; and a fever clearance time of 30 h. In four adult cohorts, the corresponding values were 70% (95% CI: 40–100%), 49 and 42 h, respectively. Data suggest that besides the partner drug, formulation determines efficacy. We advocate further clinical development of Fos-combinations.

KEYWORDS

- antimalarial drug
- clinical studies
- DOXP reducto-isomerase
- drug development
- fosmidomycin
- fosmidomycin-clindamycin
- malaria

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INTRODUCTION

Despite substantial progress with stepping-up control, malaria remains a major public health issue in endemic areas (1). According to the World Health Organization report in 2013, an estimated 584,000 deaths (with an uncertainty range of 367,000–755,000) were attributable to malaria, although an encouraging downward trend was reported recently (2). Additionally, early evidence of the emergence of Plasmodium falciparum resistance to artemisinin derivatives in southeast Asia (3) emphasizes the need for novel alternative drugs to cure or prevent the disease (4,5). One effective way to develop new drugs is to test compounds that underwent clinical development and targeting other human diseases (e.g., antibiotics), which also have antiplasmodial activity (6). Development of Fosmidomycin has been an example of how basic research can be translated fast and efficiently into clinical research on antimalarial interventions. This work aims at informing decision making on furthering the development of this potentially valuable compound.

Fosmidomycin (3-(formylhydroxy-amino)-propylphosphonic acid monosodium salt or FR31564) is a natural antibacterial agent originally isolated from Streptomyces lavendulae in the late 1970s (7). Subsequently, in the 1980s, it was evaluated in initial clinical trials as a candidate antibacterial drug (8,9). It competes with the natural substrate of 1-deoxy-D-xylulose 5-phosphate (DOXP) reduc- toisomerase, a key enzyme in the isoprenoid biosynthesis pathway. The DOXP pathway (nonme- valonate pathway or 2-C-methyl-D-erythritol 4-phosphate pathway) is essential in Plasmodium blood stages (10–12). In the late 1990s, Jomaa and colleagues demonstrated that Fos is a specific inhibitor of plasmodial DOXP (nonmevalonate pathway) (Figure 1) (13,11). In contrast to Plasmodium spp., isoprenoids are synthesized via the mevalonate pathway in humans (14,15).

Fos has an oral bioavailability of 10–30% and a plasma half-life of approximately 1.9 h. It is highly polar and deprotonated at physiological pH values (7). Besides absorption and distribution, these characteristics affect its on-target concentration, since several membranes (erythrocyte, parasitophorous vacuole, parasite and apicoplast membranes) must be crossed before it can become effective.

One review article published in 2003 summarized the up-to-then body of knowledge on both pre- and early-clinical development (10). Since then, over the past 12 years, a series of clinical trials of Fos for the treatment of malaria have been published. However, despite an encouraging accumulating body of knowledge, its clinical development slowed pending identification of suitable alternative partner drugs (i.e., in September 2014, only one active Phase II clinical trial was listed in ClinicalTrials.gov). To obtain an overview of the current situation of this candidate compound within the antimalarial drugs pipeline, we conducted a systematic review and a meta-analysis by pooling of ten clinical trials in both pediatric and adult populations in Africa (Gabon, Mozambique) and South East Asia (Thailand). For pediatric trials, since the original data sets were available to the authors, we did an in-depth analysis of individual patient data for efficacy and safety end points

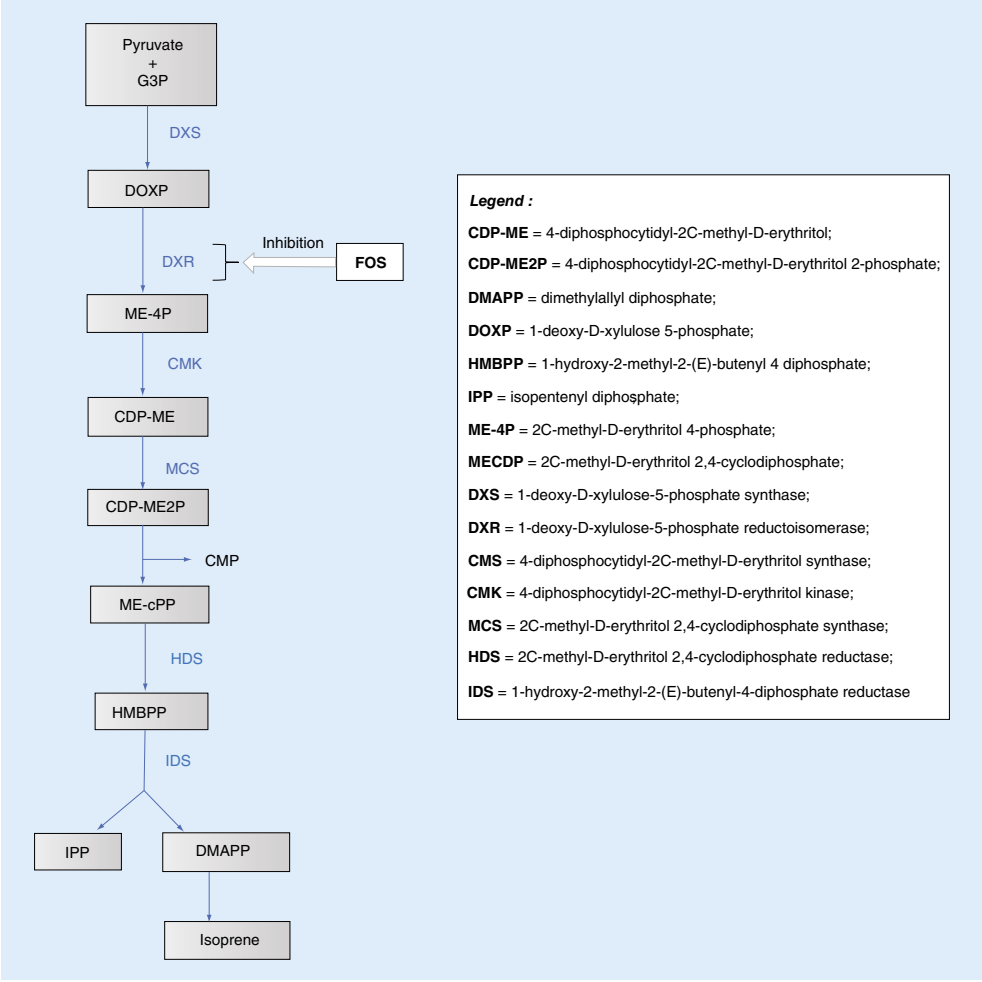

Figure 1. The nonmevalonate pathway for the biosynthesis of isoprenoids.

METHODS

This work was performed in accordance with the PRISMA proposed methods (16). Although we did not use a specific protocol in view of the small number of trials conducted, we adapted the work to comply by and large with the PRISMA check- list and registered the work under PROSPERO. Public databases (PubMed, ClinicalTrials.gov and WHO ICTRP) were screened for eligible trials originating from 2002 until 2014. Searches were restricted to English. The identified studies reported Phase I and II clinical trials in Africa and South East Asia as retrieved by the search terms 'fosmidomycin' and 'malaria'. Two investigators (JF Fernandes and RM Obiang) screened all the titles and abstracts collected and then independently assessed the eligibility criteria.

Twenty-eight studies were identified through the indexed online data bases. After removing duplicates, 23 studies were retained for detailed consideration. After assessment of eligibility, four studies were excluded because they were not related to Fos. A further nine other studies not reporting clinical trials (Figure 2) were also excluded. Thus, ten studies were included in this meta-analysis (tables 1 & 2).

Our outcomes of interest included parasitological cure rate on day 28, fever clearance time (FCT) and parasite clearance time (PCT). Pediatric studies were pooled on one hand, and adult studies on the other. All data were analyzed using R software (versions 3.0.2 and 3.1.1). Absolute risk was used for binary outcome and arithmetic mean for continuous outcome measurements.

For cure rates, the beta-binomial model (17) was used to capture the variation across studies and to estimate pooled event risk and corresponding 95% confidence intervals. The method described by Cochran and colleagues (18) was used to estimate pooled weighted mean and the corresponding 95% confidence interval for continuous outcomes (FCT and PCT). A Cox Proportional Hazard Model was used for the time-to-event analysis between the two groups of pediatric studies (the five conducted in Gabon and the one conducted in Mozambique). Finally, interstudy variability was assessed by using Cochrane's Q statistic.

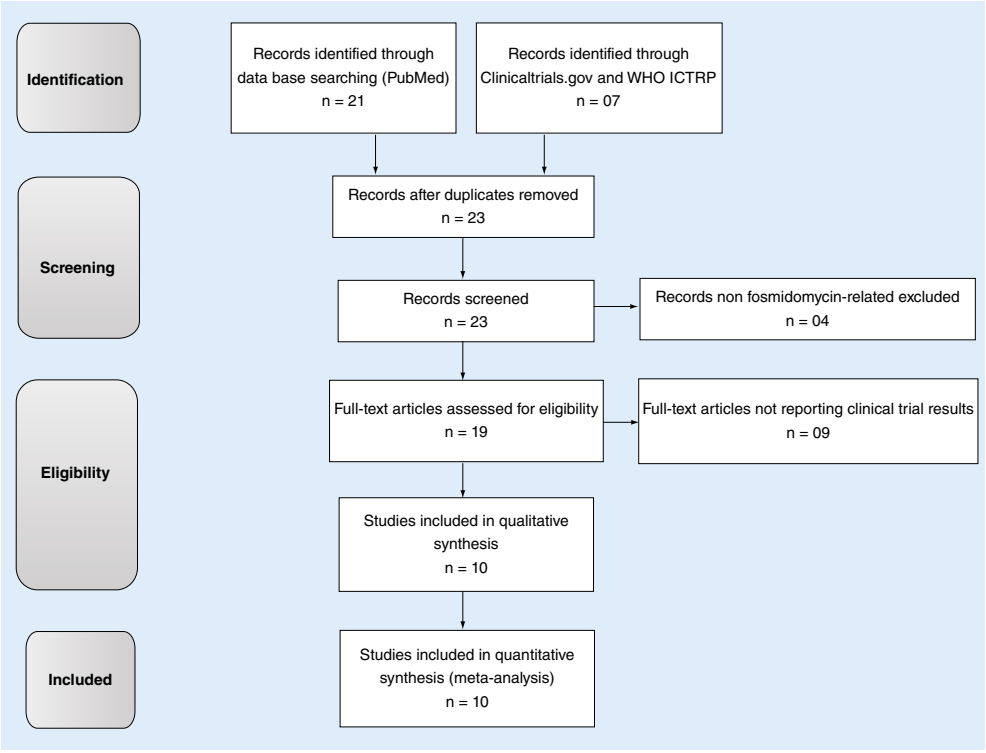

Figure 2. PRISMA flow chart.

Table 1. Overview of the pediatric studies included.

Study (year)	Country	Population	Age (years)	Interventions	Ref.
Borrmann *et al.* (2004)	Gabon	Children	7–14	Fos-Clin; Fos; Clin	[20]
Borrmann *et al.* (2004)	Gabon	Children	7–14	Fos-Clin	[30]
Borrmann *et al.* (2005)	Gabon	Children	6–12	Fos-Art	[36]
Borrmann *et al.* (2006)	Gabon	Children	1–14	Fos-Clin	[31]
Oyakhirome *et al.* (2007)	Gabon	Children	3–14	Fos-Clin	[33]
Lanaspa *et al.* (2012)	Mozambique	Children	<3	Fos-Clin	[34]

Clin: Clindamycin; Fos: Fosmidomycin; Fos-Art: Fosmidomycin + artesunate; Fos-Clin: Fosmidomycin + clindamycin.

Table 2. Overview of the adult studies included.

Study (year)	Country	Population	Age (years)	Interventions	Ref.
Missinou *et al.* (2002)	Gabon	Adults	17–42	Fos	[21]
Lell *et al.* (2003)	Gabon; Thailand	Adults	18–50	Fos	[19]
Na-Bangchang *et al.* (2007)	Thailand	Adults	18–50	Fos; Fos-Clin	[41]
Ruangweerayut *et al.* (2008)	Thailand	Adults	15–61	Fos-Clin	[40]

Clin: Clindamycin; Fos: Fosmidomycin; Fos-Art: Fosmidomycin + artesunate; Fos-Clin: Fosmidomycin + clindamycin.

SYSTEMATIC REVIEW

Fosmidomycin monotherapy

Three trials in African and Asian populations (children and adults) have been conducted with Fos monotherapy using common trial designs for early Phase II trials, with a single or multiple arms allocated to Fos (Table 1). Efficacy varied across studies and generally, recrudescence rates were higher than commonly accepted (Table 3) (19–21). This finding precludes the use of Fos alone. Therefore, further clinical development focused on the use of Fos in combination with a second suitable antimalarial.

Fosmidomycin-clindamycin combinations

A combination of antimalarials is nowadays considered standard (22). This approach may enhance efficacy, increase effectiveness and can delay development and/or spread of resistance (23–25). On the basis of their synergistic activity and matching pharmacokinetic characteristics, Fos has been combined with another antibiotic, clindamycin.

Synergy has been shown by Wiesner and colleagues by measuring the sum of the fractional inhibitory concentration (sum FIC, which is considered to indicate synergy when <0.5) in *in vitro* drug interaction assays (26). Wiesner and colleagues found a sum FIC of 0.43 ± 0.02 for Fos when added to clindamycin against *P. falciparum in vitro* (4,26). Clindamycin (7-chlorolincomycin), which is a semisynthetic derivative of lincomycin, was introduced in the 1960s as an antibiotic, showed a strong inhibitory effect on *P. falciparum*. *In vitro* and *in vivo*, it has a delayed onset of action which is seen as a major limitation for its use as a monotherapy against malaria (27,19).

During the past 10 years, the combination of Fos plus clindamycin has been evaluated in Phase II clinical trials. To date, a total of six pediatric clinical trials have been conducted in Gabon and Mozambique (28,29).

The first study was a Phase IIa clinical trial conducted in Gabon from October 2001 to January 2002. It aimed at establishing the efficacy and the safety of a 5-day regimen of the combination of Fos and clindamycin (FC), compared with Fos or clindamycin alone, in 36 Gabonese school children aged 7–14 years (20). In this study, three groups of 12 children with asymptomatic *P. falciparum* malaria were evaluated. In one group, Fos was administered at 30 mg/kg and clindamycin at 5 mg/kg orally every 12 h for 5 days. In the other two groups, Fos and clindamycin were given alone in oral doses of 30 mg/kg and 5 mg/kg, respectively. The main results obtained are summarized in Table 3.

Another study conducted in Lambaréné aimed at identifying the shortest efficacious and safe regimen of FC in 52 children, aged 7–14 years with *P. falciparum* monoinfection (30). In this study involving five groups of ten children, Fos 30 mg/kg and clindamycin 10 mg/kg were given every 12 h starting with 5 days of treatment. This was then progressively shortened to 4, 3 and 2 days when at least 85% of patients in the previous cohort showed clinical and parasitological cure on day 14. The cure rates on day 28 were 100% with regimens of 5, 4 and 3 days of treatment versus 90 and 10% with regimens of 2 days and 1 day, respectively. This confirmed the results of a satisfactory response to a 3-day regimen of Fos plus clindamycin for the treatment of children with uncomplicated malaria due to *P. falciparum* in endemic areas (31). FC showed no significant effect on gametocytes; however, its capacity to influence transmission of *P. falciparum* has not been formally addressed (32).

In 2007, Oyakhirome and colleagues compared the efficacy and safety of the combination of FC to sulfadoxine-pyrimethamine (SP) in a randomized controlled trial involving 105 children aged 3–14 years with uncomplicated malaria. In this trial, Fos (30 mg/kg) and clindamycin (10 mg/kg) were given every 12 h for three consecutive days; whereas coformulated sulfadoxine (25 mg/kg) and pyrimethamine (1.25 mg/kg) was administered as a single dose. The total population evaluated (n = 105) was allocated to two comparable treatment groups: 54 subjects received FC and 51 subjects SP. The proportion of patients cured (PCR corrected cure rate) in the per-protocol population was 94% (46 of 49) for both groups ($p = 0.2$). In the intention-to-treat population, the proportions of patients cured were 90% (46 of 51) for the SP group and 85% (46 of 54) for the FC group ($p = 0.5$) (33).

Lanaspa and colleagues conducted an open-label clinical trial from January to March 2010 in Manhiça (Southern Mozambique) to assess the efficacy and tolerability of Fos plus clindamycin for the treatment of uncomplicated *P. falciparum* malaria in Mozambican children under of 3 years of age (34) using the day-28 PCR-corrected cure rate as the primary end point. It was the first trial using an FC formulation that is suitable for infants; Fos as powder for reconstitution with water at a concentration of 250 mg/5ml and clindamycin hydrochloride as proprietary syrup at a concentration of 75 mg/5 ml. The drugs were administered in a fixed ratio of 30/10 mg/kg, twice a day for three consecutive days. The day 28 PCR-corrected cure rate was 45.9% (17/37) and PCT was notably longer than in the previous trials (121 h of mean and a median of 72 h as evaluated in 29 patients). However, the mean FCT was short (12 h evaluated

Table 3. Characteristics of the included trials.

Study (year)	Study arm	Drug	Dosage per day	Days	Doses	End point	Cured (n)	Total (n)	Cure rate (95% CI)	PCT	FCT	Ref.
Lanaspa *et al.* (2012)	Arm 1	FC	60 mg/kg + 20 mg/kg	3	2	Day 14	-	-	-	121 (24–218)‡	23.3 (0–54)‡	[34]
						Day 28	16	37	43.2 (27.2–59.2)†			
Ruengweerayut *et al.* (2008)	Arm 1	FC	3600 mg + 1800 mg	3	4	Day 28	21	23	91.3†	50 (20–80)§	40 (4–76)§	[40]
	Arm 2	FC	3600 mg + 1200 mg	3	2	Day 28	70	78	89.7†			
Na-Bangchang *et al.* (2007)	Arm1	F	3600 mg	7	3	Day 7	10	10	100	56 (8–80)§	56 (24–104)§	[41]
						Day 28	2	9	22			
	Arm 2	FC	1800 mg + 1200 mg	7	3	Day 7	12	12	100	40 (16–80)§	40 (24–72)§	
						Day 28	12	12	100			
Oyakhirome *et al.* (2007)	Arm 1	FC	60 mg/kg + 20 mg/kg	3	2	Day 14	-	-	-	38 (26–42)‡	33 (26–41)‡	[33]
						Day 28	46	54	85 (73–92)†			
Borrmann *et al.* (2006)	Arm 1	FC	60 mg/kg +20 mg/kg	3	2	Day 14	49	49	100 (93–100)†	42 (IQR: 34–49)§	38 (IQR: 18–48)§	[31]
						Day 28	42	47	89 (77–96)†			
Borrmann *et al.* (2005)	Arm 1	AF	4 mg/kg + 60 mg/kg	1	2	Day 14	7	10	70 (35–93)†	19 (18–29)‡	19 (13–29)‡	[36]
						Day 28	4	10	40 (12–74)†			
	Arm 2	AF	4 mg/kg + 60 mg/kg	2	2	Day 14	10	10	100 (69–100)†	31 (22–43)‡	14 (11–17)‡	
						Day 28	6	10	60 (26–88)†			
	Arm 3	AF	4 mg/kg + 60 mg/kg	3	2	Day 14	10	10	100 (69–100)†	17 (13–22)‡	14 (11–19)‡	
						Day 28	10	10	100 (69–100)†			
	Arm 4	AF	2–4 mg/kg + 60 mg/kg	4	2	Day 14	10	10	100 (69–100)†	35 (27–45)‡	13 (10–17)‡	
						Day 28	9	10	90 (55–100)†			
	Arm 5	AF	4–2 mg/kg + 60 mg/kg	5	2	Day 14	9	9	100 (66–100)†	22 (18–29)‡	15 (12–19)‡	
						Day 28	9	9	100 (66–100)†			
Borrmann *et al.* (2004)	Arm 1	FC	60 mg/kg + 10 mg/kg	5	2	Day 14	11	11	100 (72–100)	18 (12–47)§	-	[20]
						Day 28	11	11	100 (72–100)			
	Arm 2	F	60 mg/kg	5	2	Day 14	11	12	92 (62–100)	25 (13–46)§	-	
						Day 28	5	12	42 (15–72)			
Borrmann *et al.* (2004)	Arm 1	FC	60 mg/kg + 20 mg/kg	1	2	Day 14	5	10	50 (19–81)	63 (43–88)‡	46‡	[30]
						Day 28	1	10	10 (0–45)			
	Arm 2	FC	60 mg/kg + 20 mg/kg	2	2	Day 14	10	10	100 (69–100)	35 (25–58)‡	46‡	
						Day 28	7	10	17 (35–93)			
	Arm 3	FC	60 mg/kg + 20 mg/kg	3	2	Day 14	10	10	100 (69–100)	39 (28–42)‡	46‡	
						Day 28	9	10	90 (55–100)			
	Arm 4	FC	60 mg/kg + 20 mg/kg	4	2	Day 14	10	10	100 (69–100)	38 (32–49)‡	46‡	
						Day 28	10	10	100 (69–100)			

†PCR corrected.
‡Mean (SD).
§Median (range).
AF: Fosmidomycin + artesunate; F: Fosmidomycin; FC: Fosmidomycin + clindamycin; FCT: Fever clearance time; PCT: Parasite clearance time.

in 39 patients) (34). Several factors could explain these unexpectedly poor findings: (1) the new formulation of Fos, (2) the genetic background of the parasites leading to a prolonged PCT and low efficacy, and (3) age or genetic backgrounds of the study subjects. Unfortunately, no pharmacokinetic analyses were performed in the trial to assess if a change in absorption rate and an accelerated metabolism was present (34).

Table 3. Characteristics of the included trials (cont.).

Study (year)	Study arm	Drug	Dosage per day	Days	Doses	End point	Cured (n)	Total (n)	Cure rate (95% CI)	PCT	FCT	Ref.
Borrmann *et al.* (2004) (cont.)	Arm 5	FC	60 mg/kg + 20 mg/kg	5	2	Day 14	10	10	100 (69–100)	41 (33–49)‡	46‡	
						Day 28	10	10	100 (69–100)			
Lell *et al.* (2003)	Arm 1 (Gab)	F	3600 mg	7	3	Day 7	10	10	100	44 (±18)‡	41 (±25)‡	[19]
						Day 28	7	9	78 (40–97)			
	Arm 2 (Thail.)	F	3600 mg	7	3	Day 7	10	10	100			
						Day 28	2	9	22 (3–60)			
Missinou *et al.* (2002)	Arm 1	F	3600 mg	5	3	Day 14	8	9	89 (52–100)	49 (16)‡	23 (17)‡	[21]
	Arm 2	F	3600 mg	4	3	Day 14	7	8	88 (47–100)	45 (18)‡	15 (23)‡	
	Arm 3	F	3600 mg	3	3	Day 14	6	10	60 (26–88)	50 (20)‡	17 (20)‡	

†PCR corrected.
‡Mean (SD).
§Median (range).
AF: Fosmidomycin + artesunate; F: Fosmidomycin; FC: Fosmidomycin + clindamycin; FCT: Fever clearance time; PCT: Parasite clearance time.

Fosmidomycin-artesunate combinations

Although FC was the most frequently investigated drug combination in clinical trials, Fos has also been combined with other compounds, such as the artemisinin derivatives. For example, artesunate has been evaluated in combination with Fos for its suppressive action on gametocytes and its safety profile (35).

A Phase II study was carried out to evaluate the safety, tolerability and efficacy of successively shortened regimens of artesunate-Fos for the treatment of uncomplicated malaria secondary to infection with *P. falciparum* in 50 children aged 6–12 years from May 2002 until October 2003 in Lambaréné (36). Artesunate and Fos were coadministered orally, using the dosing schedules presented in Table 3. The end points were the day-14 cure ratio, the incidence of serious adverse events after treatment, the incidence of adverse events, PCT, FCT, and PCR-corrected day 28 cure ratios. Table 3 summarizes the results at days 14 and 28 (PCR-corrected). The main conclusion was that the 3-day regimen is highly efficacious with a cure rate of 100% (95%CI: 69–100%) (36).

Piperaquine as a partner for fosmidomycin

Piperaquine is a long-acting 4-aminoquinoline with an acceptable safety profile, tolerability and high efficacy when used in combination with fast-acting drugs such as artesunate and dihydroartemisin (37,38). The combination dihydroartemisinin-piperaquine (DHP) is recommended as a first-line artemisinin combination therapy (ACT) for *P. falciparum* and *P. vivax* infections in Southeast Asia, including areas of multidrug resistance along the Cambodian–Thai border (37).

Based on the success of DHP, it was decided to combine piperaquine with Fos, a combination currently under investigation in a clinical trial in Lambaréné, Gabon. This trial is a Phase IIa proof-of-concept study to explore the efficacy, tolerability and safety of Fos when administered with piperaquine to adults and children (aged 8–14 years) with acute uncomplicated *Plasmodium falciparum* malaria (ClinicalTrials.gov Identifier: NCT02198807). Here, Fos

sodium (30mg/kg) is given twice daily for 3 days, and piperaquine phosphate tablet (16 mg/kg) once daily for 3 days to treat subjects with uncomplicated malaria aged 8–60 years who present with a mono-infection with *P. falciparum* (results pending (G. Mombo-Ngoma, personal communication)) (39).

Pharmacokinetics & pharmacodynamics of fosmidomycin

The pharmacokinetic and pharmacodynamic profiles of Fos monotherapy and combination therapy were assessed only in two trials recruiting adult volunteers in Southeast Asia (40,41). They reported that a steady-state plasma concentration of Fos was reached between the second and third doses. The relationship between plasma drug concentration and therapeutic outcome was established and showed that patients with recrudescence tended towards lower dose-dependent pharmacokinetics parameters (40).

Hundred percent of fosmidomycin is excreted through the kidneys over 96–120 h following dosing (41).

Effect of fosmidomycin on gametocytes

The understanding of the effect of novel antimalarial drugs or drug combinations on sexual malaria parasite stages remains important. Since 2002, gametocytaemia was evaluated in clinical trials of the candidate Fos, either as monotherapy or in combination (e.g., Fosartesunate or FC). In four trials using either Fos alone or FC, an association between treatment with Fos and a high cumulative gametocyte carrier rate of around 70–80% was reported (gametocytes detected during at least one followup visit) (20,21,30,31). Only a few (8 of 44) antimalarial compounds are able to reduce gametocyte counts by at least 50% and their effect on transmission under natural exposure is not known for almost all antimalarial. Nevertheless, the identification of antimalarials that block transmission is an urgent research need (42). Interestingly, one study performed in 2005 by Borrmann and colleagues, where Fos was combined with artesunate, showed a very low cumulative gametocyte carrier ratio (36), possibly due to the partial activity of artesunate against gametocytes. In 2007, Oyakhirome and colleagues compared SP (which increases gametocytaemia) and the FC combination and found similar gametocyte carriage rates on admission (day 0) and during the follow-up period (scheduled on days 7, 14, 21 and 28) in both groups, being 4 and 35% for SP and 2 and 33% for FC, respectively (33).

The effect of Fos on gametocyte development and, more importantly, on ransmission shall be addressed in future trials and will be an important determinant for partner drug selection or even further clinical development.

Safety

In the majority of clinical trials performed first in adults and then in pediatric populations, safety and tolerability of Fos when used alone or in combination were good. The adverse event pattern included gastrointestinal disturbance, commonly during the first week of treatment with abdominal pain, diarrhea, vomiting, loose stools and nausea. Blood parameters such as hemoglobin levels and leucocyte counts did not show any significant changes. Transient increases in transaminase (ALAT) levels up to 195 U/l were reported in two subjects (20).

However, transient hematological changes (neutropenia and decrease of ≥2g/dl in the hemoglobin concentration) were reported by Borrmann and colleagues (31). This led to a reevaluation of previous data to identify potential causes. Indeed, neutropenia had already been reported in patients receiving a 4-day artesunate-Fos regimen in a previous study (36). Similarly, anemia had also been frequently reported despite this complication being a common and often coexisting complication of malaria. Moreover, Lanaspa and colleagues, in 2012, reported some adverse events, namely abnormal WBC counts and anemia thrombocytopenia and elevated liver enzyme observed in children treated with the Fos combination (34).

In terms of safety and adverse events, Oyakhirome and colleagues reported comparably good tolerability between FC and SP, with a tendency to more adverse events (including gastrointestinal symptoms) in the SP group. For example, vomiting was more frequent in the SP group than in the FC group being 26 versus 2% ($p = 0.002$). All adverse events, including one febrile convulsion (uncharacterized by the authors) on day 1 following FC, resolved spontaneously (33).

Meta-analysis

Given the partially conflicting results and the fact that individual patient data for most studies were available, we performed a meta-analysis. The Supplementary Table highlights the heterogeneity of study end points across the clinical trials.

Across the pediatric trials, the overall estimation of the cure rate on day 28 was 85% (95%CI: 71–98%) (Figure 3A). We postulated here that an optimal pediatric dosage could be calculated by using the estimated body surface or the age. When analyzing the age-versus-cure rate efficacy (Figure 4), a positive relationship does appear between both the age and the efficacy, with correlation coefficient r close to one and significantly different from zero ($r = 0.94$ (0.55–1); $p = 0.005$).

The youngest children were in the Mozambican cohort (mean age was 23 months with minimum of 6 months and maximum of 35 months); by contrast, the mean age in the Gabonese cohort was 101 months (about 8.4 years). All Mozambican children were below the first quartile of age of the Gabonese children (25 months) (Figure 5A). Interestingly, we observed at both sites a trend toward higher efficacy in the older age groups, not reaching statistical significance (HR: 0.96 (0.8–1.1); $p = 0.55$) (Figure 5B). As the association between age and efficacy is not stable when analyzing single cohorts or studies, we suggest that other variables are more important predictors of efficacy (e.g., the drug formulation). Unfortunately, no pharmacokinetic data are available to test if age or Fos formulation determined outcome.

The weighted mean of the pooled PCT was 39 h across the six studies involving African children (Figure 6A).

The mean FCT reported in three studies was 30 h but with a wide confidence interval between 0 and 66 h (Figure 6B).

We found that the overall estimate of the global cure rate on day 28, across all studies in which Fos has been combined with clindamycin to treat uncomplicated malaria in African and Asian adults, was 70% (95% CI: 40–100%) (Figure 3B).

In adults, the mean PCT and the mean FCT were 49 h (Figure 7A) and 42 h (Figure 7B), respectively.

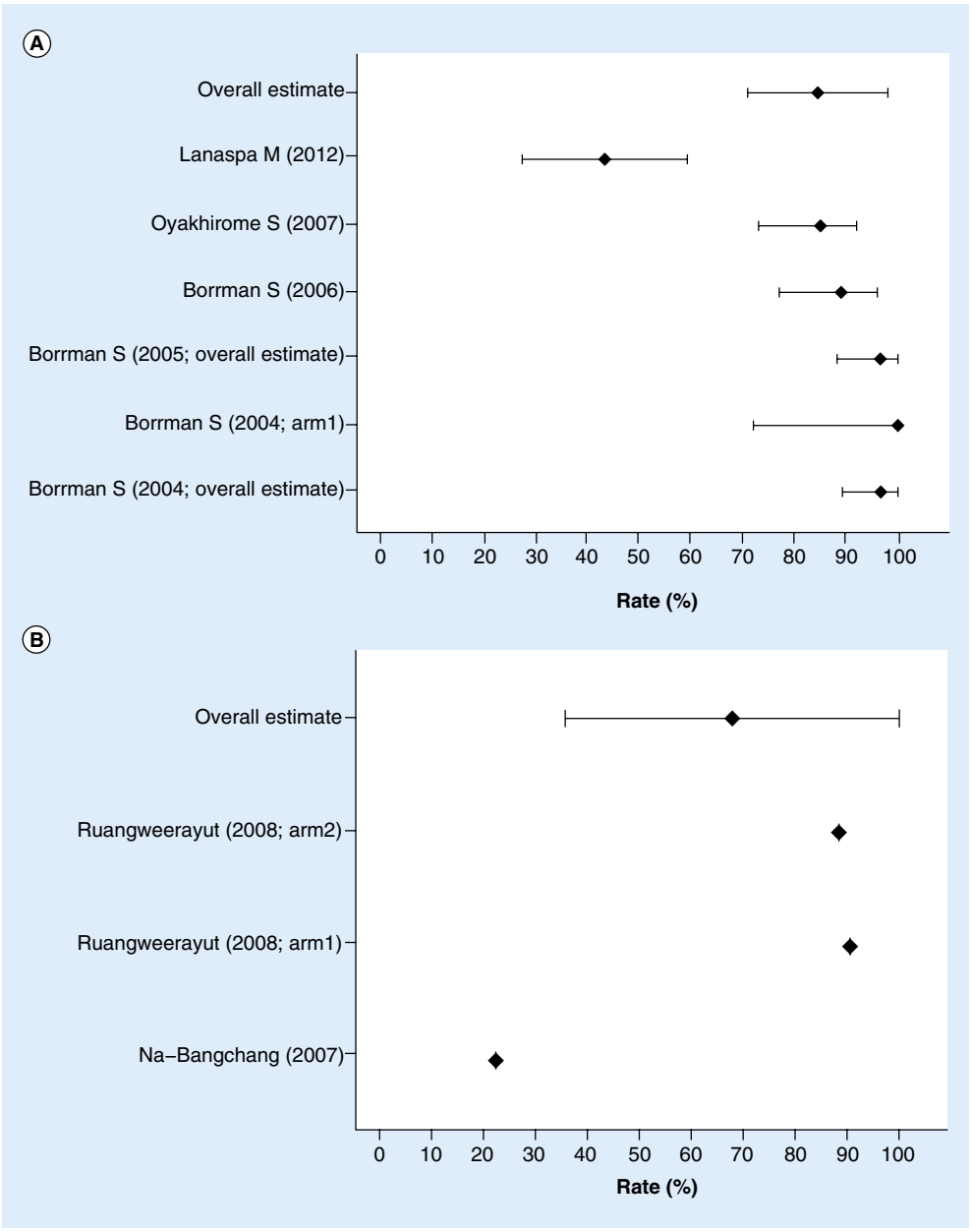

Figure 3. Cure rate of fosmidomycin combinations. **(A)** Overall estimation of the global cure rate on day 28 of fosmidomycin in combination in pediatric trials. **(B)** Overall estimation of the global cure rate on day 28 of fosmidomycin in combination in adult trials.

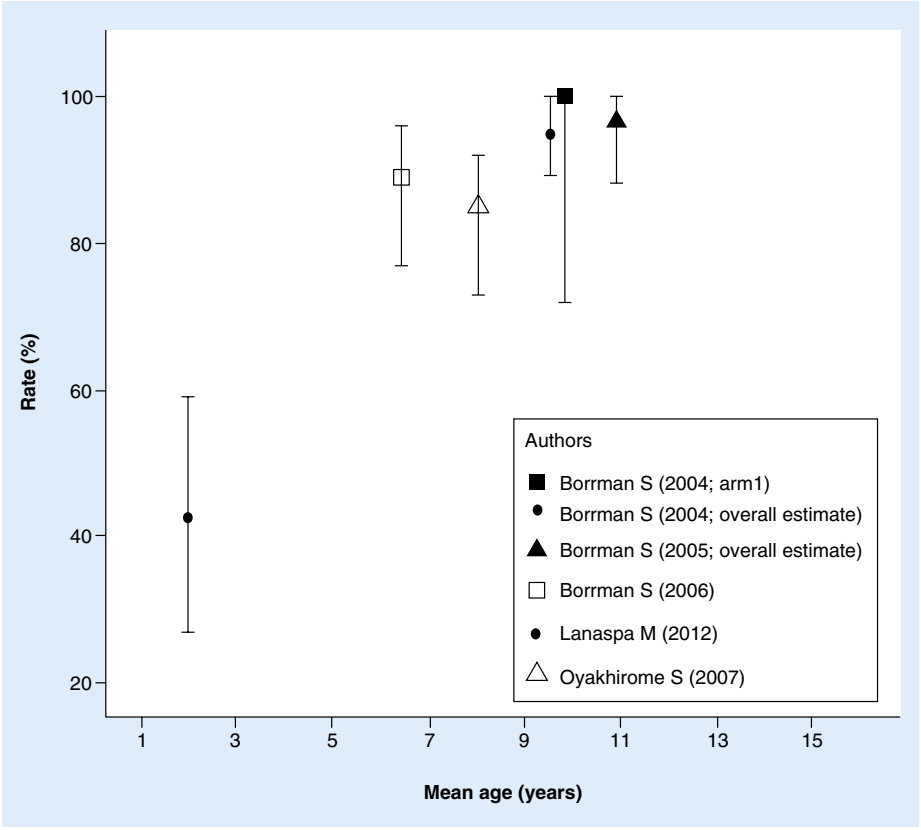

Figure 4. Scatter plot for age and cure rate efficacy.

DISCUSSION & CONCLUSION

This review presents the available data on treatment of uncomplicated malaria with Fos in adult and children since 2004. Interestingly, efficacy results are not homogeneous among the studies. Most pediatric studies have demonstrated satisfactory results with cure rates at day 28 consistently above 85–90% and good tolerability when Fos is coadministered with clindamycin for the treatment of uncomplicated malaria (20,21,26). However, the most recent Mozambican study, done in 2012, found a low cure rate on day 28 associated with an increased PCT and other laboratory changes that may be due to prolonged parasite exposure. There is no uniform and definitive explanation for these discrepancies.

First, the study populations in Gabon and Mozambique differed in the age range of participants. All Mozambican subjects were below the first quartile of age of the Gabonese study subjects. A possible explanation is that the older children already developed partial immunity, which enhances the effect of antimalarial drugs (34). Alternatively, younger children may differ in pharmacokinetic characteristics (lower absorption, accelerated metabolic rate) compared with older children and adults, which could adversely affect the pharmacokinetics and efficacy (43).

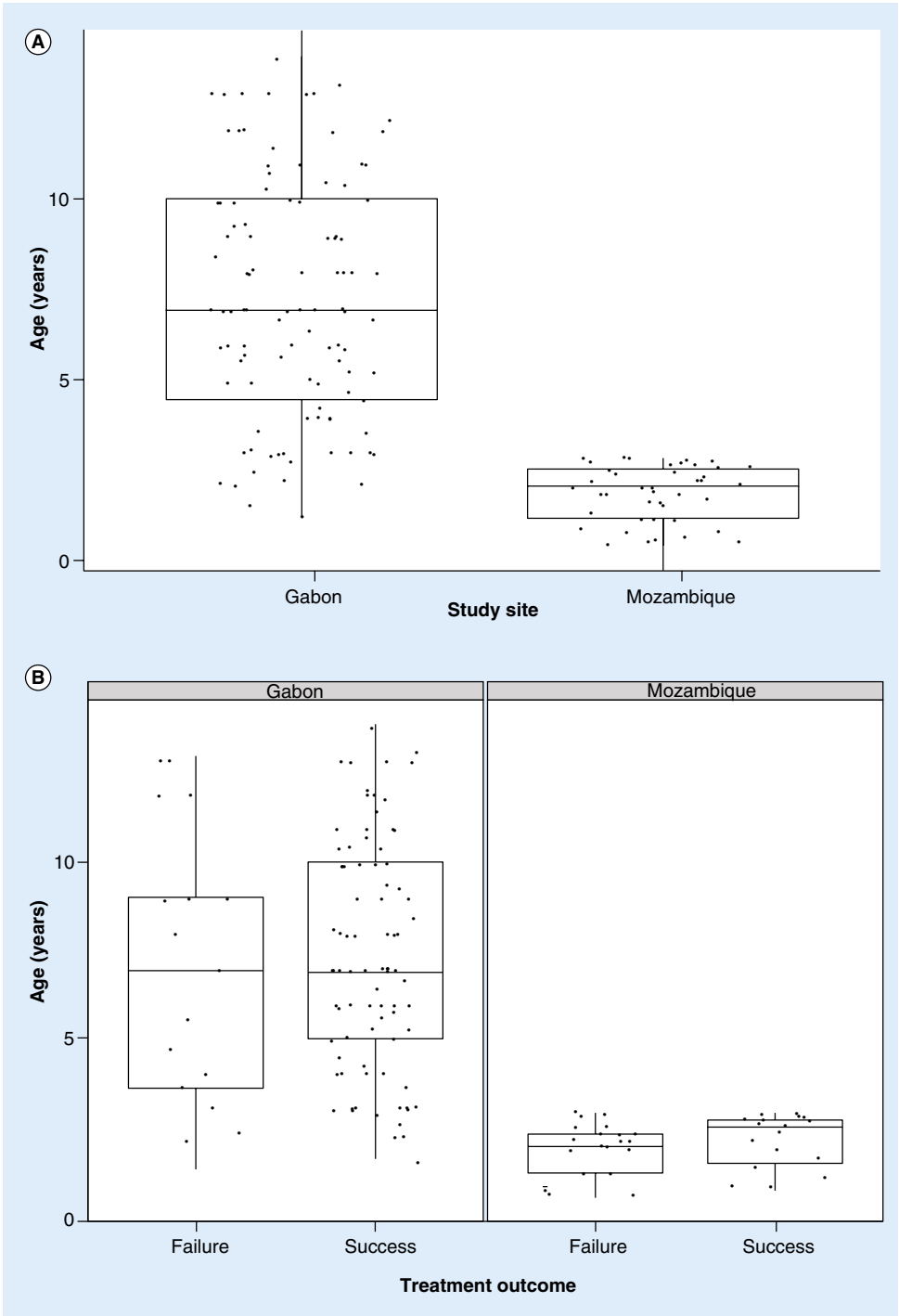

Figure 5. Study population age and treatment outcome. **(A)** Age of the study population. **(B)** Age versus treatment outcome across the two study sites.

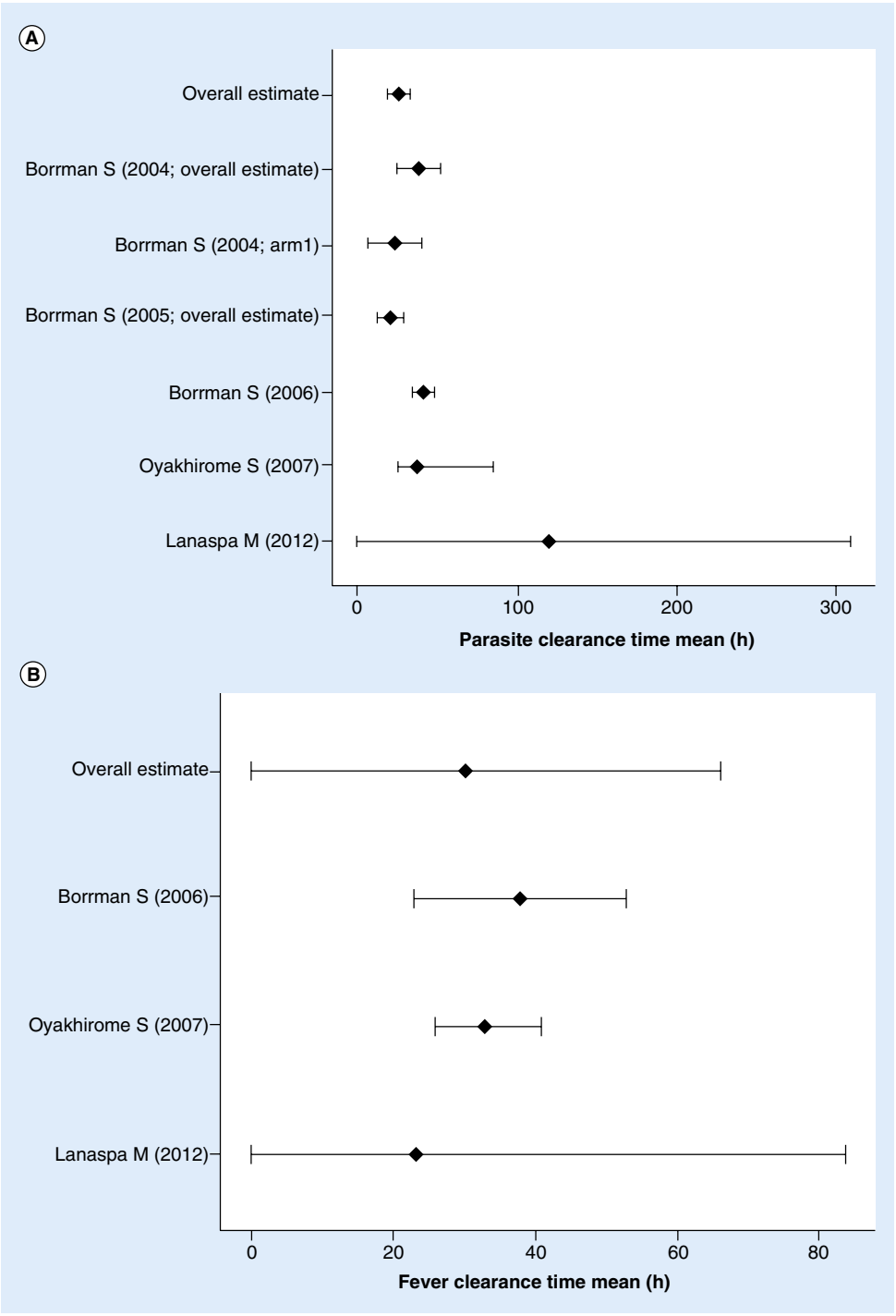

Figure 6. Parasite and fever clearance times - pediatric trials. **(A)** Overall parasite clearance time (pediatric trials). **(B)** Overall fever clearance time (pediatric trials).

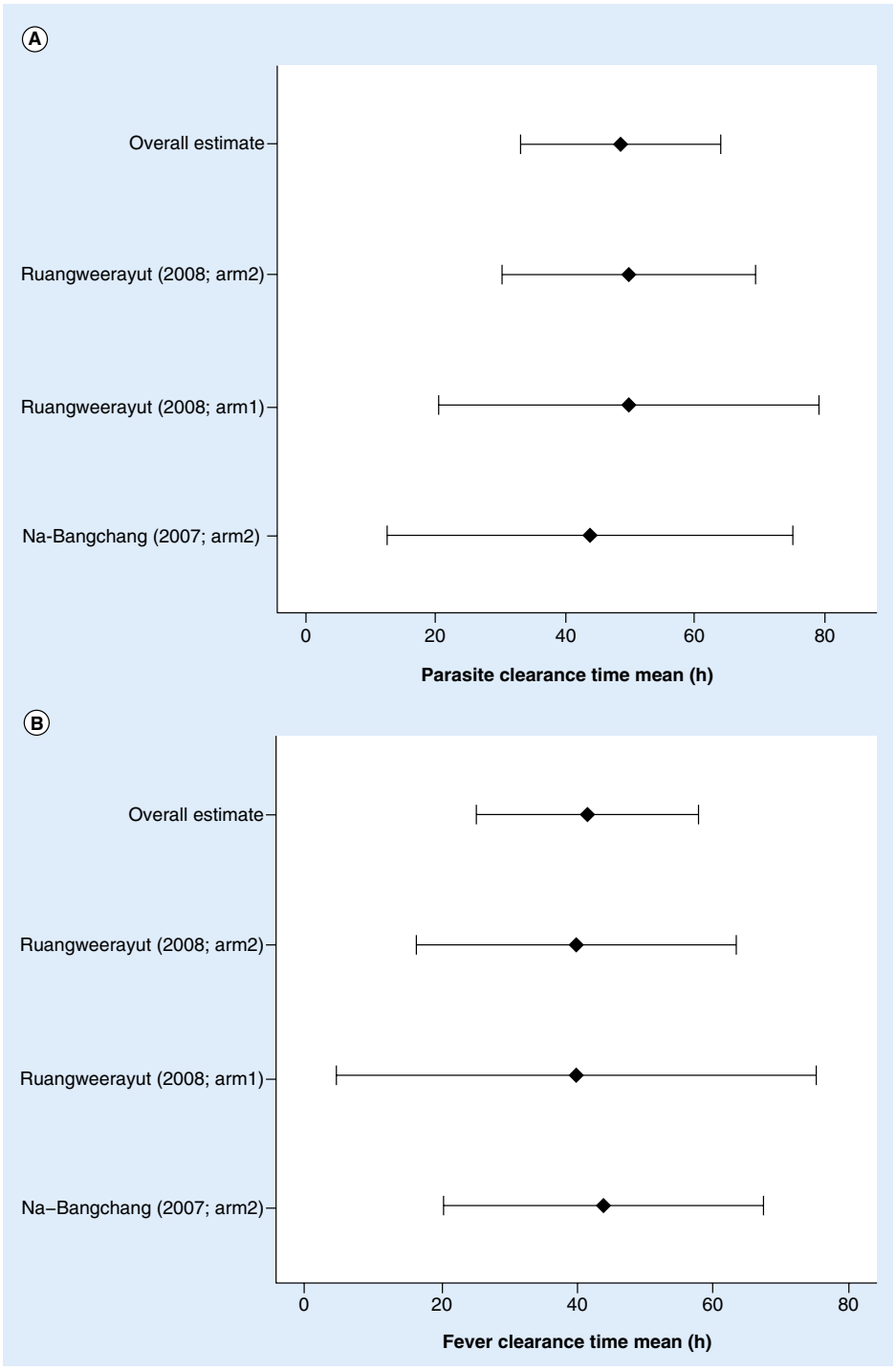

Figure 7. Parasite and fever clearance times – adult trials. (**A**) Overall parasite clearance time (adult trials). (**B**) Overall fever clearance time (adult trials).

Second, the drug formulations used were different between the studies. Capsules or crushed tablets containing the investigational drug were given in all studies in Gabon, whereas aqueous solutions reconstituted from water-soluble granules of the drugs were given in Mozambique. Indeed, reconstituted solutions requiring high volumes to reach the necessary doses of each of the two drugs in the combination (in some cases, up to 20 ml) may explain problems with tolerability, such as vomiting (34). Pharmacokinetic and pharmacodynamic studies must be conducted in this vulnerable population in order to shed light on these substantial knowledge gaps.

Our results show that both age and drug formulation may play a role in the differences observed between Gabon and Mozambique. The question to be answered is whether or not other factors, such as antimalarial pharmacokinetics, parasite polymorphisms conferring resistance, host defense and transmission intensity, have an impact on the difference in cure rates observed between the recent Mozambican trial and the five earlier studies conducted in Gabon before.

The values of mean PCT and mean FCT in pediatric populations versus adults, 39 and 30 h versus 49 and 42 h, are comparable to other registered antimalarial drugs and motivate further explorations in lack of better nonartemisinin combination therapies (21).

Results obtained after pooling the studies in adults show a low cure rate of 70%. These results could be due do the fact that data from regimen-optimizing studies were included in the analysis. Furthermore, participants come from two different regions, Gabon and Thailand, with potential differences in immunity between a population from malaria hyperendemic area (central Africa) and another from a hypoendemic area (Asia).

In addition, pharmacokinetic/pharmacodynamic differences may explain part of the variability between the trials since they are influenced by several extrinsic factors such as socioeconomic background, culture, diet and environment (41).

Moreover, those pooled cure rates should be considered with caution. PCT and FCT were 49 and 42 h, respectively, which appear to be longer than those found in pediatric cohorts with the same combination. However, this is in accordance with those of antibacterial drugs with antimalarial activity (e.g., doxycycline, clindamycin and tetracycline) (19).

Regarding the safety and tolerability profile, FC was shown to be safe and well tolerated, although definite conclusions are not possible at this stage of clinical development. Careful monitoring for safety signs shall continue; in particular, because some studies showed significant alterations in hematological parameters.

Approaches to improve the on-target concentration of Fos (10,15) and to identify novel, more potent DOXP inhibitors (44) are under active development and worth a consideration as novel development candidates. Future development needs to integrate recent data such as the association with partners other than clindamycin (e.g., piperaquine; ClinicalTrials.gov identifiers: NCT01464125, NCT02198807).

Based on the results obtained so far, it is not possible to form a definitive conclusion about whether or not this compound should be removed from the pipeline of development of antimalarial drugs. Further well-designed clinical trials that include pharmacokinetic investigations in different relevant populations are needed to inform a timely decision on whether to continue or discontinue the development of Fos combinations as future antimalarials.

FUTURE PERSPECTIVE

A part from rare exceptions, the development of new parasitic drugs in general, and especially that of antimalarials is a lengthy process, and its market appears to have little appeal for the pharmaceutical industry. Indeed, few advances have been made during the past few decades in terms of novel antimalarial development, although with some highly promising exceptions (e.g., the synthetic peroxide OZ439 and the spiroindolone KAE 609).

Innovative combinations of Fos with effective partner drugs could help diversifying the limited spectrum of available antimalarial drugs and should play a role in the short- and middle-term fight against malaria.

Further clinical trials (including PK, evaluation of effect on gametocytes and drug delivery vehicles as partners) are needed to aid the final decision on the future of Fos combinations as future antimalarials.

EXECUTIVE SUMMARY

Fosmidomycin monotherapy

› Fos monotherapy cannot be recommended.

Fosmidomycin-clindamycin combinations

› Combination of antimalarials enhances efficacy, increases effectiveness and prevents resistance development.

› Fos was combined with clindamycin due to their good synergistic profile in Phase II pediatric trials in African children.

Fosmidomycin-artesunate combinations

› Artesunate has been tested in combination with Fos and showed encouraging results of the 3-day regimen.

Piperaquine as a partner for fosmidomycin

› The combination Fos plus piperaquine is currently under investigation in a clinical trial in Gabon.

Fosmidomycin & gametocytes

› Fos-artesunate is not efficient against mature gametocytes.

› Its direct role or effect in transmission is unclear.

Safety

› Fos is safe and well tolerated, although hematologic adverse event occurred and shall be monitored.

Meta-analysis

- The overall estimation of the cure rate on day 28 of Fos was 85 and 70% in pediatric and adult population, respectively.
- Age and drug formulation may play a role in the differences of efficacy observed between trials in Gabon and Mozambique.
- The overall mean of the parasite clearance time in both adult and pediatric cohorts was 39 and 49 h, respectively.
- The overall mean fever clearance time in both adult and pediatric cohorts was 30 and 42 h, respectively.

Conclusions

- Despite the efforts during the two last decades, Fos is still in the pipeline of development as an antimalarial drug.
- The low cure rate on day 28 obtained in Mozambique explains the current hold in development of a Fos-clin combination.
- The combination Fos (and clindamycin) is still worth consideration as a potential antimalarial.

Future perspective

- Further clinical trials (including PK, evaluation of effect on gametocytes and drug delivery vehicles as partner) are needed to aid the final decision on the future of Fos combinations as future antimalarials

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CLINICAL DEVELOPMENT OF RTS,S/AS MALARIA VACCINE: A SYSTEMATIC REVIEW OF CLINICAL PHASE I-III TRIALS

4

ABSTRACT

The first clinical Phase III trial evaluating a malaria vaccine was completed in December 2013 at 11 sites from seven sub-Saharan African countries. This systematic review assesses data of Phase I–III trials including malaria-naive adults and adults, children and infants from malaria endemic settings in sub-Saharan Africa. The main endpoint of this systematic review was an analysis of the consistency of efficacy and immunogenicity data from respective Phase I–III trials. In addition, safety data from a pooled analysis of RTS/AS Phase II trials and RTS,S/AS01 Phase III trial were reviewed. The RTS,S/AS01 malaria vaccine may become available on the market in the coming year. If so, further strategies should address challenges on how to optimize vaccine efficacy and implementation of RTS,S/AS01 vaccine within the framework of established malaria control measures.

KEYWORDS

› anti-CSP
› anti-HBs
› AS01
› AS02
› geometric mean concentration
› immune responses
› RTS,S/AS
› vaccine efficacy

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INTRODUCTION

Malaria morbidity & mortality

The WHO estimates the global incidence of malaria during the past 5 years at around 200 million cases annually, resulting in about 600,000 deaths per year directly attributable to malaria (1–5). Eighty percent of total cases and 90% of deaths afflict sub-Saharan African populations. Young children, pregnant women and their offspring, and the poorest and most vulnerable communities are the most susceptible to malaria disease and deaths (5).

Overview of malaria control strategies & interventions

Insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) are currently recommended and established tools to reduce transmission of Plasmodium spp between the *anopheles* mosquito vector and human beings. Blood stage infections are prevented in pregnancy by administration of sulfadoxine-pyrimethamine (SP) during the second and third trimester of pregnancy. Amodiaquine plus SP (AQ+SP) is administered preventively to children living in areas with seasonal malaria transmission of Africa's Sahel region. Finally, prompt diagnosis by microscopy or rapid diagnostic test (RDT) and timely treatment with artemisinin-based combination therapy (ACT) of uncomplicated malaria cases is the third pillar of established malaria control tools. Altogether, existing malaria interventions are effective tools for the prevention and cure of malaria, however their consequent and universal implementation was often lacking. Their better deployment and coverage in endemic areas as consequences of increased commitment in fundraising in the last decade has significantly contributed to recent reductions in malaria incidence and mortality globally. These reductions have occurred to a degree, that even elimination and eradication as long-term goals are considered to become possible according to the WHO's vision of a 'world free from malaria' (5). For instance, in sub-Saharan Africa, malaria incidence decreased by 26% and malaria mortality rate dropped by 47% between 2000 and 2013 (5). However, these substantial gains in the battle against malaria should not hide the huge gaps remaining in the distribution of and access to proper malaria interventions, particularly in sub-Saharan Africa. Here, a third of the population at risk does not benefit from any preventive measure and in 2013, up to 87% of children experiencing malaria did not receive an ACTv (5). Thus, heterogeneity in the coverage within and between sub-Saharan Africa settings, as well as drug and insecticide resistance, pose great challenges for the implementation and access of the above sets of interventions. Hence, there is a compelling rationale that additional tools would improve the effectiveness of the current armamentarium of interventions. Among prospective malaria control tools, a malaria vaccine is arguably the most desirable beyond improved malaria diagnostic tools, drug and insecticide resistance monitoring tools and new nonartemisinin-based combinations.

Overview of malaria vaccine development rationale & strategies

In 2006, a malaria vaccine was identified as an urgent medical need to reduce the gaps left by other interventions (Malaria Vaccine Technology Roadmap 2006). Malaria morbidity and mortality have significantly reduced in many settings since 2006, due to the scaling-up of existing malaria

control measures. Nevertheless, a malaria vaccine remains an unmet medical need; the value of which could add to the existing interventions for prevention of malaria disease and deaths, and combined with goals of malaria elimination and – ultimately – global eradication (Malaria Vaccine Technology Roadmap 2013).

About 20 malaria vaccine candidates are under clinical development targeting either pre-erythrocytic stages or the blood stage of the parasite life cycle (6). Two candidates of sexual stage (transmission blocking vaccines) have reached early clinical stage development and a candidate targeting Plasmodium vivax is being evaluated in a Phase I trial (6).

Out of all potential candidates, RTS,S/AS01 is 5–10 years ahead in its clinical development compared with all other vaccine candidates. It has been successfully evaluated in Phase III and is currently under scientific and regulatory evaluation by the European Medicines Agency (EMA).

To summarize all currently available evidence, we have systematically reviewed data gathered in the clinical development of RTS,S/AS clinical trials (Phase I–III) to evaluate and put into perspective the risk-benefit assessment of RTS,S as a tool for future malaria control.

RT S, S/AS vaccine

RTS is a single polypeptide corresponding to amino acids 207–395 containing the tandemrepeats of B-cell epitopes $(NANP)_{19}$; specific to *Plasmodium falciparum* species (strain NF54, laboratory clone 3D7 is used for RT) and several T-cell epitopes from conserved region III, and from α-TSR, a sequence homologous to the thrombospondin type-1 repeat superfamily. The CSP construct (RT) is fused to the N-terminal region of hepatitis B virus surface antigen of 226 amino acids (S). The RTS and unfused S polypeptides (RTS,S) are co-expressed in yeasT cells and transform into virus-like particles (VLPs) spontaneously (7–9). The RTS,S antigen has been formulated with AS04, AS03, AS02 and AS01 adjuvants during clinical development. AS02 and AS01 are combinations of -deacylated monophosphoryl lipid A (MPL), a substantially detoxified derivative of LPS and QS-21 Stimulon® (*Quillaja Saponaria* Molina, fraction 21; Licensed by GSK from Antigenics, Inc., a wholly owned subsidiary of Agenus, Inc., MA, USA), a purified saponin from the bark of the South American tree *Quillaja saponaria* Molina, formulated either as oil-in-water (AS02) or as liposome (AS01). MPL is a Toll-like receptor 4 (TLR4) agonist which activates Th1 polarized-type responses while QS21 enhances antigen uptake and prolonged antigen retention by antigen presenting cells. It also encourages activation of dendritic cells and favors MHC class I presentation to induce both T-cell responses (10).

The repetitive and particulate structure of the RTS,S antigen formulated with combinations of adjuvants that activate multiple innate stimuli contribute to enhanced vaccine presentation to the vaccinees' immune system and induce strong antibody and T-cell responses (7).

The vaccine antigen is a lyophilized pellet containing 25 µg of RTS,S polypeptide in a single-dose vial. The final product is reconstituted with a colorless adjuvant liquid to a volume of 0.5 ml (11). Table 1 shows trials with their objectives, study design and key results.

Table 1. RTS,S/AS Phase I–III trials with their objectives, study design and key results.

Study number (e-track number)	Objectives	Study design, country, years	Key findings	Ref.
	Clinical evaluation of RTS,S formulated with MPL: safety, immunogenicity, concept of efficacy	Open label, Phase IA human challenge USA, 1994–1995	Proof of sterile protection High anti-CSP antibody concentration	[9]
WRMAL-003 (WRAIR 544)	Clinical evaluation of AS02A Clinical evaluation of AS04, AS03: safety, immunogenicity, concept of efficacy	Randomized open label Phase I–IIA human challenge 1996–1997	High rate of protection induced by RTS,S/AS02	[12]
WRMAL-004 (A-7492)	Dose finding and optimization of schedule of RTS,S/AS02A safety, immunogenicity and concept of efficacy Mechanisms underlying RTS,S/AS02 protection	Phase I human challenge study, USA 1998–2001	Three doses of RTS,S/AS02 are protective In addition, RTS,S/AS02 vaccinees harbor opsonizing CSP Abs with endocytic activity	[13] [14]
Malaria-002 (257049–002)	Clinical evaluation of RTS,S/AS02A: humoral and cell-mediated immune responses	Randomized, open-label Phase IIA trial, Belgium 1998–1999	RTS,S/AS02 indicates Th1-type immune responses	[15]
Malaria-004 (257049–004)	Clinical evaluation of RTS,S/AS02A: safety, immunogenicity of RTS,S/AS02 in adults living in a malaria endemic country	Randomized open-label Phase IB, Gambia 1997–1999	RTS,S/AS02 showed to be safe and immunogenic in adults living in a malaria endemic community	[16]
Malaria-005 (257049–005) Extended to first, second and third years: malaria 016, 017 and 018, respectively	Efficacy, safety and immunogenicity of RTS,S/AS02A in semi-immune adults Effect of a booster dose	Randomized double-blind controlled Phase IIB trial, Gambia 1998–2003	RTS,S/AS02 showed to be efficacious in adults living in a malaria endemic country In addition, the RTS,S/AS02-induced protection goes beyond the 3D7 pfcsp sequences used in the vaccines construct	[17] [18]
WRMAL-005 (A-8420)	Clinical evaluation lyophilized formulation of RTS,S/AS02A: safety, immunogenicity and efficacy	Randomized open Phase I–IIA trial, USA 1999–2001	Lyophilized formulation is stable, safe, immunogenic and confers similar level of efficacy than liquid form	[11]
Malaria-008 (257049–008)	Clinical evaluation of RTS,S/AS02A in a malaria hyperendemic area: safety, immunogenicity	Open label Phase IB trial, Kenya 1998–1999	RTS,S/AS02 remains safe and similarly immunogenic in a setting with high malaria transmission intensity	[19]
Malaria-015 (257049–015) and Malaria-020 (257049–020) Participants of Malaria 015 and 020 were followed-up for 1 year through Malaria 022 and 023, respectively	Safety and immunogenicity of RTS,S/AS02A in children 6–11 years living in a malaria endemic country Dose optimization Safety and immunogenicity of RTS,S/AS02A in children 1–5 years living in a malaria endemic country	Randomized controlled, double-blind Phase IIB trial, Gambia 2001–2003	RTS,S/AS02 is safe and immunogenic in Gambian children at 25 μg	[20]
Malaria-025 (257049–26)	Safety and immunogenicity of RTS,S/AS02A in children living in a malaria endemic area Dose (25 μg) and schedule (0, 1, 2)	Randomized controlled, double blind Phase IIB trial Mozambique 2002–2003	RTS,S/AS02 is safe and immunogenic at 25 μg and following 0, 1 and 2 months vaccination schedule	[21]
Malaria 0–26 (257049–26) Extended for 2 years as Malaria 039 (104297)	Clinical evaluation of RTS,S/AS02 for efficacy in children	Randomized controlled, double-blind Phase IIB study in children 1–4 years Mozambique, 2003–2012 (extensions)	Proof of concept of RTS,S/AS efficacy against clinical and severe malaria in African children and evidence of sustained efficacy as well as maintained anti-CSP antibody concentration	[22–25]

Table 1. RTS,S/AS Phase I–III trials with their objectives, study design and key results (cont.).

Study number (e-track number)	Objectives	Study design, country, years	Key findings	Ref.
Malaria-027 (257049–027)	First clinical evaluation of AS01B: safety, immunogenicity and concept of efficacy	Randomized double-blind Phase IA–IIA trial with Human challenge; USA, 2003–2005	RTS,S/AS01 seems to be more efficacious and immunogenic compared with RTS,S/AS02 IL-2 induced by RTS,S/AS vaccine may play as a growth factor for follicular Th cells and/or B cells	[26] [27]
Malaria-034 (257049–034)	Safety and immunogenicity of RTS,S/AS02D, a pediatric expanded immunization compatible formulation in children living in a malaria endemic area Dose (25 μg in 0.5 ml) and schedule (0, 1, 2)	Randomized controlled, double-blind Phase IIB trial Mozambique 2004–2005	RTS,S/AS02D is safe and immunogenic as RTS,S/AS02A	[28]
Malaria-038 (103967)	First clinical evaluation in infants: safety, immunogenicity and efficacy of RTS,S/AS02D when given with the other EPI vaccines in infants living in a malaria endemic area	Randomized controlled, double-blind Phase I–IIB trial Mozambique 2005–2007	RTS,S/AS02D is safe, immunogenic and prevents clinical malaria in Mozambican infants when given with other EPI vaccines	[29]
Malaria-044 (257049–044)	Clinical evaluation of both RTS,S/AS02A and RTS,S/AS01B in settings of high malaria transmission: safety, immunogenicity and efficacy	Randomized double-blind Phase IIB trial Kenya, 2005–2006	RTS,S/AS01B is safe and efficacious as RTS,S/AS02A and showed increased anti-CSP antibody concentration RTS,S/AS vaccines protects also against pfcsp alleles beyond the 3D7 pfcsp sequences used in the vaccines construct in high transmission setting	[30] [31]
Malaria-040 (104298)	Safety, immunogenicity and efficacy of RTS,S/AS02D when given with the other EPI vaccines in infants living in a malaria endemic area	Randomized controlled, double-blind Phase IIB trial Tanzania 2006–2008	RTS,S/AS02D is safe, immunogenic and prevents malaria infection in Tanzanian infants when given with other EPI vaccines	[32]
Malaria-046 (105874)	First clinical evaluation of RTS,S/AS01E, a pediatric formulation compatible with EPI standard in African children compared with RTS,S/AS02D	Randomized controlled, double-blind Phase IIB study in children, Gabon 2006–2007	RTS,S/AS01 is safe and more immunogenic than RTS,S/AS02 in Gabonese children RTS,S/AS induces CSP-specific memory B-cell and anti-CSP-specific antibodies and memory B cells persist up to 12 months after third vaccination	[33] [34]
Malaria-047 (106367)	Safety and immunogenicity of RTS,S/AS02D and RTS,S/AS01E in Ghanaian children	Randomized controlled, Phase IIB study in children, Ghana 2006–2008	Both vaccines are safe. RTS,S/AS01E showed improved humoral and cellular immune responses	[35]
	Optimization of schedule (0,1 vs 0, 1, 2vs (0, 1, 2)		The schedule 0, 1, 2 also showed improved immune responses	[36]
Malaria-049 (106464)	Efficacy, safety and immunogenicity of RTS,S/AS01E in Tanzanian and Kenyan children	Randomized controlled, double-blind Phase IIB study in children, Tanzania and Kenya 2007–2008	RTS,S/AS01 showed efficacy against clinical malaria over 18 months after vaccination	[37,38]
Malaria-050 (106369)	First clinical evaluation of RTS,S/AS01E, given with EPI vaccine in African children compared: safety, immunogenicity and efficacy Schedule optimization (0, 1, 2 vs 0, 1, 7)	Randomized controlled, double-blind Phase IIB study in infants. Gabon, Ghana, Tanzania 2007–2009	RTS,S/AS01 is compatible with co-administration with other EPI vaccine The schedule 0, 1, 2 showed better immunogenicity and efficacy against clinical malaria	[39] [40]
Malaria-055	First malaria vaccine Phase III trial: efficacy of RTS,S/AS01E in African children and infants (co-administration with EPI vaccines)	Randomized controlled, double-blind Phase III trial in African children and infants Burkina Faso, Gabon, Ghana, Kenya, Tanzania, Malawi, Mozambique, 2009–2014	RTS,S/AS01E is efficacious against clinical malaria The vaccine efficacy lasts up to 48 months after primary vaccination but declines over time There is incremental vaccine efficacy conferred by a boosted dose given 18 months after three doses RTS,S/AS01E has the potential to avert millions of cases of malaria	(CTPC 2011) (CTPC 2012) (CTPC 2014) (CTPC 2015)

METHODS

Data sources

A systematic literature search was performed using terms: malaria vaccine, clinical trials, RTS,S and Phase I, II, III trials in Medline-PubMed, and Embase.

Study selection & data extraction

Only RTS,S Phase I–III clinical trials reporting safety, immunogenicity and/or efficacy were included. Figure 1 shows diagram flow of articles selection.

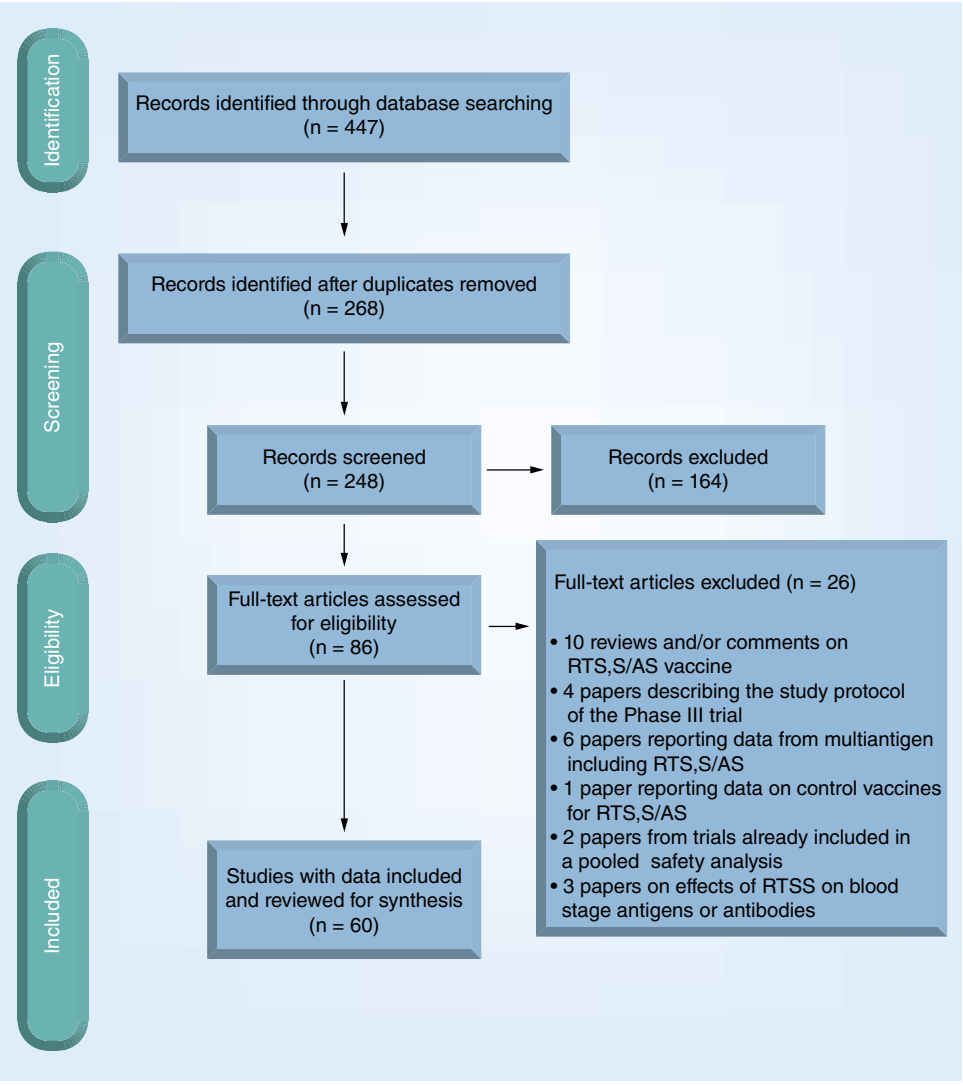

Figure 1. Flow of articles searched, selected and reviewed.

RT S,S/AS-induced immune responses

Characteristics of RT S,S/AS-specific anti-CSP & RT S,S/AS-specific anti-HB antibody responses

A single dose of 25 µg of RTS,S/AS induced seroconversion and up to 100% of recipients after having received three doses seroconvert to the epitopes of tandem repeats of CSP (9,12–13). Anti-CSP antibody concentrations vary with adjuvant components (Alum < Alum +MPL < MPL +QS21) and formulations (MPL+QS21 < MPL+QS21 in oil-in-water (AS02) < MPL+QS21 in liposome (AS01)). The highest concentrations of anti-CSP antibodies are induced by RTS,S/AS01 (9,12–13,26,30,33,35,41). Vaccination with three consecutive doses of RTS,S/AS02 or RTS,S/AS01 scheduled at 21-to 42-day intervals induces the highest peak of anti-CSP antibodies, especially in children (35) and infants (39) There is no clear age-dependent pattern of anti-CSP antibody concentration, however, children aged 5 to 17 months have higher concentrations compared with adults, children of 6–11 years and infants (20,42). Individuals living in malaria endemic areas produce higher concentration of anti-CSP antibodies following vaccination with RTS,S/AS compared with naive individuals, although important interindividual variation irrespective of being malaria naive, malaria exposed, or semi-immune exists (9). A high rate of seroconversion to CSP repeat tandem epitopes occurs upon co-administration of RTS,S/AS with other childhood vaccines in the Expanded Program of Immunization (EPI) (29,32,39). The kinetic of anti-CSP antibodies occurrence is triphasic: a peak is mounted after the primary course (three doses) of vaccination, followed by a rapid and significant waning of antibody concentration. The last Phase Is characterized by a persistence of detectable antibody concentration (17,20,22,28,43). The latter is at significantly higher levels compared with prevaccination concentrations in vaccine recipients and to pre-existing anti-CSP antibody concentration in semi-immune adults living in endemic areas (16,19). These remain at a significantly higher level for up to 4 years compared with prevaccination (23,44–45).

A booster dose of RTS,S/AS given at 12 months (43) and 18 months (46) after the third dose, induces a significant rise of anti-CSP antibody concentration, although the levels is below the peak as after dose 3 in children and infants (46). Despite anti-antibody concentration were measured in several laboratories and expressed with various units, comparison between groups and states remain consistent across studies.

The Avidity Index (AI), a surrogate of affinity function, evaluated for anti-CSP antibodies in cohorts of infants vaccinated with RTS,S/AS01 showed absolute values between 34.9 and 49.3 (ratio of the concentration of anti-CSP IgG (EU/ml) bound to the coated antigen after treatment with NH4SCN, divided by the concentration of IgG (EU/ml) (47,48) that remained bound to the coated antigen in the untreated plate). The AI increased between the second and third dose of vaccination (47,48).

RTS,S/AS-specific-anti-HBs antibody concentration reach a peak after a single dose (41) or after a continuing increase following each dose of vaccination. After a peak, the pattern of RTS,S/AS-specific anti-HBsAg antibody concentration follows a continuous decrease over time. However, at least 90% of vaccinees harbor seroprotective levels of anti-HB antibodies up to 42 months after the last dose of RTS,S/AS (12–13,16,19,23–24,26,30,33,35,39). The geometric mean of anti-HB antibody concentration following a booster dose increases above the peak obtained

after the primary vaccination course (43). RTS,S/AS01 recipients showed higher anti-HB antibody titers compared with RTS,S/AS02 vaccinees (26,33). RTS,S/AS recipients have higher anti-HB antibody concentration compared with the commercially available and registered hepatitis B vaccine (Engerix B™, GSK) (21–22,29,32,49) although the rate of seroprotected vaccinees is similar for the two vaccines up to 42 months after vaccination (23–24,50).

Interactions between pre-existing hepatitis B vaccination & RT S,S/AS specificanti-CSP antibody

Previous hepatitis B vaccination or level of anti-HB antibody concentration shows different effects on the concentration of anti-CSP antibodies. No correlation was found in malarianaive populations, although the rate of people who reached seroconversion titers of anti-HB antibodies at baseline varies substantially across studies (9,13). Increased concentration of anti-CSP antibodies were found in Gabonese children previously vaccinated against hepatitis B (33), and in a pooled analysis of Phase II trials data (51). However, this was not confirmed in Phase III (42).

CSP-specific cell-mediated immunity induced by RT S,S/AS

RTS,S formulated with alumn and MPL or MPL plus QS21 adjuvants, induced CSP-specific T-cell immune responses in volunteers including peripheral blood mononuclear cell proliferation (9,12,17,52). CD4+ T cells predominantly proliferate in response to RTS,S *in vitro* stimulation (15,44). RTS,S and RTS,S-based peptides induce Th1-type immune responses including IFN-γ (12,17,52) and IL-2 (53,54) cytokines as well as CSP-specific IFN-γ CD4+ T cells (15,55–56) and TNF-α CD4+ T cells (36,53).

Compared with RTS,S/AS02, RTS,S/AS01 generates a higher concentration of IFN-γ (26), more frequently IL-2 CD4+ T cells, TNF-α CD4+ T cells (36) and CSP-specific CD4+ T cells expressing at least two markers including IFN-γ, TNF-α, IL-2 and D40L (26).

IL-2 CD4+ T cells are the most sensitive marker of RTS,S/AS-induced T-cell responses both in malaria-naive populations (26,27), as well as in those living in endemic regions (34,36,53–54,57). RTS,S/AS induces CSP-specific IFN-γ secreting memory T cells (15,26,52,55) and RTS,S/AS-induced T-cell responses are persistent for up to 12 months after primary vaccination (15,36,53). RTS,S/AS-induced anti-HB T-cell immune responses are higher than the commercially available hepatitis B vaccine (Engerix-B, GSK) induced anti-HB T-cell immune responses (54,57).

CSP-specific humoral & T-cell immune responses & relationship with RT S,S/AS induced protection

Since its initial formulation with AS04 adjuvant (9) and further with AS02 and AS01, RTS,S/AS induced protection in malaria-naive adults and in malaria endemic populations is associated with increased concentration of anti-CSP antibodies. Similarly, RTS,S-specific anti-CSP T-cell immune responses including T-cell proliferation, cytokine production and RTS,S-specific T-cell responses are more frequently encountered in protected vaccinees.

Human challenge studies in naive adults and subjects who developed sterile immunity offer the simplest model to identify the nature and magnitude of immune markers involved in the RTS,S/AS-induced protection. Consistently, all RTS,S/AS vaccinees who developed either sterile immunity or experienced a longer period before patent infection after challenge, had higher levels of RTS,S/AS-specific-induced anti-CSP antibody concentration compared with unprotected vaccines (12–13), with a geometric mean of anti-CSP antibody concentration for protected vaccines > delayed patent malaria vaccinees > unprotected vaccinees (26,55). Protected vaccinees also showed strongest RTS,S/AS-induced anti-CSP T-cell responses compared with unprotected vaccine recipients. RTS,S-induced CSP-specific IFN-γ CD4+ T cells, and IFN-γ CD8+ T cells are associated with sterile immunity in malaria-naïve adults (26,58). The persistence of RTS,S-induced T-cell immune responses is also associated to the duration of protection (15,26,44,58). Anti-CSP antibody concentration is associated with protection in adults in Kenya and Gambia (17,30) as well as in children and infants (17,40).

Conversely in several study populations, no association between post dose three CSP-anti-antibody concentrations and vaccine efficacy (VE) was found. Importantly, the two largest trials conducted in Mozambique (22) and across 11 sites (42) do not show clear association between VE and concentration of anti-CSP antibodies. However, similar to the infant cohort from Mozambique (49) infants with the highest concentration of anti-CSP antibodies in Phase III trials correlated with higher VE (42).

The lack of association between 1 month post primary vaccination geometric mean of anti-CSP antibody concentration and VE was consistently observed in African children in Phase II (22,37) and Phase III trials (42), but not in infant cohorts (29,32,40). However, in children anti-CSP antibody concentration is correlated to VE when determined at 6.5 months postdose 3 (38) and in subpopulations of participants with high anti-CSP antibody concentration in Phase II (24) and Phase III (42). In addition, anti-CSP antibody concentration and VE are consistently higher in children compared with infants in the largest cohort of Phase III. Altogether, the clinical data emphasize anti-CSP antibody concentration as an essential component of RTS,S/AS vaccine-induced protection.

Upon vaccination with RTS,S/AS, $CD4^+$ T cells differentiate into self-renewing and long-lived $CD45RO^+CCR7^+CD4^+$ T cells known as central memory $CD4^+$ T cells (TCM), and into $CD45RO^+CCR7\text{-}CD4^+$ T cells which have a shorter half-life and are known as effector/effector memory $CD4^+$ T cells ($T_{E/EM}$) (27).

RTS,S/AS vaccinees who were protected produced higher frequencies and absolute numbers of IL-2-producing $CD4^+$ TCM and IL-2-producing $CD4^+$ TE/EM compared with nonprotected recipients (27). In contrast to IFN-y producing $CD4^+$ T cells and IFN-y producing $CD8^+$ T cells, which were inconsistently found across study populations (particularly scarcely or not found in populations living in endemic areas), IL-2-producing $CD4^+$ T cells are also strongly induced in vaccinees living in malaria endemic regions, particularly so in children (34,36,53,57).

Importantly, the frequency of IL-2-producing $CD4^+$ T cells correlates with anti-CSP antibody concentration (27).

IL-2 cytokine production upon vaccination is suggested to play several roles in the entire loop of RTS,S/AS-specific-induced immune responses. As a growth factor, IL-2 is thought to act

in an autocrine or paracrine manner to instruct expansion of cells producing effector cytokines like TNF-α and IFN-γ (26,36,53–54,57), which are known to mediate parasite killing through CD40L–CD40L interactions as IL-2$^+$ CD40$^+$ CD4$^+$-producing T cells are also generated upon RTS,S/AS vaccination (26,36).

The fact that in some individuals, IL-2-producing CD4$^+$ T cells correlate with anti-CSP antibody concentration supports either direct effect on the differentiation of

CSP-specific B-cell responses, or via growing effects on Th follicular cells which may be responsible for CSP-specific antibodies class switch, CSP-specific antibodies affinity maturation (47,48) and induction of CSP-specific memory B cells (34).

Several statistical models showed that CSP-specific IgG and CSP-specific T-cell immune responses including IL-2-producing CD4$^+$ T cells, TNF-α-producing CD4$^+$ T cells and to a lesser extent, IFN-γ-producing CD4$^+$ T cells, independently and synergistically contribute to RTS,S/AS-induced protection in malaria-naïve (59) and malaria endemic (53,60–61) settings.

Clinical efficacy

Concept of vaccine efficacy

In the RTS,S trials, VE was estimated as 1-IR where IR is the incidence ratio (total number of events/follow-up time in the RTS,S/AS01 group over the total number of events/follow-up time in the control group) against all episodes of clinical malaria, incidence of severe malaria, malaria anemia, malaria hospitalization, fatal malaria, other serious illnesses (sepsis, hospitalized pneumonia, all-cause hospitalization, all-cause mortality and blood transfusions) and prevalent end points (parasitemia, moderate and severe anemia) in children in each study group (46).

Phase I–II studies in adult populations

Phase I trials were conducted in both malaria-naïve populations (mainly USA volunteers) and semi-immune African adults located in endemic countries (Gambia and Kenya).

In naive populations, human challenges were performed to assess vaccine efficacy. The signal of RTS,S-induced efficacy was obtained after formulation with MPL adjuvant (9). Formulation of RTS,S in oil-in-water with MPL and QS21 paved the way for further clinical development as VE was substantial (12) and proved to persist until month 6 postdose 3, although in a very small number of volunteers (44). In a larger trial testing of various doses and regimens of RTS,S/AS02, a pooled VE was 41% (13). This VE was considered promising and the vaccine development was transferred to malaria endemic areas with concomitant evaluation of new formulations, dosage and schedules (13). Four months post-third dose efficacy in Gambian semi-immune adults confirmed that RTS,S/AS02 protects against malaria in the field although the protection waned over time (17). Protection was boosted upon administration of one dose the following year (17). The encouraging results from the first set of Phase I, IIA and IIB trials in adult volunteers paved the way to pursue the clinical development and extend the evaluation of RTS,S/AS in African children. Further studies in adults added to the evidence to select the RTS,S/AS01 formulation and shorter vaccine schedules for further evaluation (11,26,30,55).

Overall, RTS,S/AS consistently showed promising efficacy in both malaria naive and malaria exposed adult populations. Table 2A shows efficacy estimates reported over publications of

Phase IIA and IIB studies and Table 2B geometric mean of anti-CSP antibody concentration and their relation to vaccine efficacy.

Phase II studies in pediatric populations

Clinical development was carried out in two distinct age populations; children ≥5 months at the time of first vaccination and infants in whom RTS,S/AS vaccine was co-administered with the recommended EPI vaccines. Pivotal proof-of-concept efficacy trials in southern Mozambican children and infants initiated an extensive pediatric development program of RTS,S/AS02 and RTS,S/AS01 vaccines.

Efficacy trials in children ≥5 months at the time of first vaccination

Two Phase IIB pediatric trials tested VE in children aged ≥5 months. In Mozambique, efficacy of RTS,S/AS02 was evaluated in two cohorts of 1605 semiurban and 417 rural children aged 1–4 years. The second trial assessed efficacy of RTS,S/AS01 among 894 children living in Kilifi, Kenya and Korogwe, Tanzania. Both trials evaluated VE against time to first or only clinical malaria episode with the same primary case definition: >2500 parasites/mm3 and fever >37.5°C as the key end point starting from 2 weeks after the third dose of vaccination (22,37). VE against various secondary case definitions remains similar for both studies as well as VE against all episodes of malaria, except for 4-year VE among Kenyan children (VE against all episodes of clinical malaria was between 75 and 50% of first or only episode VE) (62).

A higher VE was obtained in Kenyan and Tanzanian cohorts after 6 to 8 months follow-up (37) or at 15–18 months follow-up (38). Vaccine efficacy was maintained over time in both study populations (24–25,38). The duration of protection lasted up to 4 years in the first Mozambican cohort and in Kenyan children (25,38). VE decayed over time at a level which remained nonsignificant in the first Mozambican cohort but highly significant in the second cohort as well as significant in Kenyan children (25,62–63). Table 3A shows VE estimates from individual Phase II studies in ≥5 months children.

Efficacy trials in infants in the context of co-administration with EPI vaccines

Three Phase IIB trials evaluated VE in infants and in case of introduction of the vaccine into routine EPI. VE was tested against time to first or only clinical malaria episode with the same primary case definition: >500 parasites/mm3 and fever >37.5°C or history of fever as a key end point starting from 2 weeks after the third dose of vaccination. VE against various secondary case definitions were also tested as well as time to any parasitemia (29,32,40). Here 212, 340 and 511 infants, respectively, were concomitantly vaccinated with RTS,S/AS and EPI vaccines in Mozambique, Tanzania and in a multicentre trial held in Gabon, Ghana and Tanzania. RTS,S/AS remains efficacious when given with other childhood vaccines. Table 3B shows VE against clinical malaria (29,32,40,49–50) from all studies. In Tanzanian infants, RTS,S/AS02 was highly protective against incident of infection up to 6 months (32) but with no confirmed protection against clinical malaria (32) and vaccine protection rapidly declined (50). In a multicentre

Table 2A. RTS,S/AS vaccine efficacy in Phase I–II trials in naive semi-immune adults.

Study population	Methods of VE evaluation	Vaccine formulations and study groups	Efficacy number of protected n/N point estimate % (95%CI)	Ref.
USA: 18–40 years Malaria naive Phase IA	Homologous sporozoite challenge: 10–14 days after third vaccination 0, 2 and 6 months vaccination schedule	RTS,S-Alum RTS,S/Alum/MPL (or RTS,S/AS04)	0/6 2/8	[9]
USA: 18–45 years Malaria-naive Phase IA	Homologous sporozoite challenge: 21 days after third vaccination 0, 1, 7 months vaccination schedule	RTS,S/Alum/MPL (or RTS,S/AS04) RTS,S in an oil-in-water emulsion (RTS,S/AS03) RTS,S/MPL/QS21 in an oil-in-water emulsion (RTS,S/AS02)	1/8 2/7 6/7	[12]
USA: 18–45 years Malaria naive Phase IA	Homologous sporozoite rechallenge of above protected volunteers: 6–7 months after third dose	RTS,S/Alum/MPL (or RTS,S/AS04) RTS,S in an oil-in-water emulsion (RTS,S/AS03) RTS,S/MPL/QS21 in an oil-in-water emulsion (RTS,S/AS02)	1/1 0/1 1/5	[44]
USA: 18–45 years Malaria-naive Phase I–IIA	Homologous sporozoite challenge: 14–28 days after third vaccination 0, 1, 9 months vaccination schedule	RTS,S/AS02: 50 μg: 1 dose RTS,S/AS02: 50 μg: 2 doses RTS,S/AS02: 50 μg: 3 doses RTS,S/AS02: 25 μg: 3 doses RTS,S/AS02: 10 μg: 3 doses Overall	3/10 7/14 3/6 3/7 1/4 41 (22–56)	[13]
Gambia: 18–45 years Phase I–IIB	Exposure to natural *P. falciparum* infections during transmission season assessed from 14 days after third dose 0, 1, 6 months vaccination schedule (passive and active cases detection) for about 4 months. Efficacy against infection	RTS,S/AS02: 50 μg: 3 doses 131 RTS,S/AS02 vs 119 controls	34 (8–53)	[17]
	Exposure to natural *P. falciparum* infections during transmission season assessed from 14 days after third dose 0, 1, 6 months regimen (passive and active cases detection) for about 4 months. Efficacy against infection	RTS,S/AS02: 50 μg: 3 doses plus a booster dose the following year 73 RTS,S/AS02 vs 85 controls	47 (4–71)	
USA: 18–45 years Malaria naive Phase IIA	Homologous sporozoite challenge: 14 days after second vaccination 0, 1 month vaccination schedule	RTS,S/AS02: 50 μg: 2 doses, lyophilized formulation 19 RTS,S/AS02 vs 6 controls	42 (5–63)	[11]
USA: 18–45 years Malaria-naive Phase IIA	Homologous sporozoite challenge: 14 days after third vaccination 0, 1, 3 months vaccination schedule	RTS,S/AS02: 50 μg: 3 doses 20 RTS,S/AS02 vs 12 controls	45 (18–62)	[55]
	Homologous sporozoite challenge: 14 days after third vaccination 0, 7, 28 days vaccination schedule	RTS,S/AS02: 50 μg: 3 doses lyophilized formulation 18 RTS,S/AS02 vs 12 controls	39 (11–56)	
USA: 18–45 years Malaria-naive Phase IIA	Homologous sporozoite challenge: 14–21 days after third vaccination 0, 1, 2 months vaccination schedule	RTS,S/AS02: 50 μg: 3 doses lyophilized formulation 44 RTS,S/AS02 vs 24 controls	31.8 (17.6–47.6)	[26]
	Homologous sporozoite challenge: 14–21 days after third vaccination 0, 1, 3 months regimen	RTS,S/AS01: 50 μg: 3 doses lyophilized formulation 36 RTS,S/AS01 vs 24 controls	50 (32.9–67.1)	
	Homologous sporozoite rechallenge of above-protected volunteers: 6–7 months after third dose	RTS,S/AS02: 50 μg: 3 doses lyophilized formulation 9 RTS,S/AS02 vs 12 controls 9 RTS,S/AS01 vs 12 controls	44.4 (10.9–79.2)	

Sterile protection: no parasitemia for 60 days.
N: Number of vaccinees challenged; n: Number of protected vaccines; VE: Vaccine efficacy.

Table 2A. RTS,S/AS vaccine efficacy in Phase I–II trials in naive semi-immune adults (cont.).				
Study population	**Methods of VE evaluation**	**Vaccine formulations and study groups**	**Efficacy number of protected n/N point estimate % (95%CI)**	**Ref.**
Kenya: 18–35 years Phase I–IIB	Exposure to natural *P. falciparum* infections during transmission season assessed from 14 days after third dose 0, 1, 2 months vaccination schedule (passive and active cases detection) for about 4 months. Efficacy against infection	RTS,S/AS02: 50 μg: 3 doses lyophilized formulation 79 RTS,S/AS02 vs 75 controls	31.7 (-11.6–58.2)	[30]
	Exposure to natural *P. falciparum* infections during transmission season assessed from 14 days after third dose 0, 1, 2 months vaccination schedule (passive and active cases detection) for about 4 months. Efficacy against infection	RTS,S/AS01: 50 μg: 3 doses lyophilized formulation 74 RTS,S/AS02 vs 75 controls	29.5 (-15.4–56.9)	
Sterile protection: no parasitemia for 60 days. N: Number of vaccinees challenged; n: Number of protected vaccines; VE: Vaccine efficacy.				

trial of infants vaccinated with RTS,S/AS01, VE against clinical malaria was high and persisted over a period of 18 months follow-up with no evidence of waning (40). In total, Phase I–II trials showed improved immune responses and protection following vaccination with RTS, S/AS01 than the RTS,S/AS02 formulation, which was therefore selected for subsequent Phase III development. Table 3B presents VE estimates for Phase II trials conducted in infant populations.

Pivotal clinical Phase III trial

Between 27 March 2009 and 31 January 2011, a total of 8922 children aged 5–17 months and 6537 infants aged 6–12 weeks were vaccinated across 11 sites including Bagamoyo and Korogwe in Tanzania, Kilifi, Kombewa and Siaya in Kenya, Agogo and Kintampo in Ghana, Lilongwe in Malawi, Manhica in Mozambique, Lambaréné in Gabon and Nanoro in Burkina-Faso. With this large sample size recruited from sites of low, moderate and high malaria transmission intensity, this pivotal Phase III trial was designed to confirm RTS,S/AS01-induced protection observed during Phase I–II trials; the duration of protection assessed up to 48 months; the incremental efficacy of a booster dose given 18 months after the primary course of vaccination; efficacy against severe malaria, malaria hospitalization and fatal malaria. The number of clinical and severe malaria cases averted over time was calculated to allow a translation of VE as public health benefit. The impact of RTS,S/AS01 on the incidence of severe anemia, blood transfusion all-cause hospitalization, all-cause mortality were also tested (42,46,64,65).

Pending questions like factors influencing VE, waning of anti-CSP antibody concentration and VE over time as well as the role of anti-CSP antibody concentration in the generation and maintenance of VE over time were addressed (46). Children and infants were randomly assigned to one of the three study groups. The first group (R3R) was given RTS,S/AS01 at 0, 1, 2 months and at 18 months after primary course. The second group received similarly RTS,S/AS01 as primary course vaccination but meningococcal serogroup C conjugate vaccine (Menjugate) at 18 months postdose 3 (R3C). Children in the control group received the rabies vaccine and infants received meningococcus C vaccine together with EPI antigens. The Phase III trial provides

Table 2B. Geometric mean concentration of anti-CSP antibodies and relation to VE in naive and semi-immune adults (Table 3B).

Study population	Vaccine Schedule	Vaccine formulations	Mean GMC/GMT of anti-CSP antibodies mean (range or 95% CI)						Ref.
			Post dose 2	Post dose 3	≤12 months post D3	Post booster 12 mo	≥24 months post booster dose	Correlation to efficacy	
USA: 18–40 years Phase IA	0.2 and 6 months regimen	RTS,S-Alum RTS,S/AS04	0.5 5.5	0.3 6.0					[9][1]
USA: 18–45 years Malaria-naive Phase IA	0, 1, 7 months regimen	RTS,S/AS04)		7 (1.38–43.19)*	2.8			*Protected tended to have higher	[12, 44][1]
		(RTS,S/AS03)		52.63 (10.31–365.7)*	30			*Protected tended to have higher	
		(RTS,S/AS02)		52.98 (5.22–595.0)*	30			*Protected tended to have higher	
USA: 18–45 years Malaria naive Phase I–IIA	0, 1, 9 months regimen	RTS,S/AS02: 50 μg: 2 doses	43 (R: 26–70)						[13][1]
		RTS,S/AS02: 50 μg: 3 doses		19 (R: 4–81)**				**	
		RTS,S/AS02: 25 μg: 3 doses		30 (R: 14–64)**				**	
		RTS,S/AS02: 10 μg: 3 doses		16 (R: 4–64)**				**	
Gambia: 18–45 years Phase I–IIB	0, 1, 6 months regimen	RTS,S/AS02: 50 μg: 3 doses	33.1 (23.1–47.4)	46.8 (33.2–66.1)				NA	[16][1]
Gambia: 18–45 years Phase I–IIB	0, 1, 6 months regimen	RTS,S/AS02: 50 μg: 3 doses		21.8 (18.4–25.8)		45.4 (35.8–57.7)	4.7 (3.5–6.4)	Parasitemia/10× increase anti-CSP OR: 0.21	[17][1] [43][1]
Kenya: 18–45 years Phase I–IIB	0, 1, 6 months regimen	RTS,S/AS02 50 μg: 3 doses	17.8 (12.6–25.2)	36.6 (24.1–55.6)				NA	[19][1]
USA: 18–45 years Malaria-naive Phase IIA	0, 1 month regimen	RTS,S/AS02: 50 μg: 2 doses, liquid	74 (20–273)					NA	[11]
		RTS,S/AS02: 50 μg: 2 doses, lyophilized	115 (68–195)						
USA: 18–45 years Malaria-naive Phase IIA	0, 1, 3 months regimen	RTS,S/AS02: 50 μg: 3 doses 20 RTS,S/AS02 vs 12 controls		72.7 (41.1–128.5)	19.7			Higher anti-CSP in protected	[55][1]

GMC: Geometric mean concentration; GMT: Geometric mean titer; R: Range.
CI are reported unless R is mentioned for range.
[1]GMT anti-CSP antibody concentration expressed in μg/ml.
[2]GMT anti-CSP antibody concentration expressed in EU/ml.
*Protected tended to have higher GMT at 1 month postdose 3.
**83% of protected vaccinees reached a seroconversion rate of 40 μg/ml vs 39% of unprotected vaccines.

Table 2B. Geometric mean concentration of anti-CSP antibodies and relation to VE in naive and semi-immune adults (Table 3B) (cont.).

Study population	Vaccine Schedule	Vaccine formulations	Mean GMC/GMT of anti-CSP antibodies mean (range or 95% CI)						Ref.
			Post dose 2	Post dose 3	≤12 months post D3	Post booster 12 mo	≥24 months post booster dose	Correlation to efficacy	
USA: 18–45 years Malaria-naive Phase IIA (cont.)	0, 7, 28 days regimen	RTS,S/AS02: 50 μg: 3 doses lyophilized		64.4 (39.9–104.3)	13.5			Higher anti-CSP in protected	
USA: 18–45 years Malaria-naive Phase IIA	0, 1, 2 months regimen	RTS,S/AS02: 50 μg: 3 doses lyophilized		82.8 (66.8–102.6)				Higher anti-CSP in protected	[26][1]
	0, 1, 2 months regimen	RTS,S/AS01: 50 μg: 3 doses lyophilized		143.5 (100.9–203.9)				Higher anti-CSP in protected	
	6–7 months after third dose	RTS,S/AS02: 50 μg: 3 doses lyophilized			58.2 (15.8214.9) in protected 18.4 (7.6–44.5) in nonprotected			Higher anti-CSP in protected	
Kenya: 18–35 years Phase I–IIB	Third dose 0, 1,2 months regimen	RTS,S/AS02: 50 μg: 3 doses lyophilized	16.7 (12.9–21.7)	21.4 (16–28.7)				Higher anti-CSP in noninfected	[30][2]
	0, 1, 2 months regimen	RTS,S/AS01: 50 μg: 3 doses lyophilized	31.6 (23.9–41.6)	41.4 (31.7–54.2)				Higher anti-CSP in noninfected	
European/ Caucasian: 18–28 Phase IIA	0, 1, 2 months regimen	RTS,S/AS02: 50 μg: 3 doses lyophilized	58.8 (33.3–103.6)	77.4 (47.3–126.7)				NA	[41][2]
		RTS,S/AS01: 50 μg: 3 doses lyophilized	93.2 (58.3–149.2)	160.3 (114.1–225.4)				NA	

GMC: Geometric mean concentration; GMT: Geometric mean titer; R: Range.
CI are reported unless R is mentioned for range.
[1]GMT anti-CSP antibody concentration expressed in μg/ml.
[2]GMT anti-CSP antibody concentration expressed in EU/ml.
*Protected tended to have higher GMT at 1 month postdose 3.
**83% of protected vaccinees reached a seroconversion rate of 40 μg/ml vs 39% of unprotected vaccines.

Table 3A. RTS,S/AS Phase IB–IIB trials, vaccine efficacy in African children ≥5 months at the time of first vaccination.

Study population	Method of VE evaluation	Vaccine formulations and study groups	Point estimate % (95% CI)	Ref.
Mozambique: 1–4 years Malaria endemic Phase IIB	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–8.5 months) 0, 1 and 2 months vaccination schedule	Cohort 1 803 RTS, S/AS02 vs 802 control	Crude ATP 26.9 (7.4–42.2) ATP Adjusted 29.9 (11–44.8) Crude ITT 30.2 (14.4–43)	[22]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–8.5 months) 0, 1 and 2 months vaccination schedule	Cohort 2 209 RTS,S/AS02 vs 208 control	Crude ATP 34.3 (3.0–55.5) ATP adjusted 35.4 (4.5–56.3) Crude ITT 9.3% (-25.3–34.3)	[63]
Mozambique: 1–4 years Malaria endemic Phase IIB	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–21 months) 0, 1 and 2 months vaccination schedule ITT from (0–21)	Cohort 1 723 RTS, S/AS02 vs 719 control	Crude ATP 35.3 (21.6–46.6) ITT 32.8 (20.1–43.4)	[24]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (8.5–21 months) 0, 1 and 2 months vaccination schedule ITT from (0–21)	Time to first infection with *P. falciparum* time to first infection Cohort 2 181 RTS,S/AS02 vs 171 control	Crude ATP 6.4 (-34–34.7) ATP adjusted 9.0 (-30.6–36.6) Crude ITT -4.2 (-46.6–25.9)	[63]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–45 months) 0, 1 and 2 months vaccination schedule	Time to first episode of symptomatic *P. falciparum* malaria Cohort 1 691 RTS,S/AS02 vs 687 control	Crude ATP 25.6 (13.4–36) ATP adjusted 30.5 (18.9–40.4)	[25]
Kenya and Tanzania 5–17 months years	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–10.5 months) 0, 1 and 2 months regimen ITT (0–10.5)	Time to first episode of symptomatic *P. falciparum* malaria Cohort 1 447 RTS, S/AS01E vs 447 control	Crude ATP 55 (31–70) ATP adjusted 53 (28–69) Crude ITT 49 (26–65)	[37]
Kenya 5–17 months years	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–14 months) 0, 1 and 2 months vaccination schedule	Time to first episode of symptomatic *P. falciparum* malaria 223 RTS, S/AS01E vs 224 control + EPI vaccines	Crude ATP 38 (19–53) ATP adjusted 39 (20–54)	[38]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–19 months) 0, 1 and 2 months vaccination schedule model	Time to first episode of symptomatic *P. falciparum* malaria 223 RTS, S/AS01E vs 224 control + EPI vaccines	Crude ATP 41 (18–58) ATP adjusted 46 (24–61)	
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–48 months) 0, 1 and 2 months vaccination schedule	Time to first episode of symptomatic *P. falciparum* malaria 223 RTS, S/AS01E vs 224 control + EPI vaccines	ATP adjusted 32.1 (11.6–47.8) Crude ITT 29.9 (10.3–45.3)	[12, 13, 30,62]

VE was adjusted for covariates of age, bed-net use, geographical area and distance to health center for both trials and to malaria exposure index for VE in Kenyan children.
Adjusted ATP/ITT: Analysis with consideration of covariates (age, bednet use, distance to health center, geographic region-may vary per trial).
VE calculated with Cox regression model.
ATP: According to protocol (only data from participants who were compliant to study protocol are analyzed); Crude ATP/ITT: Analysis without consideration of covariates; ITT: Intention to treat (data of all participants who received at least the first vaccine dose are analyzed); VE: Vaccine efficacy.

some certainties about the beneficial effects of RTS,S/AS01. It induces protection against clinical malaria up to 48 months after the primary vaccination series in both children and infants in the context of EPI (46). There was incremental efficacy induced by a booster dose delivered 18 months after the third dose at a similar rate in children and infants. The booster dose increased the number of averted cases in most study sites for both children and infants (42,46). Tables 4A & B summarized VE, cases averted and incremental VE induced by a booster dose in children ≥5 months and infants 6–12 weeks, respectively.

The beneficial effects of RTS,S/AS01 are extended to a significant reduction of all-cause hospitalization, of hospitalization due to malaria, of severe anemia and request for blood transfusion (46).

Table 3B. RTS,S/AS vaccine efficacy in Phase IB–IIB trials in African infants.

Study population	Efficacy evaluation	Vaccine formulations and study groups	Point estimate (%)	Ref.
Mozambique: 10–18 weeks malaria endemic Phase IIB	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–8.5 months) 0, 1 and 2 months vaccination schedule ITT (0–8.5)	Time to first infection with *P. falciparum* time to first infection 105 RTS, S/AS02 + EPI vaccines vs 106 control + EPI vaccines	Crude ATP 64.4 (95% CI: 22.6–83.6) ATP adjusted 65.8 (95% CI: 25.3–84.4) Crude ITT 35.5 (95% CI: -7.5–61.3)	[29]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–14 months) 0, 1 and 2 months vaccination schedule ITT (0–14)	Time to first infection with *P. falciparum* time to first infection 105 RTS,S/AS02 + EPI vaccines vs 106 control + EPI vaccines	ATP adjusted 33.3 (95% CI: -4.3–56.9) Adjusted ITT 25.9% (95% CI:-9.9–50)	[49]
Tanzania: 8–16 weeks malaria endemic Phase IIB	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–8.5 months) 0, 1 and 2 months vaccination schedule ITT from (0–8.5)	Time to first infection with *P. falciparum* time to first infection 170 RTS, S/AS02 + EPI vaccine vs 170 control + EPI vaccines	Crude ATP 53.1 (95% CI: -15–80.9) ATP adjusted 58.6- (95% CI: -1.8–83.2) ITT 41.8 (95% CI:-32.9–74.6)	[32]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–14 months) 0, 1 and 2 months vaccination schedule	Time to first infection with *P. falciparum* time to first infection 170 RTS, S/AS02 + EPI vaccine vs 170 control + EPI vaccines	ATP adjusted 53.6 (95% CI: 8.6–76.4)	[50]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–20 months) 0, 1 and 2 months vaccination schedule ITT (0–20)	Time to first episode of symptomatic *P. falciparum* malaria 170 RTS, S/AS02 + EPI vaccine vs 170 control + EPI vaccines	ATP adjusted 34.9 (95% CI: -8.8–61.1) Crude ITT 14.4 (95% CI: -41.9–48.4)	
Gabon, Ghana, Tanzania 6–10 weeks malaria endemic Phase IIB	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–8 months) 0, 1 and 2 months vaccination schedule	Time to first episode of symptomatic *P. falciparum* malaria 170 RTS, S/AS01 (0, 1, 2) + EPI vaccines vs 171 control + EPI vaccines	Crude ATP 62.0 (95% CI: 17.5–82.5) ATP adjusted 66.7 (95% CI: 27.2–84.8)	[40]
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–14 months) 0, 1 and 2 months vaccination schedule	Time to first episode of symptomatic *P. falciparum* malaria 170 RTS, S/AS01 (0, 1, 2) + EPI vaccines vs 171 control + EPI vaccines	Crude ATP 58.7 (95% CI: 30.7–75.3) ATP adjusted 61.6 (95% CI: 35.6–77.1)	
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (2.5–19 months) 0, 1 and 2 months vaccination schedule ITT (0–19)	Time to first episode of symptomatic *P. falciparum* malaria 170 RTS, S/AS01 (0, 1, 2) + EPI vaccines vs 171 control + EPI vaccines	Crude ATP 48.5 (95% CI: 19.3–67.4) ATP adjusted 52.5 (95% CI: 25.5–69.7)	

VE (vaccine efficacy) was adjusted for covariates of geographical area, and distance to health center for both Mozambican and Tanzanian trials.
VE was adjuted for site in the multicenter trial.
ATP: According to protocol): only data from participants who were compliant to study protocol are analyzed.
ITT (intention to treat) data of all participants who received at least the first vaccine dose are analyzed.
VE calculated with Cox regression model.

RTS,S/AS01 induced a higher VE when administered to children aged 5–17 months compared with infants of 6–12 weeks vaccinated together with EPI vaccines (56% (97.5% CI: 51–60%); 46% (95% CI: 42–50%); 36.3% ((95% CI: 31.8–40.5) vs 31% (97.5% CI: 24–38%); 27% (95% CI: 20–32%); 25.9% (95% CI: 19.9–31.5) at 12 months, 18 months after third vaccination and up to 30 months post booster vaccination, respectively) (42,46,64–65). Consequently, with similar access of either age population to the vaccine, the beneficial effects are more substantial when RTS,S/AS01 is given from 5 months compared with infants in the context of EPI. VE efficacy decays over time in both children and infant cohorts and this constant decrease is paralleled by the decrease over time of anti-CSP antibody concentration (42,46,64,65). Table 4C

Table 3B. RTS,S/AS vaccine efficacy in Phase IB–IIB trials in African infants (cont.).

Study population	Efficacy evaluation	Vaccine formulations and study groups	Point estimate (%)	Ref.
Gabon, Ghana, Tanzania 6–10 weeks malaria endemic Phase IIB (cont.)			Crude ITT 57.2 (95% CI: 33.1–72.7) Adjusted ITT 58.6 (95% CI: 30.2–75.4)	
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (1.5–7 months) 0, 1, 7 months vaccination schedule ITT (0–7)	Time to first episode of symptomatic *P. falciparum* malaria 170 RTS, S/AS01 (0, 1, 7) + EPI vaccines vs 171 control + EPI vaccines	Crude ATP 12.7 (95% CI: -74.9–56.4) ATP adjusted 15.2 (95% CI: -70.2–57.7)	
	Time to first episode of symptomatic *P. falciparum* malaria 14 days after third vaccination (7.5–19 months) 0, 1, 7 months vaccination schedule ITT (0–19)	Time to first episode of symptomatic *P. falciparum* malaria 170 RTS, S/AS01 (0, 1, 7) + EPI vaccines vs 171 control + EPI vaccines	Crude ATP 58.7 (95% CI: 32.0–74.9) ATP adjusted 63.8 (95% CI: 40.4–78) Crude ITT 30.6 (95% CI: 11.3–45.6) Adjusted ITT 32.0 (95% CI: 16.4–44.7)	[12, 13, 30]

VE (vaccine efficacy) was adjusted for covariates of geographical area, and distance to health center for both Mozambican and Tanzanian trials.
VE was adjuted for site in the multicenter trial.
ATP: According to protocol): only data from participants who were compliant to study protocol are analyzed.
ITT (intention to treat) data of all participants who received at least the first vaccine dose are analyzed.
VE calculated with Cox regression model.

shows geometric mean of anti-CSP antibody concentration at 1 month postdose 3 by site and for all sites over time.

Data from single Phase II trials in Manhica and Kilifi (49,62–63) and modeling of pooled efficacy data from several Phase II trials (66) showed accelerated reduction of VE in settings with high transmission intensity. Despite the fact that there is a trend toward reduced VE in settings with high transmission index in the Phase III trials, RTS,S/AS01E vaccine remains protective and significantly averts malaria cases in all settings regardless of the transmission intensity (42,46). RTS,S/AS02 (18,31,67) and RTS,S/AS01 (31) vaccine showed to protect against *P. falciparum* strains which allelic sequences that did not match with the 3D7 *P. falciparum* CSP sequences used in the RTS construct. However, these findings stem from small sample sizes and short follow-up time. Whether in some settings, genotypic diversity of *P. falciparum* parasites may contribute to reduced VE is being assessed with samples collected during the Phase III trial.

Several uncertainties remain and need to be addressed beyond the Phase I–III trials.

There is a trend toward increased risk of severe malaria around 18 months in the group of RTS,S/AS01 recipients. Whether reduced exposure to malaria infection due to vaccine protection makes children more susceptible to severe disease at the time VE wanes is unclear as there is no similar pattern for uncomplicated malaria (42,46). Relatively low mortality rates among trial participants and the good clinical care available for all vaccinees during the trial are potential reasons for an undetectable significant vaccine effect on malaria mortality and overall mortality as well as severe bacterial diseases. Although the 0, 1, 2 months followed by a booster dose at 18 months post dose 3 regimen induced sustained protection over time, there is evidence of continuous VE waning and the incremental VE specific to booster dose is transient. Whether additional booster doses or a different schedule can improve RTS,S/AS01-induced memory should also be addressed.

Table 3C. Geometric mean concentration of anti-CSP antibodies and relation to VE in Phase IB–IIB trials in African children and infants in relation to VE.

Study population	Efficacy evaluation	Vaccine formulations	Mean GMT/GMC anti-CSP antibody concentration mean (range or 95% CI)							Ref.
			Postdose 2	Postdose 3	≤12 months post D3	≥12 months post D3	>12 months post booster	≥ 12 months post booster dose	Correlation to efficacy	
Gambia 6–11 years Phase IB	0, 1, 3 months regimen	RTS,S/AS02A 10 μg		31 (18–50)					NA	[20][1]
		RTS,S/AS02A 25 μg		74 (42–128)						
		RTS,S/AS02A 50 μg		62 (39–101)						
Gambia 1–5 years Phase IB	0, 1, 3 months regimen	RTS,S/AS02A 10 μg		70 (50–97)						
		RTS,S/AS02A 25 μg		90 (61–134)						
		RTS,S/AS02A 50 μg		84 (56–125)						
Mozambique: 1–5 years Phase IB	0, 1, 2 months regimen	RTS,S/AS02		270.4 (182.7–400.3)					NA	[21][1]
Mozambique: 3–5 years Phase IB	0, 1, 2 months regimen	RTS,S/AS02A		180 (146–221)	29 (22–39)				*NA	1[28][2]
		RTS,S/AS02D		191 (150–0.242)	22 (15–32)					
Mozambique: 1–4 years Phase IIB Cohort 1	0, 1, 2 months regimen	RTS,S/AS02A		158 (142–176)	40 (36–45)	14 (12.5–15.6)			No association[c]	[22, 24][2]
Mozambique: 6–8 weeks Phase IIB	0, 1, 2 months regimen	RTS,S/AS02D		199.9 (150.9–264.7)	58.8 (41.8–82.8)	7.3			Higher anti-CSP in protected	[29][2] [49][2]
Gabon 18–48 months Phase IIB	0, 1, 2 months regimen	RTS,S/AS02D	58 (46–73)	183 (151–223)					NA	[33][2]
		RTS,S/AS01E	80 (63–101)	207 (172–250)						
Gabon, Ghana, Tanzania 6–10 weeks Phase IIB	0, 1, 2 months regimen	RTS,S/AS01E		190.3					Higher anti-CSP in protected	[39, 40]
	0, 1, 7 month regimen	RTS,S/AS01E		107.8						
Ghana 7–14 months Phase IIB	0, 1 months regimen	RTS,S/AS02D	318 (269–377)		35 (26–46)	10 (7–14)			NA	[35][2]
	0, 1 month regimen	RTS,S/AS01E	483 (395–591)		53 (41–68)	15 (11–21)				

[1]GMT (GMC) anti-CSP antibody concentration expressed in μg/ml and in EU/ml when not specified.
[2]Protected tended to have higher GMT at 1 month post dose 3.
**83% of protected vaccinees reached a seroconversion rate of 40 μg/ml vs 39% of unprotected vaccinees.
[a]Anti-CSP antibody concentration.
[b]Higher anti-CSP antibody concentration was associated with protection against infection in cohort 2.
[c]Anti-CSP antibody concentration was associated with protection from month 6.5 postdose 3.
R: Range.

Table 3C. Geometric mean concentration of anti-CSP antibodies and relation to VE in Phase IB–IIB trials in African children and infants in relation to VE (cont.).

Study population	Efficacy evaluation	Vaccine formulations	Mean GMT/GMC anti-CSP antibody concentration mean (range or 95% CI)							Ref.
			Postdose 2	Postdose 3	≤12 months post D3	≥12 months post D3	>12 months post booster	≥ 12 months post booster dose	Correlation to efficacy	
Ghana 7–14 months Phase IIB (cont.)	0, 1, 2 months regimen	RTS,S/AS02D		367 (293–459)	78 (58–106)	20 (14–29)				
	0, 1, 2 months regimen	RTS,S/AS01E		632 (554–720)	162 (134–196)	46 (37–57)				
	0, 1, 7 months regimen	RTS,S/AS02D		272 (219–339)	26 (20–34)	44 (33–58)				
	0, 1, 7 months regimen	RTS,S/AS01E		373 (311–447)	51 (40–64)	51 (40–66)				
Tanzania 8–16 weeks Phase IIB	0, 1, 2 months regimen.	RTS,S/AS02D	28.9 (22.4–37.3)	69.5 (53.9–89.6)	3.0 (2.2–4.0)	1.9 (1.4–2.6)			Higher anti-CSP in noninfected	[32, 50]
Kenya, Tanzania 5–17 months Phase IIB	0, 1, 2 months regimen	RTS,S/AS01E		539.6 (500.7–581.6)	71.9 (66–79)	31.3 (26–38)			No association[c]	[37, 38]
11 sites, 7 countries 5–17 months Phase III	0, 1, 2 months regimen	RTS,S/AS01E		621 (592–652)						(CTPC, 2015)
11 sites, 7 countries 6–12 weeks Phase III	0, 1, 2 months regimen	RTS,S/AS01E		209 (197–222)						(CTPC, 2015)

[a]GMT (GMC) anti-CSP antibody concentration expressed in μg/ml and in EU/ml when not specified.
[b]Protected tended to have higher GMT at 1 month post dose 3.
**83% of protected vaccinees reached a seroconversion rate of 40 μg/ml vs 39% of unprotected vaccinees.
[a]Anti-CSP antibody concentration.
[c]Higher anti-CSP antibody concentration was associated with protection against infection in cohort 2.
[c]Anti-CSP antibody concentration was associated with protection from month 6.5 postdose 3.
R: Range.

Table 4A. RTS,S/AS01 Phase III trial, vaccine efficacy in African children aged 5–17 months at the time of first vaccination.

Site	Study population by site	Clinical malaria VE % (95% CI)		Clinical malaria cases averted/1000 vaccinees		Clinical malaria incremental vaccine efficacy of a booster dose (M21-M32) VE % (95%CI)	Severe malaria VE % (95%CI)		Severe malaria cases averted/1000 vaccinees	
		R3C	R3R	R3C	R3R		R3C	R3R	R3C	R3R
Kilifi	600	66.0 (37.5–81.5)	74.6 (47.8–87.6)	250	303	6.9 (-133.6–62.9)	100 (-3800–100)	-3.1 (-7990–98.7)	12	6
Korogwe	912	52.0 (26.2–68.8)	46.8 (18.4–65.3)	215	205	-148.7 (-740.7–26.4)	83.4 (-36.9–99.6)	66.6 (-87–96.7)	23	19
Manhiça	1002	33.3 (7.1–52.1)	22 (-6.6–42.9)	341	236	14.6 (-36.4–46.6)	7.7 (-139.3–64.9)	62.9 (-25.3–91.4)	24	27
Lambaréné	704	36.1 (10.8–54.1)	41.1 (15.3–59.0)	498	472	30.1 (17.8–58.5)	61.7 (-14.5–89.3)	77.0 (16.4–95.8)	54	57
Bagamoyo	903	37.5 (13.5–54.9)	37.9 (12.8–55.8)	477	607	12.8 (-44.3–47.3)	45.4 (-81.5–85.6)	78.1 (-5.9–97.7)	37	37
Lilongwe	800	33.5 (8.2–51.8)	50.8 (31.4–64.7)	532	685	23.3 (-40.7–58.2)	-8.7 (-223.9–62.8)	39.4 (-110.2–84.4)	-17	6
Agogo	600	31.1 (13.3–45.2)	43.2 (29–54.6)	2060	2722	33.7 (10.3–51)	-21.4 (-166.1–43.7)	50.0 (-32.4–82.9)	8	25
Kombewa	1000	27.1 (12.9–38.9)	32.1 (18.9–43.2)	1937	2510	30.4 (9.7–46.4)	-27.6 (-131–28.7)	36.3 (-27.4–69.1)	4	17
Kintampo	1002	25.9 (15.0–35.4)	35.0 (25.5–43.4)	2663	3892	30.6 (15.3–43.2)	-48.4 (-142–7.9)	-19.4 (-98.9–27.9)	-42	-15
Nanoro	600	17.7 (7.0–27.7)	27.9 (17.9–36.8)	2897	4217	28.1 (13.8–40)	-13.0 (-182.6–54.4)	17.4 (-119.3 -69.7)	-8	-6
Siaya	799	20.2 (7.4–31.3)	37.8 (26.6–47.2)	4443	6565	21.3 (3.6–35.7)	19.9 (-27.8–50.1)	28.7 (-15.4–56.5)	3	37
Overall	8922	28.2 (23.3–32.9)	36.3 (31.8–40.5)	1363	1774	25.6 (18.2–32.3)	1.1 (–23 -20.5)	32.2 (13.7–46.9)	8	19

Vaccine efficacy (VE) against all episodes of clinical malaria and against severe malaria.
Clinical malaria case is defined as infant seen in a health facility for illness with temperature ≥37.5°C and *P. falciparum* asexual parasitaemia >5000 parasites/mm^3 (primary case definition).
Severe malaria case is defined as the occurrence of *P. falciparum* asexual parasitemia at a density of > 5000 parasites/mm^3 per with one or more markers of disease severity without a coexisting illness (primary case definition)
Cases averted (CA).
Cases averted afor R3R = RTS,S/AS01 primary schedule with booster and R3C = RTS,S/AS01 primary schedule without booster.
Clinical malaria cases averted are defined as illness in infants seen a study facility temperature of ≥37.5°C or fever within the last 24 h and *P. falciparum* asexual parasitemia at a density of >0/mm^3 (secondary case definition).
Severe malaria cases averted are defined as severe illness in infants seen with *P. falciparum* asexual parasitemia at a density of >5000 parasites/mm^3 with one or more markers of disease severity, including when the patient has a coexisting illness or when a concomitant illness could not be ruled out.
Both VE and CA are calculated from first vaccine dose to study end (up to 51 months post dose 1): intention to treat analysis (ITT).
Markers of severe disease: prostration, respiratory distress, a Blantyre coma score of ≤2 (on a scale of 0–5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycemia, acidosis, elevated lactate level or hemoglobin level of <5 g per deciliter.
Coexisting illnesses: radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteremia or gastroenteritis with severe dehydration.
Incremental vaccine efficacy of a booster dose is evaluated against all episodes of clinical (primary case definition) evaluated from day of booster dose during 1 year post booster vaccination.
Study sites are ordered from lowest (Kilifi) to highest (Siaya) incidence of clinical malaria measured in control children 5–17 years of age at enrolment during 12 months of follow-up.
Data taken from The RTS,S Clinical Trial Partneships Committee 2014; The RTS,S Clinical Trial Partneships Committee 2015.

Kinetic of anti-CS antibody concentration (61), opsonizing anti-CSP antibodies (14), central memory T cells (56), upregulation of genes correlated with efficient processing of major histocompatibility complex peptides (68), peripheral blood monocyte-to-lymphocyte ratio at study enrollment (69) and more biomarkers associated with protection in Phase I–II trials are being tested with the Phase III samples for confirmation as correlates of protection.

Safety & tolerability

RTS,S/AS vaccine showed a good safety profile during Phase I–III trials with no safety signals at the time of initiation of Phase III trial. The RTS,S/AS vaccine was consistently more reactogenic than control vaccines in trials. Local and systemic solicited symptoms were of low-to-moderate grade and transient through all trials including in adult and pediatric cohorts. Grade 3 events

Table 4B. RTS,S/AS01 Phase III trial, vaccine efficacy in African infants aged 6–12 weeks at the time of first vaccination.

Site	Study population by site	Clinical malaria VE % (95% CI)		Clinical malaria cases averted/1000 vaccinees		Clinical malaria incremental vaccine efficacy of a booster dose (M21-M32) VE % (95%CI)	Severe malaria VE % (95%CI)		Severe malaria cases averted/1000 vaccinees	
		R3C	R3R	R3C	R3R		R3C	R3R	R3C	R3R
Kilifi	304	23.1 (117.5–72.8)	-14.6 (-213.7–58.1)	27	-30	-22 (-464–73.6)	-1.9 (-7902.1–98.7)	-9.4 (-8485.6–98.6)	-11	-12
Korogwe	593	23.1 (-41.6–58.2)	44.2 (-4.6–70.3)	114	190	81.3 (37.1–94.4)	-98.0 (-11 579.6–89.7)	3.0 (-7515.4–98.8)	-9	3
Manhiça	635	33.6 (-2.8–57.1)	7.1 (-41.4–39.0)	218	179	-4.6 (-89.5–42.2)	-26.2 (-535.9–72.8)	-24.4 (-527–73.2)	17	0
Lambaréné	226	–2.8 (-106–48.7)	43.9 (-11.6–71.8)	-140	268	65.2 (6.9–87)	-74.4 (-1827.5–75.0)	15.0 (-1072.7–93.8)	-31	0
Bagamoyo	802	28.0 (-21.8–57.4)	50.4 (13.8–71.5)	277	309	40.6 (-37.9–74.4)	85.5 (-12.9–99.7)	85.7 (–11.6 to 99.7)	40	40
Lilongwe	826	28.6 (2.8–47.5)	38.9 (16.8–55.1)	493	772	31.4 (-6.4–55.8)	26.2 (-101.4–74.2)	25.4 (–103.6–74.0)	3	3
Agogo	688	10.2 (-11.3–27.5)	34.6 (17.9–48.0)	585	1077	46.1 (25.3–61.1)	-50.0 (-412.1–52.3)	-17.7 (-323.9–66.1)	-17	-12
Kombewa	631	21.7 (2.0–37.4)	19.8 (-0.5–35.9)	1144	1404	-12.5 (-48.2–14.5)	52.0 (0.9–78.0)	20.4 (-49.3–58.1)	59	51
Kintampo	331	4.1 (-20.8–23.8)	0.1 (-23.3–19.1)	-172	726	23.4 (-4.6–43.9)	-0.9 (-115.6–52.8)	1.8 (-109.8–54.0)	-43	-62
Nanoro	681	11.4 (0.9–20.7)	17.4 (8.0–25.9)	1367	2428	19.5 (7.1–30.3)	8.3 (-126.9–63.4)	57.8 (-28.8–88.3)	10	19
Siaya	820	21.5 (8.1–33.0)	30.6 (18.2–41.1)	2178	3406	22 (3–37.3)	-12.9 (-89.3–32.4)	3.6 (-64.7–43.6)	13	56
Overall	6537	18.3 (11.7–24.4)	25.9 (19.9–31.5)	558	983	22.3 (14–29.8)	10.3 (-17.9–31.8)	17.3 (-9.4–37.5)	8	12

Vaccine efficacy (VE) against all episodes of clinical malaria and against severe malaria.

Clinical malaria case is defined as infant seen in a health facility for illness with temperature ≥37.5°C and *P. falciparum* asexual parasitemia >5000 parasites/mm^3 (primary case definition).

Severe malaria case is defined as the occurrence of *P. falciparum* asexual parasitemia at a density of >5000 parasites/mm^3 per with one or more markers of disease severity without a coexisting illness (primary case definition).

Cases averted (CA).

Cases averted are for R3R = RTS,S/AS01 primary schedule with booster and R3C = RTS,S/AS01 primary schedule without booster.

Clinical malaria cases averted are defined as illness in infants seen a study facility temperature of ≥37.5°C or fever within the last 24 h and *P. falciparum* asexual parasitemia at a density of >0/mm^3 (secondary case definition).

Severe malaria cases averted are defined as severe illness in infants seen with *P. falciparum* asexual parasitemia at a density of >5000 parasites/mm^3 with one or more markers of disease severity, including when the patient has a coexisting illness or when a concomitant illness could not be ruled out.

Both VE and CA are calculated from first vaccine dose to study end (SE = up to 39 months post dose 1).

Markers of severe disease: prostration, respiratory distress, a Blantyre coma score of ≤2 (on a scale of 0–5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycemia, acidosis, elevated lactate level or hemoglobin level of <5 g per deciliter.

Coexisting illnesses: radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteremia or gastroenteritis with severe dehydration.

Incremental vaccine efficacy of a booster dose is evaluated against all episodes of clinical (primary case definition) evaluated from day of booster dose during 1 year post booster vaccination.

Study sites are ordered from lowest (Kilifi) to highest (Siaya) incidence of clinical malaria measured in control infants 6–12 weeks of age at enrolment during 12 months of follow-up.

Data taken from The RTS,S Clinical Trial Partnerships Committee 2014; The RTS,S Clinical Trial Partnerships Committee 2015.

were rare. Before the Phase III trial initiation, two cases of simple febrile seizures related to RTS,S/AS01 were reported in Ghanaian (35) and Kenyan (37) children, and in the multicenter Phase II trial of RTS,S/AS01 administered with EPI vaccines, one site reported an increased rate of diaper dermatitis in the RTS,S/AS01 group (39,40). Safety databases of all pediatric Phase II trials were pooled and confirmed the good safety profile of RTS,S/AS vaccine observed (70). Higher rate of rash and diaper dermatitis within a window of 30 days postvaccination was observed (70). Safety data from Phase III confirmed the increased reactogenicity of RTS,S/AS01 over comparator vaccines in children (64) and infants (65) including the booster dose of RTS,S/AS01 being associated with a slight increase of generalized convulsive seizures within 7 days (42,46). An imbalance of skin lesions between groups observed in Phase II trials was not confirmed when skin lesions were collected in a standardized manner during the Phase III trial (42,46).

Table 4C. Geometric mean concentration of anti-CSP antibodies from Phase III trial by site at 1 month post 3 and at 5 study time points for the first 200 vaccinees of all sites.

Study population and sites	Mean GMT/GMC anti-CSP antibody concentration mean (95% CI)				
	Post post dose 3	18 months post dose 3 per RTS,S/S recipients groups	One month post booster dose per RTS,S/S recipients groups	12 months post booster dose per RTS,S/S recipients groups	Study end (39–48 months) per RTS,S/S recipient groups
Overall 11 sites, 7 countries 5–17 months Phase III	621 (592–652)	R3R: 34.4 (30.7–38.6) R3C: 35.4 (31.7–39.5)	318.2 (295.1–343.0) 34.2 (30.5–38.3)	52.4 (47.8–57.6) 19.3 (17.2–21.8)	25.4 (20.6–38.6) 14.4 (11.4–18.1)
Kilifi	685.2 (606.1–774.6)				
Korogwe	534./ (477.6–598.5)				
Manhica					
Lambaréné	385.0 (322.6–459.5)				
Bagamoyo	514.0 (441.0–599.0)				
Lilongwe	348.4 (270.2–449.2)				
Agogo	665.5 (591.4–749.0)				
Kombewa	745.1 (648.1–856.6)				
Kintampo	787.1 (682.6–907.6)				
Nanoro	705.1 (628.6–791.0)				
Siaya	708.6 (573.8–875.0)				
Overall 11 sites, 7 countries 6–12 weeks Phase III	210.5 (198.2–223.6)	R3R: 5.9 (5.2–6.7) R3C: 6.6 (5.8–7.5)	169.9 (153.8–187.7) 6.2 (5.4–7.0)	15.9 (13.8–18.3) 3.7 (3.3–4.2)	8.9 (6.5–12.3) 2.6 (2.0–3.4)
Kilifi	254.4 (206.8–313.2)				
Korogwe	252.1 (217.7–292.0)				
Manhica	335.3 (289.5–388.5)				
Lambaréné	287.6 (248.8–332.3)				
Bagamoyo	179.1 (143.1–224.0)				
Lilongwe	235.5 (200.9–276.0)				
Agogo	158.6 (129.1–194.8)				
Kombewa	242.3 (199.7–294.1)				
Kintampo	151.0 (128.5–177.4)				
Nanoro	116.9 (92.5–147.9)				
Siaya	244.1 (189.2–315.0)				

GMC: Geometric mean concentration.
Anti-CSP antibody concentration measured for the first 200 participants of each age group from each site.
Per protocol data are reported here.
Study sites are ordered from lowest to highest clinical malaria incidence measured in the control groups.
Data taken from The RTS,S Clinical Trial Partnerships 2014; The RTS,S Clinical Trial Partnerships 2015.

Meningitis was reported in 22 children and 18 infants. Meningitis occurred more frequently in RTS,S/AS01 recipient children compared with control groups (11, 10 and 1 in the R3R, R3C and C3C groups, respectively). Out of 22 cases in children, 5 occurred after booster dose (2 vs 3 for R3R and R3C, respectively). The causality link with RTS,S/AS01 remains uncertain because of a lack of temporal association with vaccination and no imbalance in infant cohorts (5, 7 and 6 cases for R3R, R3C and C3C, respectively). In addition, 20 cases of meningitis occurred in only two sites (15 for Lilongwe and 5 in Kombewa) (42,46).

Postmarketing surveillance

Postregistration studies are needed to address the imbalance of meningitis cases observed in children. Uncertainty concerning increased susceptibility to severe malaria following vaccine protection decay is being investigated through long-term follow-up of Phase III vaccinees in a few sites (Korogwe, Tanzania, Nanoro, Burkina-Faso and Kombewa, Kenya). The benefit of a single booster dose is transient; an improved schedule with additional booster dose (s) needs to be investigated. In case of further deployment, VE against overall mortality, malaria mortality and impact on other severe illnesses should be addressed. Improvement of RTS,S/AS01 induced

immune memory and efficacy is considered as part of the agenda of the development of a second-generation vaccine.

Regulatory affairs

A dossier of RTS,S/AS01 as a vaccine to prevent malaria in African children has been submitted to the European Medicine Agency. The dossier is under scientific evaluation according to article 58 procedures which allow delivery of 'a European scientific opinion' to a medicinal product developed by an EU manufacturer but targeting disease and recipients outside the EU, as it is for malaria in African children (71). This evaluation is performed in association with WHO, which in parallel may decide to prequalify the RTS,S/AS01 vaccine. Proper licensure or registration would then take place by African national regulatory authorities under their own jurisdictions.

WHO established a joint technical expert group to continuously review the progress of RTS,S/AS trials and assist WHO in their recommendation for the use of RTS,S/AS01 as a public health intervention to prevent malaria (71). WHO recommendations will guide both national decision-making authorities to seek introduction of the vaccine in their control programs in Africa, as well as international donor agencies like GAVI (Global Alliance for Vaccines and Immunisation) Alliance to support prefunding procurement of RTS,S/AS01 for eligible countries.

Ideally, a new vaccine will be introduced into the existing schedules of EPI and require coadministration with several antigens. While RTS,S/AS protects infants and averts cases of clinical malaria when given with the existing EPI vaccine, pooled analysis of Phase II data and Phase III data showed reduced anti-CSP antibody concentration (51,65) and lower VE (42,46,64) in infants vaccinated through the existing EPI schedule. Recommendation of RTS,S/AS01 use for children of ≥5 months of age is a key topic which should be addressed by regulatory stakeholders, at WHO and national decision-makers as well as for funding agencies.

CONCLUSION

CSP-based malaria antigen RTS,S formulated with AS01 adjuvant shows improved immunogenicity and efficacy compared with formulated formulation with AS02 adjuvant. A large Phase III trial enrolled and successfully followed-up 15,459 infants and young children in seven sub-Saharan African countries including Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and the United Republic of Tanzania. This trial confirmed a good safety profile and protective effect of RTS,S/AS01 against clinical malaria but also against all-cause hospitalization, malaria hospitalization, severe anemia and need for blood transfusion over the study period which lasted up to 51 months when the vaccine is administered at 5–17 months. A booster dose given at 18 months post primary vaccination induced incremental VE. Concerns raised about meningitis cases and increased susceptibility to severe malaria with the constant decrease of VE over time are currently uncertain, and while being addressed in postregistration studies, they should not prevent to consider the optimal use of RTS,S/AS01 which has the potential to avert millions of cases of clinical malaria. If EMA issues a favorable opinion, and WHO prequalification and regulatory clearance at national levels is issued, further challenges should focus on how to optimally implement the RTS,S/AS01 vaccine into the existing malaria

control measures to address malaria elimination, taking into account transmission intensity in each setting.

FINANCIAL& COMPETING INTERESTS DISCLOSURE

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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EXECUTIVE SUMMARY

RT S,S/AS vaccine

- RTS,S antigen is a recombinant polypeptide construct of B-cell epitopes and T-cell epitopes from CSP fused to the N-terminal region of hepatitis B virus surface antigen (S). The RTS and unfused S polypeptides (RTS,S) are co-expressed in yeasT cells and transform into virus-like particles (VLPs) spontaneously.
- RTS,S is adjuvanted with combination of -deacylated monophos- phoryl lipid A (MPL), and QS21, a purified saponin formulated either as oil-in-water (AS02) or as liposome (AS01).

Characteristics of RT S,S/AS specific anti-CSP

- Vaccination with three consecutive doses of RTS,S/AS02 or RTS,S/AS01 scheduled at 21- to 42-day intervals induces the highest peak of anti-CSP antibodies.
- The highest concentration of anti-CSP antibodies are induced by RTS,S/AS01.
- A peak of anti-CSP antibodies is generated after the three vaccine doses; followed by a rapid waning of antibody concentration and a persisting of detectable antibody concentration.

CSP-specific cell-mediated immunity induced by RT S,S/AS

- RTS,S/AS vaccine induces Th1-type immune responses.
- IL-2 $CD4^+$ T cells are the most sensitive marker of RTS,S/AS-induced T-cell responses.

CSP-specific humoral and T-cell immune responses and relationship with RT S,S/AS-induced protection

- Geometric mean of anti-CSP antibody concentration for protected vaccines > delayed patent malaria vaccinees > unprotected vaccines.
- Anti-CSP antibody concentration is higher in protected malaria naive adults and children vaccinees living in malaria endemic areas.
- IL-2-producing $CD4^+$ T cells correlate with anti-CSP antibody concentration supports, either via direct effect on the differentiation of CSP-specific B-cell responses, or via growing effects on Th follicular cells which may be responsible
- for CSP-specific antibodies class switch, CSP-specific antibodies affinity maturation and induction of CSP-specific memory B cells.

› CSP-specific antibodies and CSP-specific T-cell immune responses including IL-2-producing $CD4^+$ T cells, TNF-α-producing $CD4^+$ T cells and to a lesser extent, IFN-γ-producing $CD4^+$ T cells, independently and synergistically contribute to RTS,S/AS-induced protection.

Phase I–II studies in adult populations

› Overall, RTS,S/AS consistently showed efficacy in both malaria naive and malaria exposed adult populations.

Efficacy trials in children ≥5 months at the time of first vaccination

› RTS,S/AS consistently induced protection against clinical malaria in African children.
› RTS,S/AS01 is associated with higher VE after 6–8 or at 15–18 month follow-ups.
› The duration of protection lasted up to 4 years.
› VE decayed over time.

Efficacy trials in infants in the context of co-administration with EPI vaccines

› Phase II in infant trials showed improved immune responses and protection following vaccination with RTS, S/AS01, which was selected for the large Phase III trial.

Phase III trial

› RTS,S/AS01 protects against clinical malaria up to 48 months after the primary vaccination series in both children and infants in the context of EPI.
› There was incremental efficacy induced by a booster dose delivered 18 months after the third dose at a similar rate in children and infants.
› RTS,S/AS01 induced a higher VE when administered to children aged 5–17 months compared with infants of 6–12 weeks vaccinated together with EPI vaccines.
› RTS,S/AS01 significantly averts malaria cases and the booster dose increased the number of averted cases in most study sites for both children and infants.
› Several uncertainties remain and need to be addressed beyond the Phase I–III trials.
› There is a trend toward increased risk of severe malaria around 18 months in the group of RTS,S/AS01 recipients.

Safety & tolerability

› Meningitis occurred more frequently in RTS,S/AS01 recipient children compared with control groups.
› The causality link with RTS,S/AS01 remains uncertain because of a lack of temporal association with vaccination and no imbalance in infant cohorts

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A NEW VACCINE CANDIDATE AGAINST EBOLA VIRUS

SAFETY AND IMMUNOGENICITY OF RVSVΔG-ZEBOV-GP EBOLA VACCINE IN ADULTS AND CHILDREN IN LAMBARÉNÉ, GABON: A PHASE I RANDOMIZED TRIAL

5

ABSTRACT

Background

The rVSVΔG-ZEBOV-GP vaccine prevented Ebola virus disease when used at 2×10^7 plaque-forming units (PFU) in a trial in Guinea. This study provides further safety and immunogenicity data.

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Methods and findings

A randomised, open-label phase I trial in Lambaréné, Gabon, studied 5 single intramuscular vaccine doses of 3×10^3, 3×10^4, 3×10^5, 3×10^6, or 2×10^7 PFU in 115 adults and a dose of 2×10^7 PFU in 20 adolescents and 20 children. The primary objective was safety and tolerability 28 days post-injection. Immunogenicity, viraemia, and shedding post-vaccination were evaluated as secondary objectives. In adults, mild-to-moderate adverse events were frequent, but there were no serious or severe adverse events related to vaccination. Before vaccination, Zaire Ebola virus (ZEBOV)–glycoprotein (GP)–specific and ZEBOV antibodies were detected in 11% and 27% of adults, respectively. In adults, 74%–100% of individuals who received a dose 3×10^4, 3×10^5, 3×10^6, or 2×10^7 PFU had a ≥4.0-fold increase in geometric mean titres (GMTs) of ZEBOV-GP-specific antibodies at day 28, reaching GMTs of 489 (95% CI: 264–908), 556 (95% CI: 280–1,101), 1,245 (95% CI: 899–1,724), and 1,503 (95% CI: 931–2,426), respectively. Twenty-two percent of adults had a ≥4-fold increase of ZEBOV antibodies, with GMTs at day 28 of 1,015 (647–1,591), 1,887 (1,154–3,085), 1,445 (1,013–2,062), and 3,958 (2,249–6,967) for the same doses, respectively. These antibodies persisted up to day 180 for doses $\geq 3 \times 10^5$ PFU. Adults with antibodies before vaccination had higher GMTs throughout. Neutralising antibodies were detected in more than 50% of participants at doses $\geq 3 \times 10^5$ PFU. As in adults, no serious or severe adverse events related to vaccine occurred in adolescents or children. At day 2, vaccine RNA titres were higher for adolescents and children than adults. At day 7, 78% of adolescents and 35% of children had recombinant vesicular stomatitis virus RNA detectable in saliva. The vaccine induced high GMTs of ZEBOV-GP-specific antibodies at day 28 in adolescents, 1,428 (95% CI: 1,025–1,989), and children, 1,620 (95% CI: 806–3,259), and in both groups antibody titres increased up to day 180. The absence of a control group, lack of stratification for baseline antibody status, and imbalances in male/female ratio are the main limitations of this study.

Conclusions

Our data confirm the acceptable safety and immunogenicity profile of the 2×10^7 PFU dose in adults and support consideration of lower doses for paediatric populations and those who request boosting.

Trial registration:

Pan African Clinical Trials Registry PACTR201411000919191

AUTHOR SUMMARY

Why was this study done?

- The worst Ebola outbreak in history ended in 2016 after killing about 11,323 individuals and infecting 28,650 individuals worldwide.
- This public health emergency accelerated efforts to develop a vaccine as part of the strategy to contain the outbreak.
- Two vaccine candidates with preclinical safety and efficacy data obtained from non-human primates entered human trials.
- The one used in our study is the rVSVΔG-ZEBOV-GP vaccine, containing a non-infectious portion of a gene from the Zaire Ebola virus introduced into a recombinant vesicular stomatitis virus (rVSV), which itself is unlikely to cause disease in humans.
- To generate data for deployment of the vaccine, several dose-ranging phase I trials were initiated across centres in the United States, Europe, and Africa.

What did the researchers do and find?

- We allocated 115 adults aged 18–50 years to receive 1 of the 5 doses used in the trial. A single intramuscular dose ranging from 3×10^3 to 2×10^7 plaque-forming units (PFU) was given, and participants were followed up until 6 months post-injection for safety and immunogenicity.
- Preliminary results led to the selection of the 2×10^7 PFU dose for further development.
- We also included 20 adolescents (13–17 years) and 20 children (6–12 years), who received the 2×10^7 PFU dose and were followed-up in a similar way as the adults.
- No vaccine-related serious or severe adverse event was reported by any participant.
- A high proportion of our population—even though residing in an area with no history of Ebola outbreak—had pre-vaccination antibodies specific to the Zaire Ebola virus.
- In adults, antibodies persisted up to 6 months post-injection at doses of 3×10^5 to 2×10^7 PFU.
- In participants with baseline antibodies, a dose as low as 3×10^4 PFU could induce high antibody titres up to day 56 post-injection.
- Higher vaccine replication, leading to shedding of the vaccine in saliva and urine, occurred in children and adolescents.

What do these findings mean?

- Our results and other findings show that this vaccine is safe and immunogenic.
- Lower vaccine doses may be needed in paediatric populations as well as for boosting after primary vaccination or naturally acquired immunity.

INTRODUCTION

The western African Ebola virus disease (EVD) public health emergency of international concern ended in June 2016 (1), after infecting approximately 28,650 individuals, of whom 11,323 died (2,3). Global commitment led to landmarks in vaccine development against EVD, with 8 candidates out of 15 undergoing evaluation in phase I–III clinical trials worldwide by the end of 2015 (4–6). A live-attenuated recombinant vaccine consisting of the vesicular stomatitis virus (VSV), strain Indiana, with the gene for the Kikwit-95 Zaire Ebola virus (ZEBOV) glycoprotein (GP) replacing the VSV glycoprotein (G) had given acceptable results in non-human primate challenge models and was selected for accelerated clinical development. In European and African populations, the VEBCON Consortium (VSV-EBola CONsortium) carried out parallel dose-escalation phase I trials of the recombinant VSV (rVSV)–ZEBOV candidate vaccine in Germany (NCT02283099), Kenya (NCT02296983), and Gabon (PACTR2014000089322) and a double-blind phase I/II randomised controlled trial in Switzerland (NCT02287480). Three further phase II/III trials were later launched in Guinea, Sierra Leone, and Liberia. Results from phase I trials in the US (7) and preclinical data supported selection of the 2×10^7 plaque-forming units (PFU) dose as the most immunogenic for phase IIb/III trials in Guinea, Sierra Leone, and Liberia. A final analysis of the Guinea trial showed that a single dose of 2×10^7 PFU given immediately after contact with an index case was 100% (95% CI: 70%–100%, $P = 0.0045$) efficacious in preventing EVD in individuals, and protected the population through a ring vaccination strategy 10 days or more post-vaccination (8).

Detailed dose-ranging studies (3×10^5, 3×10^6, 1×10^7, 2×10^7, and 5×10^7 PFU) at the 4 VEBCON sites showed acceptable safety, dose-dependent reactogenicity (9), and high seroconversion rates among all participants on day 28 after vaccination (9,10).

In Gabon, 2 seroprevalence studies in epidemic and non-epidemic regions showed varying proportions of participants with pre-vaccination ZEBOV-specific IgG antibodies (11,12). In Lambaréné, with no reported EVD outbreak, ZEBOV-GP-specific antibody responses after vaccination were similar at 2 tested doses (3×10^5 and 3×10^6 PFU) (9). This finding contrasted with that in vaccinees in Geneva, where antibody titres at 3×10^5 PFU were significantly lower than responses to higher vaccine doses, including 1×10^7 PFU and 5×10^7 PFU (10). Additionally, irrespective of vaccine dose, delayed oligoarthritis and skin and mucous membrane lesions emerged as vaccine-related adverse events in a proportion of recipients more than 1 week after vaccination in Geneva (10). These delayed complications were not observed in Gabon, despite the fact that the same vaccine batch and similar doses were used (9).

Because of these divergent site-specific observations, we need further assessments of the vaccine in Ebola virus endemic areas as well as in children. We present a comparison of safety and immunogenicity outcomes in participants vaccinated with (1) 2×10^7 PFU, the dose used in the efficacy trial; (2) 2 previously reported doses, 3×10^6 and 3×10^5 PFU (9); and (3) 2 lower doses, 3×10^4 PFU and 3×10^3 PFU, in African adults. Furthermore, we report, to our knowledge for the first time, on children and adolescents aged 6 to 17 years vaccinated with 2×10^7 PFU.

METHODS

Study design and participants

The trial protocol was approved by the Scientific Review Committee of Centre de Recherches Médicales de Lambaréné (CERMEL), the Institutional Ethics Committee of CERMEL, the National Ethics Committee of Gabon, the World Health Organization (WHO) Ethics Committee, and the Institutional Ethics Committee of the Universitätsklinikum Tübingen. The trial was registered with the Pan African Clinical Trials Registry (PACTR201411000919191).

The study was a randomised, open-label, dose-escalation phase I trial at CERMEL in Gabon. The trial was initially designed to escalate doses to 3×10^5, 3×10^6, and 2×10^7 PFU in 60 adults. After successive protocol amendments, a total of 115 adults (18–50 years), 20 adolescents (13–17 years), and 20 children (6–12 years) were enrolled, between 17 November 2014 and 7 July 2015. Written informed consent was obtained from adults and parents/guardians of adolescents/children, and written assent from minors aged 11–17 years, prior to study-related procedures (details are in S3 Text).

Healthy consenting volunteers who were aged 6–50 years and resident in the study area—which had no history of an Ebola outbreak—and willing to minimise blood/body fluid exposure to their relatives for 5 days post-vaccination were included. Field workers used the door-to-door approach to invite individuals from the Lambaréné community to screen for the study. After screening, individuals with a history of severe local or systemic allergic reaction to vaccination, known allergy to constituents of the rVSVΔG-ZEBOV-GP vaccine, or any acute or chronic clinically significant medical or psychiatric condition were excluded. All pregnant and lactating women were excluded. Volunteers who received a licensed vaccine within 14 days (or 30 days for a live vaccine), had a history of blood donation within 60 days prior to vaccination, were positive for HIV and/or hepatitis B or C virus infection, or had an immunocompromised member in the family were also excluded from the study.

Randomisation and treatment allocation

Randomisation and allocation was performed by an independent investigator from 17 November 2014 until 13 April 2015 using a web-generated sequence. Randomisation in permuted blocks was performed in 2 stages. In the first, participants were assigned in a ratio of 1:1:1 to 3×10^5, 3×10^6, and 2×10^7 PFU, and in the second stage, in a ratio of 1:1 to 3×10^3 and 3×10^4 PFU. On 9 December 2014, a temporary consortium-wide safety hold was placed on doses above 1×10^7 PFU due to adverse events reported at the Swiss site with doses of 1×10^7 and 5×10^7 PFU. In Gabon, only 1 participant had been allocated to the 2×10^7 PFU dose. In all, 20, 20, 1, 20, and 20 adults were randomised to a vaccine dose of 3×10^5, 3×10^6, 2×10^7, 3×10^3, and 3×10^4 PFU, respectively. Preliminary data from the 20 participants vaccinated with 3×10^5 PFU and the initial 19 vaccinated with 3×10^6 PFU were previously reported (9).

An unblinded safety review of VEBCON Consortium trials by the data and safety monitoring board lifted the safety hold on 5 January 2015. After this and during the third stage of the study, 19 adults were vaccinated with 3×10^6 PFU without randomisation. In addition, Merck Sharp & Dohme selected the 2×10^7 PFU dose for further development, as being the most immunogenic

dose with an acceptable safety profile (S. Gupta, oral presentation at the WHO Ebola Research and Development Summit, 11–12 May 2015, Geneva) (13,14). A subsequent amendment included 20 adolescents and 20 children aged 13 to 17 years and 6 to 12 years, respectively, to be vaccinated with 2×10^7 PFU. The National Ethics Committee of Gabon recommended that adults from this population should be vaccinated with the intended dose before administration to the paediatric cohorts, so an additional 15 adults were included in the study (S3 Text).

Vaccine and vaccination procedures

The rVSVΔG-ZEBOV-GP vaccine, developed by the Canadian National Laboratory under the patent number WO2004011488 A2 and licensed to BioProtection Systems (NewLink Genetics), was the unique intervention in this trial. The vaccine was subsequently sublicensed to Merck and was manufactured at IDT Biologika (Dessau-Rosslau, Germany). WHO supplied single-dose vials of 1×10^8 PFU (lot no 0030513) to conduct the trial at CERMEL, from a donation of rVSVΔG-ZEBOV-GP by the Canadian government to WHO. The dispensed vials were reconstituted in serial dilutions for vaccination. A single injection of 1 ml of the reconstituted vaccine for the required dose was administered intramuscularly into the deltoid muscle of volunteers at vaccination (S1 Fig).

Safety assessments

The nature, frequency, and severity of adverse events constituted the primary safety endpoint of the trial. Local and systemic reactogenicity symptoms and signs (solicited adverse events) were recorded for 14 days post-injection. Unsolicited adverse events, including laboratory anomalies, were recorded up to 28 days post-injection. Detailed descriptions of all serious adverse events were recorded throughout the study follow-up visits, as a secondary safety endpoint.

Solicited adverse events (pain, swelling, redness) were obtained by direct examination of the injection site, or direct questioning when follow-up occurred by telephone. Arthralgia and arthritic symptoms were later added as a solicited adverse event upon the request of the data and safety monitoring board. Participants were asked specifically if they were experiencing these symptoms.

rVSVΔG-ZEBOV-GP viraemia and shedding

Plasma, saliva, and urine samples (at screening and days 1, 2, and 7 post-vaccination) were processed and stored in Trizol LS at the study site, until rVSVΔG-ZEBOV-GP viral load determinations were performed by reverse transcriptase quantitative PCR as a secondary outcome. The lower limit of detection for rVSVΔG-ZEBOV-GP RNA was 30 copies/ml, and the lower level of quantification was 100 copies/ml (9).

Immunological assessments

As a secondary objective, enzyme-linked immunosorbent assays (ELISAs) were performed on days 0, 28, 56, 84, and 180 after injection. ZEBOV-specific antibody assays were conducted at the Institute for Virology, Marburg. Antibodies were detected using an antibody capture

ELISA based on inactivated Ebola Zaire Makona virus particles (15). ELISA for ZEBOV-GP-specific antibodies was performed at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) using the Kikwit-95 ZEBOV strain GP (standard operating procedure AP-03-35-00). Antibodies were reported as geometric mean titres (GMTs), or geometric mean concentrations, of arbitrary ELISA units (AEU) per millilitre with 95% confidence intervals, as indicated.

Neutralising antibodies (Nabs) were detected using either particles of Ebola virus (Zaire isolate Mayinga,AF086833), with the assays being performed in a BSL4 laboratory (Institute for Virology, Marburg), or VSV pseudovirions expressing the luciferase reporter gene complemented by GP from the Kikwit-95 ZEBOV strain, with assays being performed at USAMRIID.

All 4 assays were previously reported by our team (9,15) and other researchers working on this candidate vaccine in the US (7).

Statistical analysis

WHO estimated that a sample size of 74–124 participants would be needed across the VEBCON Consortium sites to show a 2-fold change in ZEBOV-specific antibody titres between vaccine doses and proposed a target sample size of approximately 250 participants for all sites (10). We described the frequency and intensity of adverse events using counts and percentages, means and standard deviations, or medians and interquartile ranges (IQRs), for skewed continuous variables. Chi-squared test or Fisher's exact test was used to compare pairwise proportions. Seropositivity rates were defined as the percentage of participants having AEU above a cutoff per vaccine group. Seroconversion rates were defined as the percentage of converted participants in each group. McNemar's test was used to compare the seropositivity between day 0 and other days. We used Fisher's test to perform inter-group comparisons and to determine the association between the seroconversion rate and seropositivity rate at each time point. Antibody concentrations or units were normalised using log transformations, and responses are reported as GMTs with 95% confidence intervals or geometric mean of AEU per millilitre with 95% confidence intervals. Student's test or Wilcoxon's paired test was used to compare magnitudes of antibody induced between day 0 and other days. All statistical analyses were conducted in R statistical software version 3.1.2 (16), except for viraemias (copies/millilitre of plasma), which were analysed with a Kruskal–Wallis test combined with Dunn's multiple comparison test using GraphPad Prism version 6.

RESULTS

From 21 November 2014 to 13 April 2015, 115 adults were vaccinated with a single injection of rVSVΔG-ZEBOV-GP at 5 different doses. Twenty adolescents and 20 children were vaccinated between 8 May and 7 July 2015 (Fig 1). Safety and immunogenicity data are reported until month 6 for adults, adolescents, and children for all 155 participants. In all, 108 (93%) adults and 36 (90%) adolescents and children attended all planned immunogenicity visits. Mean age and body mass index were similar among the 5 adult cohorts, with 21 adult women enrolled in the trial (Table 1).

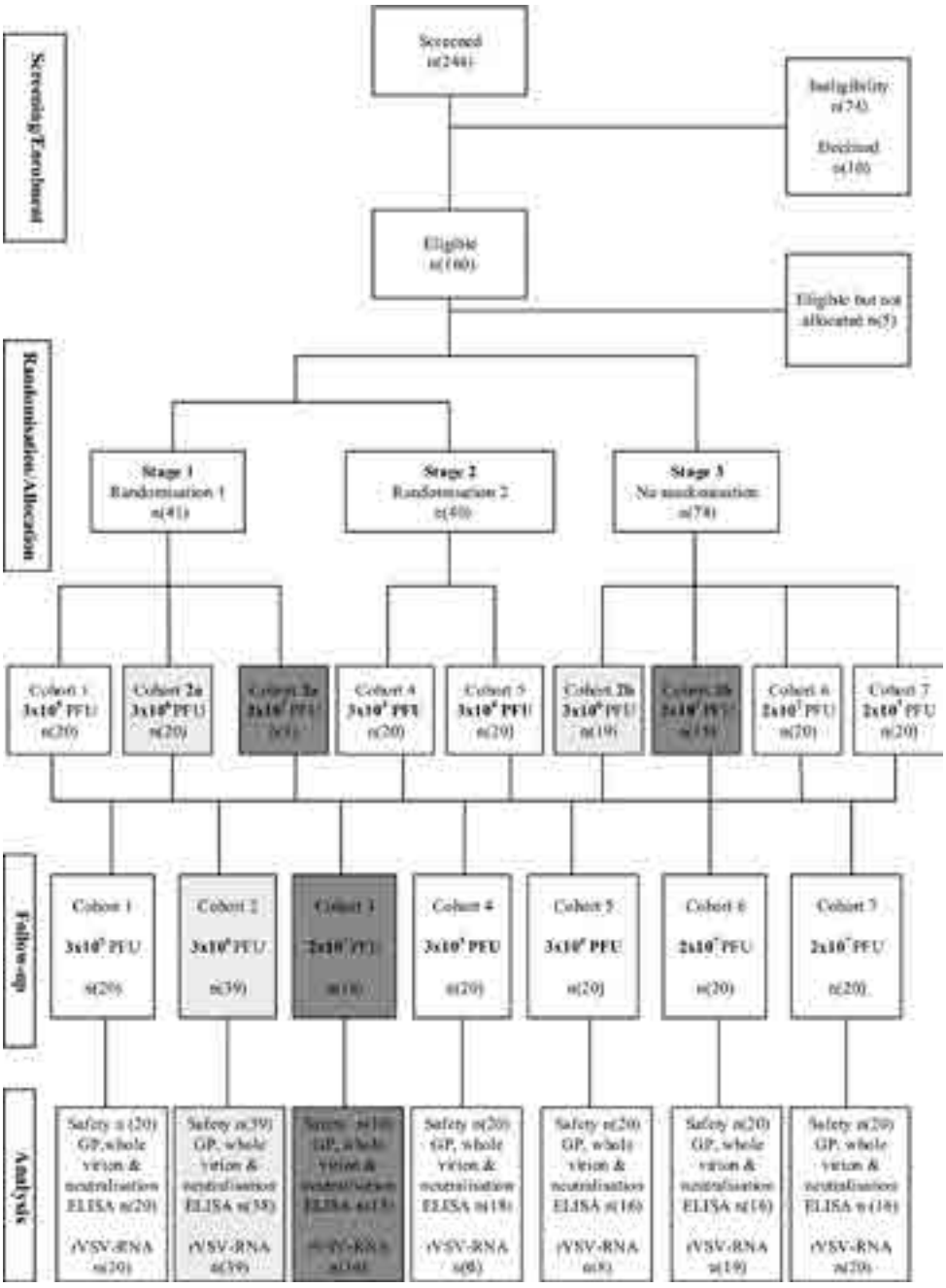

Figure 1. Participant flow diagram. Randomisation and flow of participants over a period of 6 months for adults (cohorts 1 to 5), adolescents (cohort 6; 13±17 years), and children (cohort 7; 6±12 years). Similar dose groups are matched with shading (light grey, 3 × 106 PFU; dark grey, 2 × 107 PFU). GP, glycoprotein; ELISA, enzyme-linked immunosorbent assay; PFU, plaque-forming units; rVSV, recombinant vesicular stomatitis virus.

Table 1. Baseline characteristics of study participants.

Characteristic	Adults (18–50 years)					Adolescents (13–17 years): 2×10^7 PFU ($N = 20$)	Children (6–12 years): 2×10^7 PFU ($N = 20$)
	3×10^3 PFU ($N = 20$)	3×10^4 PFU ($N = 20$)	3×10^5 PFU* ($N = 20$)	3×10^6 PFU* ($N = 39$)	2×10^7 PFU ($N = 16$)		
Sex, *n* (percent)							
Male	13 (65)	17 (85)	14 (70)	35 (90)	15 (94)	19 (95)	16 (80)
Female	7 (35)	3 (15)	6 (30)	4 (10)	1 (6)	1 (5)	4 (2)
Age (years), mean (SD)	27 (8)	23 (5)	28 (7)	27 (7)	25 (6)	15 (1)	9 (1)
BMI (kg/m^2), mean (SD)	23 (3)	23 (3)	23 (3)	23 (3)	23 (2)	18 (2)	16 (1)

*Already reported in The New England Journal of Medicine (doi: 10.1056/NEJMoa1502924) [9]: 3×10^5 PFU, N = 20, and 3×10^6 PFU, N = 19.
BMI, body mass index; PFU, plaque-forming units.

Assessment of 5 vaccine doses in adult volunteers

Reactogenicity and tolerability

Headaches, fatigue, pain at injection site, gastrointestinal symptoms, and subjective fever were the most frequent symptoms. Of these, 68% were mild and 32% moderately intense, with similar frequencies up to day 28 across cohorts in adults. There were no vaccine-related severe adverse vents (Tables 2 and S1).

Monocytes increased and lymphocytes decreased in the first week after vaccination in a dose-dependent fashion (S5 Table).

Mild-to-moderate symptoms reported at days 56, 84, and 180 and during unscheduled visits were considered unrelated to the study vaccine (S4 Table). Few haematological or biochemical changes of clinical significance were captured as adverse events, and these were followed up until resolution without sequelae.

A total of 11 adult participants experienced a serious adverse event. Six participants had malaria requiring hospitalisation, 2 underwent surgery due to appendicitis, and 1 was diagnosed with glaucoma. The last probably had the condition prior to enrolment after a detailed history was obtained, and is now receiving specialised care. Two individuals were hospitalised for bleeding after dental surgery and gastritis, respectively. All of these events were judged unrelated to the vaccine. Three women became pregnant after vaccination; they were monitored until delivery. Their neonates had no safety complications.

Immunogenicity

Eleven adults were excluded from either sampling and/or analysis of immunogenicity data: a male participant was HIV positive, 3 women became pregnant beyond day 28 after vaccination, and 7 participants received anti-tetanus vaccine/immunoglobulin. These participants were not sampled on subsequent visits (days 56, 84, and 180).

ZEBOV-GP-specific and ZEBOV antibodies

In all, 70%–100% of adult participants vaccinated with all doses $\geq 3 \times 10^4$ PFU reached a greater than 4.0-fold increase of ZEBOV-GP-specific GMT at day 28. ZEBOV-GP antibody GMTs peaked at day 56, with antibody levels persistently higher than baseline up to 6 months post-vaccination (Table 3).

Table 2. Reactogenicity to rVSVΔG-ZEBOV-GP vaccine until day 28 post-vaccination.

Adverse events/ grading	Number (percent) of participants						
	Adults					Children: 2×10^7 PFU ($N = 20$)	Adolescents: 2×10^7 PFU ($N = 20$)
	3×10^3 PFU ($N = 20$)	3×10^4 PFU ($N = 20$)	3×10^5 PFU* ($N = 20$)	3×10^6 PFU* ($N = 39$)	2×10^7 PFU ($N = 16$)		
Adverse events (highest grade)							
None	6# (30%)	8 (40%)	2 (10%)	5 (13%)	1 (6%)	0 (0%)	0 (0%)
Mild	9# (45%)	7 (35%)	11 (55%)	20 (51%)	10 (63%)	15 (75%)	14 (70%)
Moderate	5 (25%)	5 (25%)	7 (35%)	14 (36%)	5 (31%)	5 (25%)	6 (30%)
Solicited injection site reactions							
Pain							
None	17 (85%)	16 (80%)	17 (85%)	17 (44%)	7 (44%)	9 (45%)	10 (50%)
Mild	3 (15%)	3 (15%)	3 (15%)	22 (56%)	6 (37%)	9 (45%)	8 (40%)
Moderate	0 (0%)	1 (5%)	0 (0%)	0 (0%)	3 (19%)	2 (10%)	2 (10%)
Swelling							
None	20 (100%)	20 (100%)	20 (100%)	39 (100%)	15 (94%)	20 (100%)	19 (95%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	1 (5%)
Solicited systemic reactions							
Headache							
None	11 (55%)	15 (75%)	11 (55%)	17 (44%)	8 (50%)	10 (50%)	7 (35%)
Mild	6 (30%)	4 (20%)	6 (30%)	14 (36%)	6 (37%)	9 (45%)	10 (50%)
Moderate	3 (15%)	1 (5%)	3 (15%)	8 (20%)	2 (13%)	1 (5%)	3 (15%)
Myalgia							
None	17 (85%)	18 (90%)	18 (90%)	29 (74%)	14 (87%)	16 (80%)	14 (70%)
Mild	2 (10%)	1 (5%)	1 (5%)	6 (15%)	2 (13%)	3 (15%)	5 (25%)
Moderate	1 (5%)	1 (5%)	1 (5%)	4 (10%)	0 (0%)	1 (5%)	1 (5%)
Subjective fever							
None	18 (90%)	15 (75%)	19 (95%)	28 (72%)	9 (56%)	12 (60%)	12 (60%)
Mild	1 (5%)	3 (15%)	1 (5%)	9 (23%)	7 (44%)	7 (35%)	7 (35%)
Moderate	1 (5%)	2 (10%)	0 (0%)	2 (5%)	0 (0%)	1 (5%)	1 (5%)
Fatigue							
None	16 (80%)	14 (70%)	10 (50%)	20 (51%)	12 (75%)	10 (50%)	13 (65%)
Mild	3 (15%)	5 (25%)	7 (35%)	11 (28%)	3 (19%)	8 (40%)	3 (15%)
Moderate	1 (5%)	1 (5%)	3 (15%)	8 (20%)	1 (6%)	2 (10%)	4 (20%)
Objective fever							
None	17 (85%)	19 (95%)	19 (95%)	33 (85%)	12 (75%)	13 (65%)	15 (75%)
Mild	3 (15%)	1 (5%)	1 (5%)	3 (8%)	4 (25%)	7 (35%)	5 (25%)
Moderate	0 (0%)	0 (0%)	0 (0%)	3 (8%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal symptoms							
None	17 (85%)	13 (65%)	10 (50%)	28 (72%)	10 (63%)	8 (40%)	14 (70%)
Mild	3 (15%)	6 (30%)	7 (35%)	10 (26%)	6 (38%)	10 (50%)	3 (15%)
Moderate	0 (0%)	1 (5%)	3 (15%)	1 (3%)	0 (0%)	2 (10%)	3 (15%)
Chills							
None	20 (100%)	20 (100%)	20 (100%)	35 (90%)	16 (100%)	15 (75%)	17 (85%)
Mild	0 (0%)	0 (0%)	0 (0%)	4 (10%)	0 (0%)	5 (25%)	3 (15%)
Arthralgia							

(*Continued*)

About 11% (13/114) of adult participants had ZEBOV-GP-specific ELISA antibody concentrations > 200 AEU/ml at baseline. The proportions of individuals with high concentrations at baseline were inconsistent across vaccine groups and ranged from 0% to 25%. Antibody concentrations were significantly higher at day 56 post-vaccination in individuals with prior antibodies following vaccination with doses of 3×10^3, 3×10^4, and 3×10^6 PFU (Table 4).

The whole-virion assay is a less sensitive method to detect vaccine-induced antibody responses, which are directed against GP; 34% and 63% of vaccinees who received a dose equal to or more than 3×10^5 PFU reached a greater than 2.0-fold increase in ZEBOV antibody GMT

Table 2. (*Continued*)

Adverse events/ grading	Number (percent) of participants						
	Adults					Children: 2×10^7 PFU ($N = 20$)	Adolescents: 2×10^7 PFU ($N = 20$)
	3×10^3 PFU ($N = 20$)	3×10^4 PFU ($N = 20$)	3×10^5 PFU* ($N = 20$)	3×10^6 PFU* ($N = 39$)	2×10^7 PFU ($N = 16$)		
None	17 (85%)	17 (85%)	18 (90.0%)	26 (67%)	12 (75%)	17 (85%)	16 (80%)
Mild	0 (0%)	0 (0%)	0 (0%)	8 (21%)	1 (6%)	2 (10%)	3 (15%)
Moderate	3 (15%)	3 (15%)	2 (10%)	5 (13%)	3 (19%)	1 (5%)	1 (5%)
Mouth ulcer							
None	20 (100%)	20 (100%)	19 (95%)	37 (95%)	14 (88%)	18 (90%)	20 (100%)
Mild	0 (0%)	0 (0%)	1 (5%)	2 (5%)	1 (6%)	1 (5%)	0 (0%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	1 (5%)	0 (0%)
Skin lesion							
None	20 (100%)	19 (95%)	19 (95%)	38 (97%)	11 (69%)	19 (95%)	18 (90%)
Mild	0 (0%)	1 (5%)	1 (5%)	0 (0%)	4 (25%)	1 (5%)	2 (10%)
Moderate	0 (0%)	0 (0%)	0 (0%)	1 (3%)	1 (6%)	0 (0%)	0 (0%)
Blister							
None	20 (100%)	20 (100%)	20 (100%)	39 (100%)	15 (94%)	20 (100%)	20 (100%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Unsolicited adverse events							
Malaria							
None	19 (95%)	20 (100%)	15 (75%)	36 (92%)	8 (50%)	18 (90%)	17 (85%)
Mild	1 (5%)	0 (0%)	0 (0%)	0 (0%)	4 (25%)	0 (0%)	2 (10%)
Moderate	0 (0%)	0 (0%)	5 (25%)	3 (8%)	4 (25%)	2 (10%)	1 (5%)
Rhinitis							
None	18 (90%)	19 (95%)	18 (90%)	38 (97%)	12 (75%)	19 (95%)	17 (85%)
Mild	2 (10%)	1 (5%)	2 (10%)	1 (3%)	4 (25%)	1 (5%)	3 (15%)
Cough							
None	14 (70%)	17 (85%)	20 (100%)	39 (100%)	12 (75%)	17 (85%)	20 (100%)
Mild	3 (15%)	2 (10%)	0 (0%)	0 (0%)	4 (25%)	2 (10%)	0 (0%)
Moderate	3 (15%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Other							
None	14 (70%)	11 (55%)	3 (15%)	17 (44%)	0 (0%)	10 (50%)	14 (70%)
Mild	2 (10%)	4 (20%)	10 (50%)	13 (33%)	17 (68%)	8 (40%)	6 (30%)
Moderate	4 (20%)	5 (25%)	7 (35%)	9 (23%)	8 (32%)	2 (10%)	0 (0%)

*Already reported in The New England Journal of Medicine (doi: 10.1056/NEJMoa1502924) [9]: 3×10^5 PFU, N = 20, and 3×10^6 PFU, N = 19.
#Total number of participants reporting at least 1 event within 28 days after vaccination with rVSVΔG-ZEBOV-GP vaccine. Only events with the highest grade are reported per cohort.
PFU, plaque-forming units.

at day 28 and 56, respectively. Only 40% (30/74) of participants in the dose groups 3×10^5, 3×10^6, and 2×10^7 PFU had a greater than 2.0-fold increase in antibody persisting up to 6 months post-injection (Table 5).

About 27% (31/115) of adults had ZEBOV antibody concentrations > 500 AEU/ml at baseline, with inconsistent frequencies (5% to 56%) across dose levels. In adults with pre-vaccination antibodies, a dose as low as 3×10^4 PFU yielded a 2-fold increase in ZEBOV antibodies post-injection. Regardless of baseline status, the highest antibody titres were observed with the 2×10^7 PFU dose (Table 6).

Neutralising antibodies

Nabs for doses of 3×10^4, 3×10^5, 3×10^6, and 2×10^7 were detected in 52%, 55%, 82%, and 62% of recipients against VSV-based Ebola pseudovirions (pseudovirion neutralisation assay 50% (PsVNA50)), respectively, and in 70%, 84%, and 56% of recipients against ZEBOV virus particles. The highest Nab GMTs were observed in recipients of 2×10^7 PFU. About 35%, 13%, and 25% of participants in the dose groups 3×10^5, 3×10^6, and 2×10^7 PFU, respectively, had

Table 3. Endpoint geometric mean titres, seropositivity rates, and proportions of seroresponders to rVSVΔG-ZEBOV-GP measured by ZEBOV-GP ELISA in adults.

Dose	Time*	*N*	GMT (95% CI)	Seropositivity (>200 AEU/ml), *N* (percent)	Seroresponse (≥4×), *N* (percent)	*P* value			
						Early change in GMT[†]	Change in seropositivity[‡]	Seropositivity and seroresponse[Ω]	Later change in GMT[β]
3×10^3 PFU	D0	20	24 (9–60)	4 (20)	0 (0)	—	—	—	—
	D28	19	81 (35–184)	5 (26)	7 (37)	0.08	1	**0.03**	0.5
	D56	18	43 (14–131)	5 (28)	8 (44)	0.3	1	**0.006**	0.5
3×10^4 PFU	D0	20	23 (9–63)	2 (10)	0 (0)	—	—	—	—
	D28	19	489 (264–908)	14 (74)	16 (84)	**<0.001**	**0.001**	**0.01**	—
	D56	16	633 (305–1,314)	14 (88)	15 (94)	**<0.001**	**0.001**	0.1	—
3×10^5 PFU	D0	19	10 (4–22)	0 (0)	0 (0)	—	—	—	—
	D28	20	556 (280–1,101)	15 (75)	18 (90)	**<0.001**	**<0.001**	0.05	0.1
	D56	17	676 (246–1,859)	13 (77)	15 (88)	**<0.001**	**0.001**	**0.04**	**0.01**
	D84	17	536 (215–1,338)	14 (82)	15 (882)	**<0.001**	**<0.001**	**0.02**	**0.01**
	D180	16	365 (187–713)	12 (75)	15 (94)	**<0.001**	**0.002**	0.2	—
3×10^6 PFU	D0	39	16 (9–27)	3 (8)	0 (0)	—	—	—	—
	D28	39	1,245 (899–1,724)	39 (100)	39 (100)	**<0.001**	**<0.001**	1	**<0.001**
	D56	37	1,331 (977–1,813)	36 (973)	37 (100)	**<0.001**	**<0.001**	1	**<0.001**
	D84	35	994 (731–1,352)	33 (943)	35 (100)	**<0.001**	**<0.001**	1	**<0.001**
	D180	37	685 (546–858)	35 (95)	36 (97)	**<0.001**	**<0.001**	0.05	—
2×10^7 PFU	D0	16	47 (19–115)	4 (25)	0 (0)	—	—	—	—
	D28	16	1,503 (931–2,426)	16 (100)	16 (100)	**<0.001**	**0.001**	1	0.3
	D56	13	2,590 (1,604–4,182)	13 (100)	12 (92)	**<0.001**	**0.007**	1	**0.004**
	D84	14	1,826 (1,134–2,940)	14 (100)	13 (93)	**<0.001**	**0.004**	1	0.09
	D180	15	1,514 (997–2,301)	15 (100)	15 (100)	**<0.001**	**0.002**	1	—

ZEBOV-GP-specific antibodies are expressed in GMTs with 95% confidence intervals. Seropositivity is defined by geometric mean concentration > 200 AEU/ml. Seroresponse is defined by a ≥4-fold rise in GMT. P values < 0.05 are given in bold.

*Time point in day(s) since vaccination.

[†]Wilcoxon's test for paired data. P < 0.05 indicates a statistical difference in antibody titre between day 0 and other days.

[‡]McNemar's test. P < 0.05 indicates a statistical difference in seropositivity rate between day 0 and other days (28, 56, and 84 days post-vaccination).

[Ω]Fisher's test. P < 0.05 indicates a statistical association between seropositivity and seroresponse for each time point.

[β]Wilcoxon's test for paired data. P < 0.05 indicates a statistical difference in antibody titre between day 180 post-vaccination and days 28, 56, and 84 post-vaccination.

AEU, arbitrary enzyme-linked immunosorbent assay units; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titre; PFU, plaque-forming units; ZEBOV, Zaire Ebola virus.

baseline Nabs against ZEBOV virus particles (defined as GMT > GMT + SD at D0). Higher Nab GMTs were observed at day 28 regardless of baseline antibody status (Tables7, S8 and S9).

Table 4. Endpoint geometric mean titres measured by USAMRIID ZEBOV-GP ELISA in adults with and without baseline specific antibodies.

Dose	Time *	With baseline GP-specific antibodies		Without baseline GP-specific antibodies		*P* value
		N	GMT (95% CI)	*N*	GMT (95% CI)	
3×10^3 PFU	D0	4	346 (244–492)	16	12 (59–30)	**0.002**
	D28	4	305 (157–592)	15	57 (22–148)	0.08
	D56	4	295 (157–554)	14	25 (7–90)	**0.04**
3×10^4 PFU	D0	2	549 (328–919)	18	16 (6–43)	**0.02**
	D28	2	3,489 (1,083–11,245)	17	388 (215–701)	**0.04**
	D56	2	5,229 (2,435–11,232)	14	468 (234–937)	**0.03**
3×10^5 PFU	D0	—	—	19	10 (4–22)	—
	D28	—	—	20	556 (280–1,101)	—
	D56	—	—	17	676 (246–1,859)	—
	D84	—	—	17	536 (215–1,338)	—
	D180	—	—	16	365 (187–713)	—
3×10^6 PFU	D0	3	310 (181–532)	36	12 (7–20)	**0.004**
	D28	3	6,307 (1,125–35,368)	36	1,088 (813–1,454)	**0.06**
	D56	3	4,263 (1,885–9,640)	34	1,201 (882–1,634)	**0.01**
	D84	3	2,984 (1,720–5,175)	32	897 (657–1,223)	**0.004**
	D180	3	1,616 (1,225–2,133)	34	635 (505–797)	**0.004**
2×10^7 PFU	D0	4	375 (223–632)	12	23 (10–56)	**<0.001**
	D28	4	1,466 (761–2,824)	12	1,516 (820–2,801)	0.8
	D56	4	2,174 (1,713–2,760)	9	2,799 (1,401–5,590)	0.7
	D84	4	1,514 (882–2,597)	10	1,968 (1,036–3,738)	0.6
	D180	4	1,013 (672–1,526)	11	1,753 (1,028–2,990)	0.1

Seropositivity at day 0 (D0) defined by a GMT > 200 AEU/ml. P values < 0.05 are given in bold.

*Time point in day(s) since vaccination.

†Wilcoxon's test. P < 0.05 indicates a statistical difference in antibody titre at each time point between the adults with and without the antibodies at D0.

AEU, arbitrary enzyme-linked immunosorbent assay units; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titre; GP, glycoprotein; PFU, plaque-forming units; USAMRIID, US Army Medical Research Institute of Infectious Diseases; ZEBOV, Zaire Ebola virus.

The vaccine dose of 2 × 107 PFU in adult, adolescent, and child volunteers

Reactogenicity and tolerability

Twenty adolescents aged 13–17 years and 20 children aged 6–12 years were vaccinated with 1 intramuscular dose of 2×10^7 PFU. Adolescents and children reported mostly headaches, fatigue, pain at injection site, gastrointestinal symptoms, and subjective fever. All reported symptoms were of mild (81% adolescents, 82% children) to moderate (19% adolescents, 18% children) intensity (Table 2).

As in adults, a general reduction in leukocyte counts was observed in adolescents and children within the first 2 days post-injection; leukocytes gradually restored to baseline values by day 28. An increase in monocyte and lymphocyte counts was observed between days 2 and 7, with lymphocytes rapidly restoring to baseline values by day 7 (S5 Table). No vaccine-related serious or severe adverse events occurred. One child was hospitalised for malaria.

rVSVΔG-ZEBOV-GP viraemia and shedding

Compared to adults vaccinated with 2×10^7 PFU, rVSVΔG-ZEBOV-GP RNA copy numbers in both adolescents and children were significantly higher at 1,592 (IQR 1,019–2,704) and 1,109 (IQR 663–1,963), respectively, versus 532 (IQR 373–898) in adults ($P = 0.001$) at day 2 post-injection (Table 8).

For viral shedding, there was a low percentage of positive samples at day 2, albeit below the level of quantification. At day 7, there was 1 child and 1 adolescent who had quantifiable

Table 5. Geometric mean titres, seropositivity rates, and proportions of seroresponders to rVSVΔG-ZEBOV-GP measured by whole-virion ELISA in adults.

Dose	Time point*	*N*	GMT (95% CI)	Seropositivity (>500 AEU/ml), *N* (percent)	Seroresponse, *N* (percent)		*P* value			
					≥2×	≥4×	Change in GMT[†]	Change in seropositivity[‡]	Seropositivity and seroresponse (≥2×)[Ω]	Seropositivity and seroresponse (≥4×)[β]
3×10^3 PFU	D0	20	718 (529–975)	5 (25)	—	—	—		—	—
	D28	20	673 (975–896)	4 (20)	0 (0)	0 (0)	0.28	—	1	1
	D56	18	803 (565–1,141)	6 (33)	1 (6	0 (0)	0.40	—	0.33	1
3×10^4 PFU	D0	20	949 (627–1,435)	7 (35)	—	—	—	—	—	—
	D28	20	1,015 (647–1,591)	7 (35)	1 (5)	0 (0)	0.20	—	0.35	1
	D56	16	1,029 (628–1,686)	5 (31)	1 (6)	0 (0)	0.40	—	0.31	0.31
3×10^5 PFU	D0	19	575 (440–751)	1 (5)	—	—	—	—	—	—
	D7	20	641 (481–851)	3 (15)	2 (11)	0 (0)	0.42	0.47	**0.02**	1
	D14	18	674 (502–905)	4 (22)	2 (12)	1 (6)	0.58	0.24	**0.04**	**1**
	D28	20	1,887 (1,154–30,853)	13 (65)	11 (58)	10 (53)	**0.01**	**0.002**	**<0.001**	**<0.001**
	D56	17	1,402 (842–2,333)	8 (47)	7 (44)	7 (44	**0.02**	**0.02**	**<0.001**	**<0.001**
	D84	17	1,667 (1,098–2,531)	12 (71)	10 (63)	6 (38)	**0.03**	**0.004**	**0.001**	**0.09**
	D180	16	1,194 (809–1,762)	9 (56)	5 (33)	5 (33)	0.18	**0.02**	**0.02**	**0.02**
3×10^6 PFU	D0	39	693 (565–850)	9 (23)	—	—	—	—	—	—
	D7	38	726 (573–920)	8 (21)	2 (5)	0 (0)	0.18	1	**0.04**	1
	D14	38	1,037 (734–1,463)	15 (40)	11 (29)	4 (11)	**0.003**	0.07	**<0.001**	**0.01**
	D28	39	1,445 (1,013–2,062)	21 (54)	17 (44)	11 (28)	**<0.001**	**0.001**	**<0.001**	**<0.001**
	D56	37	1,824 (1,316–2,527)	27 (73)	24 (65)	10 (27)	**<0.001**	**<0.001**	**<0.001**	**0.03**
	D84	36	1,586 (1,179–2,133)	25 (69)	19 (53)	8 (22)	**<0.001**	**<0.001**	**<0.001**	**0.07**
	D180	38	1,450 (1,105–1,903)	26 (68)	19 (50)	7 (18)	**<0.001**	**<0.001**	**<0.001**	**0.07**
2×10^7 PFU	D0	16	1,625 (879–3,006)	9 (56)	—	—	—	—	—	—
	D7	16	1,220 (695–2,142)	7 (44)	0 (0)	0 (0)	**0.01**	0.47	1	1
	D14	16	2,153 (1,140–4,067)	10 (63)	2 (13)	1 (6)	0.16	1	0.5	1
	D28	16	3,958 (2,249–6,967)	13 (81)	7 (44)	4 (25)	**0.003**	0.22	0.21	0.5
	D56	13	4,402 (2,888–6,711)	12 (92)	6 (46)	4 (31)	**0.002**	0.22	1	1
	D84	14	3,638 (2,372–5,580)	13 (93)	7 (50)	3 (21)	**0.01**	0.13	1	1
	D180	15	2,963 (1,769–4,962)	13 (87)	6 (40)	3 (20)	0.3	0.22	0.48	1

ZEBOV antibodies are expressed in GMTs with 95% confidence intervals. Seropositivity is defined by a GMT > 500 AEU/ml. Seroresponse is expressed as a ≥2-fold or ≥4-fold increase in titre. P values < 0.05 are given in bold.

*Time point in day(s) since vaccination.

[†]Wilcoxon's test for paired data. P < 0.05 indicates a statistically significant difference in antibody titre between day 0 and other days.

[‡]McNemar's test. P < 0.05 indicates a statistically significant difference in seropositivity rate between day 0 and other days.

[Ω]Fisher's test. P < 0.05 indicates a statistical association between seropositivity and seroresponse (≥2×) for each time point.

[β]Fisher's test. P < 0.05 indicates a statistical association between seropositivity and seroresponse (≥4×) for each time point.

AEU, arbitrary enzyme-linked immunosorbent assay units; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titre; PFU, plaque-forming units.

RNA in urine. Saliva investigations showed that 42% and 30%, respectively, of adolescents and children had detectable RNA, corresponding with peak viraemia at day 2. At day 7, a considerably higher proportion of adolescents and children, 78% and 35% respectively, had RNA-positive saliva, with most samples being quantifiable (Figs 2 and S2; S13 – S15 Tables).

Immunogenicity

ZEBOV-GP-specific and ZEBOV antibodies

In all, 90% and 100% of children and adolescents, respectively, receiving 2×10^7 PFU had ZEBOV-GP-specific antibodies at day 28. Antibody titres were similar between adolescents, adults, and children using GP ELISA regardless of baseline antibody status (Figs 3, 4, S3 and S4).

Table 6. Geometric mean titres of ZEBOV antibodies in adults by baseline antibody status measured by whole-virion ELISA.

Dose	Time*	With baseline ZEBOV antibodies			Without baseline ZEBOV antibodies		
		N	GMT (95% CI)	*P* value†	*N*	GMT (95% CI)	*P* value†
3 × 10^3 PFU	D0	5	2,129 (1,276–3,552)	—	15	500 (—)	—
	D28	5	1,637 (780–3,435)	0.31	15	500 (—)	—
	D56	5	2,196 (1,064–4,532)	0.81	13	545 (466–638)	—
3 × 10^4 PFU	D0	7	3,119 (2,106–4,621)	—	13	500 (—)	—
	D28	7	3,778 (2,642–5,403)	0.22	13	500 (—)	—
	D56	6	3,428 (1,618–7,260)	0.43	10	500 (—)	—
3 × 10^5 PFU	D0	1	7,085 (—)	—	18	500 (—)	—
	D7	1	6,325 (—)	—	19	567 (479–672)	0.37
	D14	1	6,110 (—)	—	17	592 (495–708)	0.18
	D28	1	4,372 (—)	—	19	1,805 (1,084–3,007)	**0.003**
	D56	—	—	—	17	1,402 (831–2,364)	**0.02**
	D84	1	3,977 (—)	—	16	1,579 (1,027–2,427)	**0.006**
	D180	1	2,587 (—)	—	15	1,134 (758–1,695)	**0.02**
3 × 10^6 PFU	D0	9	2,055 (1,458–2,896)	—	30	500 (—)	—
	D7	9	2,108 (1,184–3,753)	0.30	29	521 (481–565)	1
	D14	9	4,129 (1,986–8,585)	**0.04**	29	675 (534–854)	**0.02**
	D28	9	4,686 (2,905–7,559)	**0.01**	30	1,015 (714–1,445)	**0.002**
	D56	9	3,936 (2,009–7,711)	**0.02**	28	1,424 (1,021–1,987)	**<0.001**
	D84	9	3,371 (1,834–6,196)	**0.04**	27	1,234 (920–1,654)	**<0.001**
	D180	9	3,046 (1,758–5,276)	0.07	29	1,152 (882–1,504)	**<0.001**
2 × 10^7 PFU	D0	9	4,065 (2,274–7,266)	—	7	500 (—)	—
	D7	9	2,440 (1,180–5,048)	**0.007**	7	500 (—)	—
	D14	9	4,422 (2,339–8,361)	0.42	7	854 (383–1,900)	0.37
	D28	9	5,633 (2,973–1,067)	**0.05**	7	2,515 (977–6,470)	0.06
	D56	8	4,365 (2,286–8,332)	0.07	5	4,463 (2,634–7,561)	0.06
	D84	8	4,179 (2,169–8,054)	0.31	6	3,024 (1,792–5,102)	**0.03**
	D180	9	3,691 (1,877–7,255)	0.50	6	2,131 (956–4,752)	0.06

Results are expressed in GMTs of AEU/millilitre with 95% confidence intervals. Seropositivity is defined by a GMT > 500 AEU/ml. Values below the threshold were given arbitrary units of 500 AEU/ml. P values < 0.05 are given in bold.

*Time point in day(s) since vaccination.

†Wilcoxon's test for paired data used to compare antibody titres between time points; a P value < 0.05 indicates a statistically significant difference in antibody titre between day 0 and other days (D7, D14, D28, D56, D84, and D180). Samples from D84 for doses 3 × 10^3 and 3 × 10^4 PFU were not analysed.

AEU, arbitrary enzyme-linked immunosorbent assay units; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titre; PFU, plaque-forming units; ZEBOV, Zaire Ebola virus.

By day 28, 70% and 60% of adolescents and children, respectively, were seropositive with whole-virion ELISA, compared to 81% of adults injected with 2 × 10^7 PFU. Using a more sensitive GP ELISA, we obtained higher seropositivity rates, 100%, 100%, and 90% for adults, adolescents, and children, respectively, at day 28. We observed a ≥4-fold increase in ZEBOV-GP-specific antibody titres in about 90%–100% of adults, adolescents, and children consistently from day 28 to 180 post-injection. However, ZEBOV-GP-specific antibodies increased up to day 180 in children and adolescents (Table 9), while in adults, it peaked at day 56, and there was a decline until day 180 (Tables 3 and 4).

Lower proportions of adults, adolescents, and children had a ≥4-fold increase in ZEBOV antibodies with whole-virion ELISA. Considering a ≥2-fold increase for this less sensitive ELISA, the yielded proportions were still much lower than those seen with GP ELISA. However, the proportion of adolescents and children with a ≥2- or ≥4-fold increase in ZEBOV antibodies increased from day 28 to 56, in contrast to the lack of difference between these time points in adults (Tables 5, 6 and S6). Thirteen percent of the children, but none of the adolescents, were seropositive for ZEBOV antibodies at baseline. None of the children and 7% of the adolescents were seropositive for ZEBOV-GP antibodies at baseline. As in adults, children with ZEBOV

antibodies at baseline had higher GMTs at days 28 and 56 compared to those without baseline antibodies (Tables 6 and S7).

Table 7. Geometric mean titres, seropositivity rates, and proportions of seroresponders to rVSVΔG-ZEBOV-GP measured by ZEBOV PsVNA50 in adults.

Dose	Time point*	*N*	GMT (95% CI)	Seropositivity (>20 titre), *N* (percent)	Seroresponse (≥4×), *N* (percent)	*P* value		
						Change in GMT†	Change in concentration‡	Change in seropositivityΩ
3 × 10^3 PFU	D0	19	19 (—)	0 (0)	0 (0)	—	—	**—**
	D28	19	24 (19–32)	3 (16)	2 (11)	0.1	0.2	**0.01**
3 × 10^4 PFU	D0	19	19 (—)	0 (0)	0 (0)	—	—	**—**
	D28	19	70 (36–135)	10 (53)	8 (42)	**0.005**	**0.004**	**0.001**
3 × 10^5 PFU	D0	19	19 (—)	0 (0)	0 (0)	—	—	**—**
	D28	20	66 (34–128)	11 (55)	7 (35)	**0.005**	**0.004**	**0.004**
	D180	16	21 (17–27)	1 (6)	1 (6)	1	1	0.06
3 × 10^6 PFU	D0	39	19 (—)	0 (0)	0 (0)	—	—	**—**
	D28	39	81 (56–119)	32 (82)	18 (46)	**<0.001**	**<0.001**	**0.009**
	D180	37	20 (19–22)	6 (16)	0 (0)	**0.03**	**0.04**	1
2 × 10^7 PFU	D0	16	19 (—)	0 (0)	0 (0)	—	—	**—**
	D28	16	126 (56–285)	10 (63)	10 (63)	**0.005**	**0.004**	**<0.001**
	D56	13	102 (52–202)	9 (69)	9 (69)	**0.009**	**0.007**	**0.001**
	D84	14	30 (23–41)	8 (57)	1 (7)	**0.01**	**0.01**	1
	D180	15	26 (21–34)	6 (40)	1 (7)	**0.03**	**0.04**	0.4

Results are expressed as geometric mean PsVNA50 neutralisation titres with 95 CIs. Seropositivity was defined as GMT > 20. Values below the threshold were given arbitrary titres of 19. Seroresponse was defined as a ≥4-fold increase. P values < 0.05 are given in bold.

*Time point in day(s) since vaccination.

†Wilcoxon's test for paired data. P < 0.05 indicates a statistical difference in antibody titre between day 0 and other days.

‡McNemar's test used to compare concentration between day 0 and other days. P value < 0.05 indicates a statistical difference in seropositivity rate between day 0 and other days.

ΩFisher's test used to compare seropositivity rate between day 0 and each time point. P value < 0.05 indicates a statistical difference between tested time points.

GMT, geometric mean titre; PFU, plaque-forming units; PsVNA50, pseudovirion neutralisation assay 50%; ZEBOV, Zaire Ebola virus.

Table 8. Description of viraemia by dose and age.

Time point*	Adults										Children: 2 × 10^7 PFU		Adolescents: 2 × 10^7 PFU		*P* value†
	3 × 10^3 PFU		3 × 10^4 PFU		3 × 10^5 PFU		3 × 10^6 PFU		2 × 10^7 PFU						
	N	Median copy number (IQR)	*N*	Median copy number (IQR)	*N*	Median copy number (IQR)	*N*	Median copy number (IQR)	*N*	Median copy number (IQR)	*N*	Median copy number (IQR)	*N*	Median copy number (IQR)	
D0	5	0 (0–0)	6	0 (0–0)	19	0 (0–0)	35	0 (0–0)	16	0 (0–0)	20	0 (0–0)	20	0 (0–0)	0.2
D1	6	0 (0–0)	8	0 (0–0)	18	3 (0–13)	33	228 (150–481)	16	334 (301–1,001)	4	731 (507–2,142)	19	655 (412–912)	0.5
D2	5	0 (0–0)	6	1 (0–12)	19	4 (0–30)	35	793 (401–1,286)	16	532 (373–898)	20	1,109 (663–1,963)	19	1,592 (1,019–2,704)	**0.001**
D7	5	0 (0–0)	6	4 (2–51)	12	1 (0–6)	32	7 (0–22)	16	4 (0–29)	19	0 (0–17)	17	0 (0–1)	0.1

All viraemia values expressed as median (IQR). P values < 0.05 are given in bold.

*Time point in day(s) since vaccination.

†Kruskal Wallis test. P < 0.05 indicates a significant statistical difference in viraemia values between the 3 groups (adults, children, and adolescents) at each time point at the 2 × 10^7 PFU dose.

IQR, interquartile range; PFU, plaque-forming units.

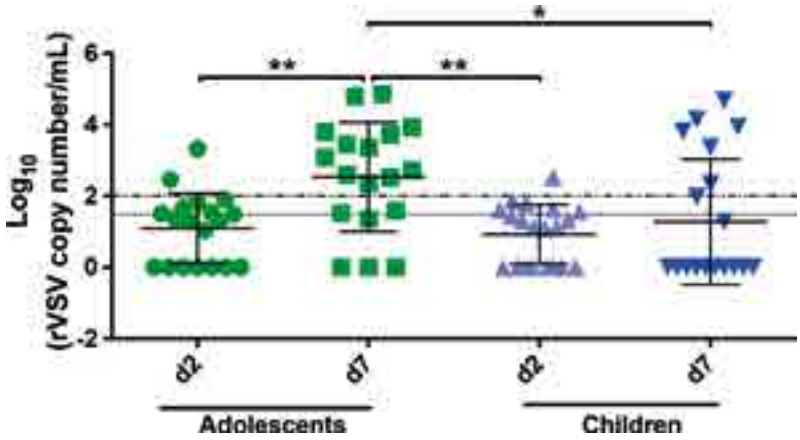

Figure 2. Viral load in saliva for children and adolescents. rVSVΔG-ZEBOV-GP (rVSV) RNA copy numbers in saliva presented as log10 rVSV RNA copies/ml from day 2 and 7 (d2 and d7) post-injection in adolescents and children vaccinated with 2 × 107 PFU. The broken line denotes the limit of quantitation, and the dotted line denotes the limit of detection. About 67% (12/18) and 30% (6/20) adolescents and children, respectively, had samples above the limit of quantification at day 7. *P < 0.05; **P < 0.01.PFU, plaque-forming units.

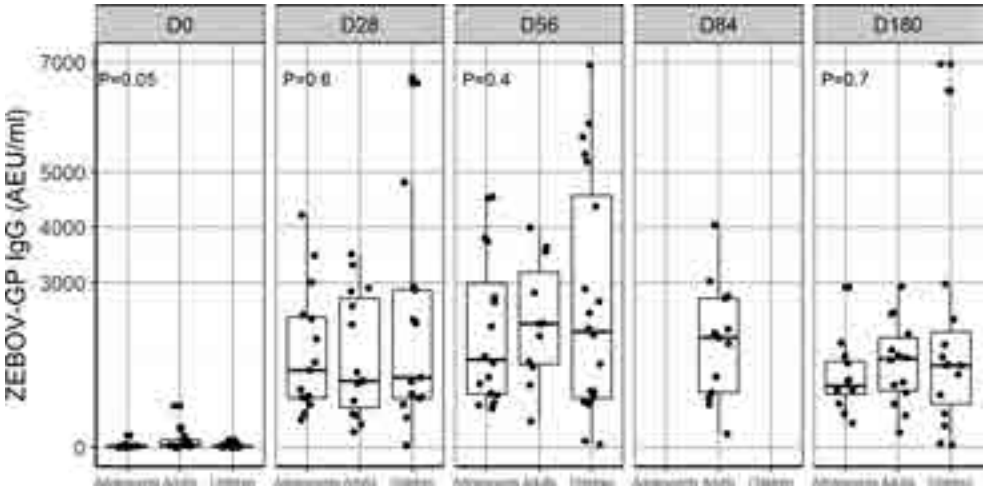

Figure 3. Glycoprotein antibody distribution by age group: Comparison of distribution of ZEBOV-GP IgG antibodies (AEU/ml) measured by USAMRIID ZEBOV-GP ELISA for dose 2 × 107 PFU administered to children, adolescents, and adults from day 0, 28, 56, 84, and 180. Data were not available for children and adolescents at D84. P < 0.05 indicates a statistical difference in ZEBOV-GP IgG between children, adolescents, and adults. AEU, arbitrary enzyme-linked immunosorbent assay units; ELISA, enzyme-linked immunosorbent assay; GP, glycoprotein; PFU, plaque-forming units; USAMRIID, US Army Medical Research Institute of Infectious Diseases; ZEBOV, Zaire Ebola virus.

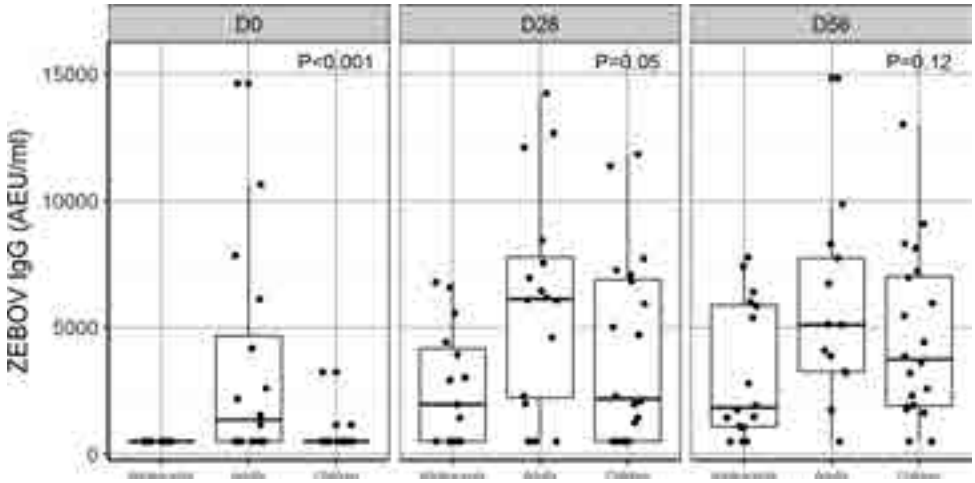

Figure 4. Antibody responses to whole-virion ELISA (AEU/ml) by age group: Comparison of geometric mean concentration of IgG antibodies for children, adolescents, and adults vaccinated with the 2 × 107 PFU dose at day 0, 28, and 56. P < 0.05 indicates a statistical difference in antibody concentrations between age groups at the measured time points. AEU, arbitrary enzyme-linked immunosorbent assay units; ELISA, enzyme-linked immunosorbent assay; PFU, plaque-forming units; ZEBOV, Zaire Ebola virus.

Table 9. Geometric mean titres, seropositivity rates, and proportions of seroresponders to rVSVΔG-ZEBOV-GP measured by ZEBOV-GP ELISA in children.

Cohort (2×10^7 PFU)	Time point*	*N*	GMT (95% CI)	Seropositivity (>200 AEU/ml), *N* (percent)	Seroresponse (≥4×), *N* (percent)	*P* value		
						Change in GMT†	Change in seropositivity‡	Seropositivity and seroresponseΩ
Children	D0	15	15 (7–35)	0 (0)	0 (0)	—	—	**—**
	D28	20	1,620 (806–3,259)	18 (90)	19 (95)	**<0.001**	**<0.01**	**0.1**
	D56	20	1,599 (921–2,777)	18 (90)	20 (100)	**<0.001**	**<0.01**	1
	D180	20	2,069 (1,005–4,258)	18 (90)	18 (90)	**<0.001**	**<0.01**	**<0.01**
Adolescents	D0	15	12 (5–28)	1 (7)	0 (0)	—	—	**—**
	D28	15	1,427 (1,024–1,989)	15 (100)	15 (100)	**0.001**	**0.002**	1
	D56	16	1,744 (1,264–2,407)	16 (100)	16 (100)	**<0.001**	**<0.001**	1
	D180	17	2,541 (1,317–4,906)	17 (100)	17 (100)	**<0.001**	**<0.001**	1

Results are presented as GMTs with 95% confidence intervals. Seropositivity is defined by geometric mean concentration > 200 AEU/ml. Seroresponse is defined by a ≥4-fold rise in GMT. P values < 0.05 are given in bold.

*Time point in day(s) since vaccination.

†Wilcoxon's test for paired data. P < 0.05 indicates a statistical difference in antibody titre between day 0 and other days.

‡McNemar's test. P < 0.05 indicates a statistical difference in seropositivity rate between day 0 and other days.

ΩFisher's test. P < 0.05 indicates a statistical association between seropositivity and seroresponse for each time point.

AEU, arbitrary enzyme-linked immunosorbent assay units; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titre; GP, glycoprotein; PFU, plaque-forming units; ZEBOV, Zaire Ebola virus.

Neutralising antibodies

Against VSV pseudovirions, about 73% of children and adolescents elicited Nabs, with higher GMTs occurring at day 56 compared to day 28. In all, 95% and 80% of children and adolescents, respectively, had ZEBOV Nabs at day 28. Overall, children produced significantly higher GMTs of Nabs against ZEBOV particles (20 (95% CI: 13–32) compared to adolescents and adults, 10 (95% CI: 8–14) and 10 (95% CI: 6–14), respectively, $P = 0.04$) (S10–S12 Tables).

DISCUSSION

Although the 2014–2016 EVD emergency in western Africa has ended, the increasing mobility of people between remote and urban areas and the weak health systems in Ebolavirus endemic countries suggest that a future outbreak could reassert itself as a major international threat (17,18). Risks include increased human-to-human secondary transmission as in the recent epidemic (19) as well as continuing transmission after recovery. Halting transmission by vaccination will be key in curbing future outbreaks (20). The rVSVΔG-ZEBOV-GP and ChAd3-ZEBOV vaccine candidates were selected by WHO in August 2014 for fast track clinical evaluation (6). As part of these efforts, we examined a range of doses for rVSVΔG-ZEBOV-GP in adults as well as safety and immunogenicity in children.

As reported earlier, rVSVΔG-ZEBOV-GP doses of 3×10^5 and 3×10^6 PFU were well tolerated by 39 Lambaréné participants until day 28 and were safe up to 6 months (9). Comparable to studies in Guinea (21) and US adults (7), transient cases of arthralgia were reported after vaccination (9,21,22), but no case of arthritis. In Kilifi, Kenya, there were 2 self-limiting, low-severity, and short-duration cases of arthritis (9,23). This contrasts with a higher frequency of vaccine-induced arthritis (24%), dermatitis (9.8%), and vasculitis (2%) in Geneva (9,10,24) and more recently in Canada, the US, and Spain (25). There may be similarities between rVSVΔG-ZEBOV-GP vaccine and rubella vaccine, which also causes transient arthritides in some populations (26–28).

Ongoing studies are investigating the potential mechanisms by which rVSVΔG-ZEBOV-GP vaccine might disseminate into peripheral tissues and induce arthritides in specific hosts. The magnitude of innate immune responses to rVSVΔG-ZEBOV-GP vaccine correlated with the peak of rVSV RNA at day 1 in vaccinees of both Geneva and Lambaréné cohorts (29). Importantly, high-dose vaccinees who experienced arthritis in Geneva had a significantly lower magnitude of early immune response compared to high-dose vaccinees who did not experience arthritis. These findings suggest that early and appropriate (in nature and magnitude) innate immune responses play a key role in limiting viral replication and dissemination to tissues and thus prevent the risk of arthritis. With lower vaccine dose (3×10^5 PFU), the strength of early innate immune responses was similar in cases both with and without arthritis. Thus, rVSV-ZEBOV-induced arthritis may occur through mechanisms related to either vaccine dose or underlying factors that influence immune responses in vaccinees (29).

We observed higher and persistent viraemia in children and adolescents as well as shedding in saliva and urine, in contrast to the very low proportions or no shedding previously reported in the saliva of American and European adults vaccinated with 3×10^6 to 5×10^7 PFU (7,9,10). The shedding in saliva did not correlate with oral symptoms. Although no alarming symptoms have been detected so far, our finding suggests that a vaccine dose of 2×10^7 PFU exposed the paediatric population to prolonged or uncontrolled viraemia, with potential to disseminate to peripheral tissues. Specific studies are needed to elucidate the underlying mechanisms prolonging viraemia and causing shedding, such as differences in innate responses to vaccine between adults and younger participants. It is also necessary to assess any potential dissemination of rVSV-ZEBOV among household members of vaccinated children.

We observed dose-dependent antibody responses to the rVSVΔG-ZEBOV-GP vaccine. A very low dose (≤3×10^3 PFU) did not generate antibodies measured with either whole-virion or ZEBOV-GP-specific ELISA. In all individuals vaccinated with 3×10^4, 3×10^5, 3×10^6, and 2×10^7 PFU, the vaccine induced significant increases in ZEBOV-GP-specific antibodies measured by ZEBOV-GP ELISA alone for 3×10^4 PFU and by both whole-virion and GP ELISAs for the other vaccine doses. The highest GMTs were observed with 2×10^7 PFU irrespective of the ELISA method used.

As previously reported (9,11,12), our participants harboured naturally acquired antibodies against ZEBOV, or possibly related viruses. Western blot analysis of sub-samples showed that these antibodies were directed more often against nucleocapsid and matrix proteins of ZEBOV and not against GP. Nonetheless, 11% of our adults had ZEBOV-GP-specific antibodies before vaccination using the GP-specific ELISA. Individuals with baseline antibodies developed higher antibody titres with a dose as low as 3×10^4 PFU compared to those without. The vaccine may have elicited higher titres of antibodies in the presence of natural GP-specific antibodies but also in the presence of antibodies directed against other viral components including nucleocapsid and matrix proteins (detected in baseline sera of some study participants) (9).

In adults, vaccine-induced antibodies peaked at day 56 and declined slowly by day 180. In children and adolescents, who showed high viraemia at day 2 and shed the vaccine until day 7, antibody titres increased until day 180. The kinetics of antibodies after vaccination may be affected by the specificity of pre-existing antibodies, and persistent vaccine replication may enhance immunogenicity. Also, the highest titres of Nabs against Ebola virus, which paralleled those against VSV pseudovirions, were generated at day 28 post-injection, regardless of baseline seropositivity. The relative roles of neutralising, GP, and non-GP antibodies in protection against EVD remain undefined, so it is difficult to draw conclusions on the clinical significance of correlations between GP-binding and neutralising antibodies produced after vaccinations.

The vaccine dose of 2×10^7 PFU showed the optimal safety versus immunogenicity balance in our adult cohorts as well as in the Geneva and Hamburg cohorts (29,30). These findings support the choice to use this dose in the context of outbreaks (8). However, our data cannot explain the protection induced by the vaccine within 10 days observed in a phase III trial in Guinea (8) as the seroconversion rates and antibody titres were very weak before day 28 irrespective of the vaccine dose. Innate immune components induced immediately after vaccination may have played an important role in early protection. A recent study demonstrated the direct influence of innate immune responses on this vaccine's safety and immunogenicity (29), a finding which supports the interest in assessing the efficacy of this vaccine beyond *Zaire ebolavirus* spp. as innate mechanisms can be cross-reactive.

Lower doses could be considered in vaccination strategies for children and individuals with impaired innate immune responses to control early rVSV replication. The dose of 3×10^5 PFU generated significantly fewer rVSV RNA copies and shorter rVSV replication cycles but high antibody titres, so is of interest. As an incidental finding in our area, where Ebola virus transmission is endemic, a proportion of participants had antibodies directed against the whole-virus or GP-specific antigen before vaccination (11,12,31). In those participants, a vaccine dose

as low as 3×10^4 PFU induced high antibody titres, suggesting lower vaccine doses should be considered in boosting strategies.

There are some limitations of our observations. For example, we cannot relate viral shedding in saliva with the oral symptoms reported by adolescents and children, suggesting that further studies are needed to evaluate this finding. We did not stratify participants based on antibody status at enrolment; future studies in Ebola virus endemic areas where such stratification is inherent in the design will provide insights into the relationships between naturally acquired antibodies and vaccine-induced immune responses and safety. We enrolled very few women across cohorts, leading to imbalances in the male/female ratio in our trial, which may reflect the general reluctance of women to enrol in phase I studies.

Our study confirms the acceptable safety and immunogenicity profile of the 2×10^7 PFU dose in adults. However, considering the persistent replication of the rVSVΔGP-ZEBOV-GP vaccine in children and adolescents, further studies investigating lower doses in this population are warranted. In addition, lower vaccine doses should be considered when boosting individuals with pre-existing antibodies.

SUPPORTING INFORMATION

(available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5630143/)

S1 Fig. Study vaccine reconstitution.

S2 Fig. Viral load and viraemia in children, adolescents, and adults.

S3 Fig. ZEBOV-GP-specific antibodies by age group in individuals without baseline antibodies.

S4 Fig. ZEBOV antibodies measured by whole-virion ELISA by age group in individuals without baseline antibodies.

S1 Table. Reactogenicity to rVSV-ZEBOV-GP vaccine until day 28.

S2 Table. Reactogenicity to rVSV-ZEBOV-GP vaccine until day 28 in vaccinees with baseline ZEBOV-specific antibodies.

S3 Table. Reactogenicity to rVSV-ZEBOV-GP vaccine until day 28 in vaccinees without baseline ZEBOV-specific antibodies.

S4 Table. Frequency of symptoms after day 28 in adults.

S5 Table. Haematology and biochemistry parameters.

S6 Table. ZEBOV antibodies in geometric mean titres measured by whole-virion ELISA in children and adolescents.

S7 Table. ZEBOV antibodies by baseline status in children measured by whole-virion ELISA.

S8 Table. Neutralising antibodies to infectious ZEBOV isolate in adults.

S9 Table. Neutralising antibodies to infectious ZEBOV isolate classified by baseline ZEBOV antibody status in adults.

S10 Table. Neutralising antibodies to ZEBOV in children and adolescents.

S11 Table. Neutralising antibodies to VSV peudovirions measured by PsVNA50 in children and adolescents.

S12 Table. Comparison of antibodies in adults, children, and adolescents.

S13 Table. rVSV RNA shedding and proportion of adolescents and children with detectable and quantifiable viral RNA.
S14 Table. Viraemia in participants with baseline ZEBOV antibodies.
S15 Table. Viraemia in participants without baseline ZEBOV antibodies.
S1 Text. Trial protocol.
S2 Text. CONSORT checklist.
S3 Text. Dose escalation and randomisation.
S4 Text. Data collection, management, and safety assessment.

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The opinions, interpretations, conclusions, and recommendations contained herein are those of the authors and are not necessarily endorsed by the US Department of Defense.

ABBREVIATIONS

AEU	arbitrary enzyme-linked immunosorbent assay units
CERMEL	Centre de Recherches Médicales de Lambaréné
ELISA	enzyme-linked immunosorbent assay
EVD	Ebola virus disease
GMT	geometric mean titre
GP	glycoprotein
IQR	interquartile range
Nab	neutralising antibody
PFU	plaque-forming units
PsVNA50	pseudovirion neutralisation assay 50%
rVSV	recombinant vesicular stomatitis virus
USAMRIID	US Army Medical Research Institute of Infectious Diseases
VSV	vesicular stomatitis virus
WHO	World Health Organization
ZEBOV	Zaire Ebola virus

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DATA AVAILABILITY

Metadata used to generate the results of this manuscript have been submitted to the Dryad Digital Repository: https://datadryad.org/resource/doi:10.5061/dryad.n515p. http://dx.doi.org/10.5061/dryad.n515p.

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GENERAL DISCUSSION OF STUDY FINDINGS AND FUTURE PERSPECTIVES

6

José Francisco Fernandes

GENERAL DISCUSSION OF STUDY FINDINGS

The place of malaria among pathogens causing febrile infections in Gabon

Screening with a predefined algorithm a set of clinical and biological data from hospitalised febrile patients, identified the possible cause of fever in 94% of the 600 children included in our study. When present at admission, grade 3 fever (body temperature between 39.4°C and 40°C), lethargy, unconsciousness, convulsions, anemia, malnutrition, and thrombocytopenia were significantly associated with malaria (95) as reported in Congo as well (96). Pre-existing conditions and co-morbidities, such as malnutrition, sickle cell anemia, HIV infection had no strong negative impact on the outcome of the febrile diseases (95), conversely to what described from other populations (97,98), although our methodology might not have been ideal to identify discrete signs.

In terms of final diagnosis, the burden of malaria is still high and was found in more than half of the febrile children in Gabon and in Ghana (99), where malaria was diagnosed in 52% and 59% of hospitalised children, respectively. Consistent with the known trend malaria has, for a long time, been the major cause of febrile illness in sub-Saharan Africa (100). Despite that, the prevalence of malaria in febrile children decreased in Gabon, between 2000 and 2008 (100), and reports on decreasing malaria incidence from several sub-Saharan countries (101). There is an exigency to maintain the best strategies of control and/or elimination possible (102,103). The given strategies which include prompt and effective treatment with artemisinin-based combination therapies, use of insecticide-treated bed nets, and indoor residual spraying with insecticide to control the mosquitoes (104). A combination of those interventions proved to be highly effective at reducing prevalence and incidence across the continent (101).

However, it is uncertain how representative our findings are, and how they can be generalised to the nation as a whole and, to an extent, to other countries in sub-Saharan Africa, knowing that malaria transmission intensity varies even within a country, between countries, and also between regions within a continent (105). Another explanation of this variation in malaria prevalence/distribution could lead to changes in the distribution of this vector-borne infection (106,107).

Besides malaria, respiratory tract infections (namely lower respiratory tract infections) were the second-most frequent cause of fever, thus increasing the risk of respiratory distress in young children which could be life-threatening, especially in low resource settings. Despite of their low numbers, respiratory viruses isolated from nasopharyngeal throat swabs were consistent with the common viral spectrum seen in the same age population in other African settings (108). Lasty, the PCR positive Hib cases found from pharyngeal swabs were most likely colonization rather than true infections.

In terms of other pathogens encountered in blood; for viruses: cytomegalovirus (CMV) found in a relatively large number in non-immunocompromized children was considered most likely not all acute cases, but rather potentially due to delayed sample preparation. Epstein Barr virus (EBV) is less common in young children but rather in young adults (109). Human Herpesvirus-6 positive cases were not accompanied by exanthema maybe that is due to acute infection without visible skin lesions or related to previous infections.

Despite previous reports of their circulation in Lambaréné (110), we identified neither dengue nor chikungunya. This absence might reflect a predominant sylvatic life cycle of both viruses in the Moyen-Ogooué region which hosted several small outbreaks (111). Whereas for bacteria, we found 0.7% of invasive non-typhoidal salmonella (iNTS), although very low but in accordance with the trend showing that iNTS diseases are emerging in Africa (112). Also, only a very low proportion (0.3%) of meningitis was found which could be explained by the fact that in Gabon, the vaccine policy against Haemophilus influenzae b (Hib) one decade ago as seen elsewhere (113), plus the geographic location of Gabon out of the meningitis belt (114).

At the end of our explorations, no pathogens were found in 6% of the children. A number that we presume to be likely much higher, if considering causal pathogens, since a few may have not been captured, for any reason, through the study screening processes despite their thoroughness. For instance, measles could be considered as one possible differential diagnosis in some cases even in patients without mucosal and/or skin lesions. In fact, despite a vaccination coverage over 80% for all vaccines of the expanded programme on Immunization (EPI) administered to new borns and infants from 0 to 14 weeks of age, in our cohort, the measles vaccination rate was 54% far below that 95% recommended by WHO, to prevent measles epidemics and to eradicate the disease (115).

Everything included, for such a screening study it is essential to ensure that the causality between a potential pathogen and fever can be established and to differentiate between colonization, infection and disease.

Fosmidomycin for the treatment of uncomplicated malaria

Fosmidomycin (3-[formylhydroxy-amino]-propylphosphonic acid monosodium salt) is an antibiotic that inhibits the isoprenoid biosynthesis, and its activity against *P. falciparum* malaria has been investigated in both children and adults in Africa and Southeast Asia.

Efficacy results from the available data on the treatment of uncomplicated malaria with Fosmidomycin (FOS) in both adults and children since 2004, presented in this review, are not homogenous. Indeed, most pediatric studies demonstrated good tolerability and satisfactory day 28 cure rates (above 85-90%) of the combination FOS+Clindamycin for treating uncomplicated malaria (116–118). However, without a definitive clear explanation, a Mozambican study showed, in 2012, a low cure rate at day28 plus an increased parasite clearance time (PCT), along with other laboratory changes possibly due to prolonged parasite exposure. The Mozambican children were younger than the Gabonese ones, thus a possible explanation could be that older children might have developed partial immunity that enhanced antimalarial effect (119). Also, pharmacokinetic characteristics (*e.g.* lower absorption, accelerated metabolic rate) may differ in younger children compared with older children and adults, which could adversely affect the pharmacokinetics and efficacy (120). Moreover, drug formulations used in Mozambique and Gabon were different. Aqueous solutions reconstituted from water-soluble granules oft he drugs versus capsules or crushed tablets containing the investigational drug respectively.

In adults, a low cure rate of 70% was found after pooling all the studies available. These findings could be due to the fact that data from regimen optimizing studies were included in the analysis and/or because participants came from two different continents, Gabon and Thailand,

with implicit immunological differences between populations of hyperendemic (Central Africa) and hypoendemic (South East Asia) malaria regions. Also, both pharmacokinetics and pharmacodynamics differences might explain partly the variability between the studies since they are impacted by several factors (*i.e.* socioeconomic, cultural, dietetic, environmental) (121). Moreover, carefulness should be taken while considering pooled cure rates with the same combination, fever clearance time and fever clearance time of 49 and 42h respectively have been found, which appear to be longer than those in pediatrics. Howbeit this is in agreement with those of antibacterial drugs with antimalarials activity (*e.g.* doxycycline, clindamycin, and tetracycline) (122).

Among novel candidates worth considering there are approaches aiming to improve the on-target concentration of FOS (123,124) and to identify novel more potent 1-deoxy-D-xylulose 5-phosphate (DOXP) inhibitors (125). Also, next development strategies should integrate recent data such as the association with partners other than clindamycin (e.g. piperaquine).

Still in this search for alternatives, several antibacterial drugs (e.g. sulphonamides and sulphones, tetracyclines, clindamycin, macrolides, and chloramphenicol) are also known to have antiplasmodial activities. However, significant resistance has been reported to the sulphonamides but not the other classes of antibiotics (although macrolide resistance is readily induced in the laboratory) (8). *Until now no conclusion is made regarding the future and progress of fosmidomycin within the current pipeline of development of antimalarial drugs for uncomplicated malaria. This situation allows advocating, based on our finding for reactivation of such a drug and assess what could be its best partner drug. Making potential* novel chemotherapies that target multiple enzymes within the methylerythritol phosphate (MEP) pathway that should minimize the chance for resistance mutations to develop and survive in variant strains of pathogenic species (116,117).

The state-of-the-art of phase 1 – 3 trials of the RTS, S/AS01 malaria vaccine

Efficacy and safety of the RTS, S/AS01 malaria vaccine are the two relevant points to be discussed.

In regard to efficacy: in a phase 2 trial in children, a period of increased risk of clinical malaria was observed during the fifth year after the primary vaccination (126). This may be consistent with a rebound effect occurring when vaccine-induced protection decreases after an initial period of reduced risk of *P. falciparum* infection, leading to a delay in the acquisition of natural immunity. However, in the phase 3: differences in both anti-CS antibody geometric mean titre (GMT) and the intensity of malaria transmission (measured by the incidence of clinical malaria in control groups and which may be influenced by various genetic and environmental factors) across study sites could not explain the variation in vaccine efficacy (VE) between the study sites. The causes of lower efficacy in infants are not yet well understood but could include either one or several of the following mechanisms: (*i*) an inhibitory effect of maternal antibodies, (*ii*) some immune interferences due to simultaneous administration of others routine paediatric vaccines, (*iii*) the suppressive effect of exposure to malaria antigens in utero (127).

Concerning the safety; overall the incidence of serious adverse events (SAE) was similar in each group, but as reported in previous studies there are higher reports of meningitis in the RTS, S group. Also, for children who did not receive the 4th dose (booster dose), a higher risk of severe malaria appeared from month 21 until the end of the study, especially in study areas of high malaria transmission intensity (128).

An increased risk of febrile seizures in children vaccinated with RTS, S / AS01 during the large, double-blind, randomized phase 3 trial in Sub-Saharan Africa has previously been described (129). Indeed, during the first 2-3 days after vaccination with RTS, S / AS01, the incidence of febrile seizures in children was higher compared to the one seen in the control group. This time window corresponds to the time to onset of post-vaccination febrile reactions (in particular the day after vaccination) observed in this study. Thus confirming that the convulsions would be mainly triggered by a fever induced by vaccination, as also observed with other pediatric vaccines (*e.g.* pertusis, measles, mumps, rubella or varicella) (130–132).

Another matter of concern, was meningitis more frequently reported in children in the RTS, S / AS01 groups compared to those of the control group before the fourth dose and persisted throughout follow-up (127,128,133). However, this trend was no longer observed when comparing the same two groups after the fourth dose of vaccine. The etiology of the meningitis cases was heterogeneous, including different pathogens common in this population and outside the context of a meningitis epidemic in any of the clinical trial countries throughout the follow-up period (134). In addition, more than a third of meningitis cases (38%) were reported at one single study site (namely Lilongwe, Malawi), but were not associated with any recognized meningitis outbreak in Malawi during the period. study. This imbalance has not been observed in other RTS, S / AS01 trials (135). This could be related to the difference in the strict definition of meningitis cases and to the fact that the investigators also reported suspected cases of meningitis without laboratory confirmation (134). Worthy of note, data from animal studies on RTS, S and AS01 did not reveal any clinical or histological signs related to the treatment of meningitis, encephalitis, seizures, neurotoxicity or inflammation of the brain in any of the subjects examined (136,137). It is currently not known whether a causal link between the vaccine and meningitis is biologically plausible. The most probable hypothesis to explain the signal of meningitis seems to be a fortuitous finding. A hypothesis also evoked by investigators and external experts from WHO and EMA. Nevertheless, these meningitis cases will be closely monitored during the phase 4 studies (134).

Last but not least, in girls who received the RTS, S / AS01, all-cause mortality was higher than in girls in the control group (2.4% vs. 1.3%, for all age class). Showing that the effect of RTS, S / AS01 vaccination on mortality was modified by sex. However, these results should be interpreted with caution, given that no deaths were considered vaccine-related by the investigators and various causes of death were reported during the trial, including trauma, malaria, and others infectious diseases (134). The fact that girls in the RTS, S/AS01 group had a higher mortality risk could be explained by the lower mortality rate seen in girls of the control group. An interesting potential confounding factor to take into account could have been parental behaviors influenced by gender while seeking medical care for their children (138) – which might have confounded these gender-specific mortality outcomes – could have been of great support for interpretating this observation (134).

In November 2015, the European Medicines Agency (EMA) reviewed both the efficacy and safety of RTS, S/AS01 and using article 58 gave its approval for use in both infants and children (104). In contrast, the WHO's Strategic Advisory Group of Experts on Immunization and Malaria Policy Advisory Committee only recommended the use of RTS, S/AS01 in the children group (139).

The absence of impact of RTS, S / AS01 on mortality in phase 3 does not, however, exclude a potential impact in field conditions. Large phase 4 studies are needed to assess RTS, S/AS01 impact on mortality, for which phase 3 trials are too small, prior to large-scale deployment to malaria-endemic areas (140,141).

In the end, the final recommendation on this vaccine implementation would need to consider several factors such as malaria transmission intensity, the cost-effectiveness and coverage of other malaria interventions, health priorities, financing, and the capacity of the health system to deliver the vaccine (142). In this perspective, a pilot implementation of the RTS,S/AS01 vaccine (Mosquirix®) began in 2019, in three malaria-endemic countries (*i.e.* Ghana, Kenya, and Malawi) (77,143).

The rVSV-ZEBOV vaccine: a phase 1 in Gabon and its deployment in the field

Following the largest outbreak ever which occurred in West Africa of 2013-2014 (144,145), a public health emergency of international concern (PHEIC) was declared by the WHO (146,147). Ebola virus disease gains in notoriety and becomes the fourth most studied infectious disease after malaria (20%), HIV/AIDS (15%), tuberculosis (7%) (148). In response to the West African epidemic, trials of several candidate vaccines were fast-tracked (149). Thus, in Lambaréné we conducted a dose-escalation phase 1 randomized trial, assessing the safety and immunogenicity of the rVSV-ZEBOV-GP Ebola vaccine in adults and children, where five different doses – expressed in plaque forming units (PFU), namely: $3x10^3$, $3x10^4$, $3x10^5$, $3x10^6$ and $2x10^7$ PFU – were evaluated.

Transient cases of arthralgia without arthritis were reported after vaccination (150,151) comparable to studies in Guinea (152) and US adults (153). Whereas in Kilifi, Kenya two self-limiting, low-severity, and short-lasting cases of arthritis were reported (150). Conversely higher frequency of vaccine-induced arthritis (24%), dermatitis (9.8%) and vasculitis (2%) were found in Geneva, Switzerland (150,154,155), Canada, the US and Spain (156). Potential similarities between the rVSVΔG-ZEBOV-GP vaccine and the rubella vaccine, which also causes transient arthritides in some populations (157,158), could explain observed phenomena.

Higher and persistent viraemia as well as shedding in saliva and urine were observed in children and adolescents in contrast with very low proportion or absence of shedding reported in saliva in adults receiving $3x10^6$ to $5x10^7$ PFU in America and Europe (150,153,154). The underlying mechanisms prolonging viraemia and causing shedding are still needed to e elucidate by specific study designs.

In adults cohorts of Lambaréné, Geneva and Hamburg (159,160), the vaccine dose of $2x10^7$ PFU showed the optimal safety versus immunogenicity balance, what is then supporting the choice to use this dose in the context of outbreaks (161). Following those findings, the Ebola

Ça Suffit ring vaccination phase 3 cluster-randomized trial using the rVSV-ZEBOV vaccine took place in Guinea, and showed the first evidence that the rVSV-ZEBOV vaccine is efficacious in a trial setting – vaccine efficacy of 100% (95% CI 74·7–100·0; p=0·0036) – and that it might be effective in real-life scenarios (70)

Up to now, no Ebola vaccine has been licensed and has been prequalified by WHO, including the rVSV-ZEBOV. Nonetheless, based on interim trial results suggesting high safety and efficacy, Nonetheless, in case of an Ebola virus disease outbreak may occur before the registration of an Ebola vaccine the WHO Strategic Advisory Group of Experts on Immunization recommended a speedy deployment and use of the rVSV-ZEBOV vaccine – that demonstrated high safety and efficacy profiles during interim analyses – with informed consent and following good clinical practice (GCP) (139,149). For instance, the rVSV-ZEBOV vaccine is used for the current ongoing epidemic in the Democratic Republic of Congo 2018 – 2019 (162)

Our phase 1 trial has some limitations such as the absence of a control group, lack of stratification for baseline antibody status, and imbalances in male/female ratio (163).

Another potential challenge is a logistical constraint for the deployment on the field (i.e. study sites or epidemic regions) is the availability of a reliable cold chain -80°C (-112 F) freezers for vaccine storage or transport at -80°C (-112°F) (164). In a small phase 1 study, this was not an issue, under real-life situations, in rural/remote areas, then conditions become very different (165).

PERSPECTIVES FOR FUTURE WORK

All the above presents the humble contribution of our results to the medical and scientific community. Nonetheless, it opens further interesting perspectives. Indeed, some remaining gaps are potential directions for research:

1. Establishment of causality between pathogens in febrile diseases: either in single isolate/infection or multiple isolate/infection, remains challenging. Not even mentioning the question of the best appropriate laboratory method to be chosen in due time adapted to the type of samples.
2. Also, in case one of the candidate drug and/or vaccines (*i.e.* RTS, S/AS01 or Mosquirix®) assessed during this thesis is licensed or prequalified by WHO, no clear policy is in place in Gabon to have it implementation for Gabonese population as part of health coverage, through the Malaria Vaccine Implementation Programme (MVIP).
3. And finally, for better control of subsequent Ebola virus epidemics, future works should include strategies to protect vulnerable populations (*i.e.* pregnant, breastfeeding, severely ill, or younger than 6 years old) not eligible for ring vaccination and for whom no safety data is available yet.

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SUMMARY

S

Fever is a key sign or symptom exhibited during infectious diseases. Hence, understanding of fever and identification of its causative pathogens are pivotal steps for efficient prevention and/or treatment of the majority of infectious febrile diseases. This research project aimed to assess the causes of fever and to contribute to the development of medicines for two locally important infectious diseases: malaria and ebola virus disease. It also describes the results of global strategies meant to tackle fever-related health with both novel drug and vaccine candidates. This work has been performed in Lambaréné, in the Gabonese population between 2013 and 2018. In a hospital-based survey including 600 children aged 0 to 15 years old, ill enough to be hospitalised with a body temperature equal or above 38° C, malaria parasites (*Plasmodium falciparum* with co-infections with *P. malariae*, *P. ovale wallikeri* and *P. ovale curtisi*) were present in 52% of children. Viral infections were heterogeneous, mainly impacting the respiratory and gastrointestinal tract, on one hand, with viruses such as adenovirus, coronavirus, enteroviruses, influenzae and parainfluenza virus, rhinovirus, respiratory virus, and astroviruses, noroviruses, rotaviruses, sapoviruses, respectively. And on the other hand, systemic infections caused by *herpesviridae* (*i.e.* cytomegalovirus, Epstein Barr virus, and HHV6). Bacterial pathogens were, mainly in the blood, with 3% of sepsis well distributed between gram-negative and gram-positive bacilli, of which 1/4 was invasive non-typhoidal salmonella (iNTS) disease. Whereas in urine, infections were caused mainly by gram-negative bacilli with *E. coli* and *K. pneumoniae* far more present, with some found to be extended-spectrum beta-lactamase (ESBL) producing. Finally, a small portion of invasive gram-negative bacilli was seen in the stool. However, at the end of our explorations, no pathogens were found in 6% of the children; a number presumably likely higher if considering *stricto sensu* the detection of causative pathogens. Despite the diversity of pathogens encountered, malaria is still by far the first cause of feverish illnesses leading to hospitalizations in Gabon (Chapter 2).

Integrating the important burden of malaria, it is imperative to remain devoted to optimising its curative treatment. Especially in the context of the ever-growing threat antimalarial drug resistance. Thus, we performed a meta-analysis on the clinical development of a potential antimalarial drug, fosmidomycin. Indeed, fosmidomycin, a natural antibacterial agent also coded FR31564 (3-[formylhydroxy-amino]-propylphosphonic acid monosodium salt) has been tested in both children and adults in Africa and Asia. A total of six paediatric studies were carried out in Gabon and Mozambique in children aged 0-14 years. Adult studies, meanwhile, were conducted in Gabon and Thailand, in age groups ranging from 15 to 61 years old. In six clinical trials of fosmidomycin against uncomplicated malaria in African children yielded an overall day 28 cure rate of 85% (95%CI: 71-98%); a parasite clearance time 39 hours; and a fever clearance time of 30 hours. Whereas, the four adult cohorts, found that the corresponding values were 70% (95% CI: 40–100%), 49 and 42 h, respectively. Fosmidomycin is safe well-tolerated, despite some hematologic adverse events that need to be monitored (Chapter 3). Beyond the curative aspect, preventive measures by vaccines have been sought for the last 40 years, to be a key preventive tool against malaria.

The RTS, S vaccine candidate (a recombinant polypeptide construct of B-cell epitopes and T-cell epitopes from CSP fused to the N-terminal region of hepatitis B virus surface antigen (S) where the RTS and unfused S polypeptides (RTS, S) are co-expressed and transform into

virus-like particles) is currently the most advanced in the pipeline. It, therefore, seemed to us important to read through the chronological history of its clinical development by carrying out a systematic review covering all phases 1, 2, and 3 and with the main endpoint being an analysis of the consistency of efficacy and immunogenicity data from respective trials. As well, safety data from a pooled analysis of RTS/AS Phase 22 trials and RTS, S/AS01 Phase 33 trials were reviewed. A total of 60 studies has been included, covering a period from 1994 to 2014. And phase 1 - 2 trials done in different populations across the world; adults' studies were conducted either in malaria naïve (USA and Europe) or in malaria semi-immune populations (Gambia and Kenya). Paediatric studies phase 1 - 2B were also conducted first in children ≥ 5 months at the first dose of vaccination in three African countries (i.e. Kenya, Mozambique, and Tanzania), and later in infants in four African countries (i.e. Gabon, Ghana, Mozambique, and Tanzania) with overall encouraging safety, tolerability and immunogenicity profile, which finally, allowed the progress into the pivotal phase 33 trial that took place from 2009 to 2014 in 11 research centres over seven African countries (Burkina-Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and Tanzania) vaccinating an unprecedented total of 8,922 children and 6,537 infants aged 5 to 17 months and 6 to 12 weeks respectively at the time of the first injection of the candidate vaccine. Efficacy phase 22 trials in infants in the context of co-administration with expanded programme on immunization (EPI) vaccines showed improved immune responses and protection following vaccination with RTS, S/AS01. Also, the highest concentration of anti-CSP antibodies are induced by RTS, S/AS01, hence it was selected for the large Phase 33 trial. Analysis of the Phase 33 trial allowed to see that although RTS, S/AS01 induced a higher vaccine efficacy (VE) when administered to children aged 5–17 months compared with infants of 6–12 weeks, it protects against clinical malaria up to 48 months after the primary vaccination with an incremental efficacy induced by a booster dose delivered 18 months after the third dose at a similar rate in children and infants. Lastly, regarding safety and tolerability: meningitis occurred more frequently in RTS, S/AS01 recipient children compared with control groups, but the causality link with RTS, S/AS01 remains uncertain (Chapter 4). Still, in the field of vaccines for the prevention of serious/fatal febrile infections, there was the development of an Ebola vaccine candidate. During the largest outbreak ever which occurred in West Africa (i.e. Guinea, Liberia, and Serra Leone) that started in December 2013, and in August 2014 World Health Organization (WHO) considered it as a public health emergency of international concern and urged the scientific community in developing an effective vaccine that could be deployed in areas of crisis to halt the epidemic. Therefore, within the frame of the VEBCON consortium (VSV-Ebola CONsortium), our work at the Centre de Recherches Médicales de Lambaréné (CERMEL) served to evaluate, in phase 1 randomized trial, the safety and immunogenicity of rVSV-ZEBOV-GP ebola in adults and children in Lambaréné. Our results and other findings show that this vaccine is safe and immunogenic at the dose of $2x10^7$ PFU in adults, and support that the lower doses may be needed in pediatric populations as well as for boosting after primary vaccination or naturally acquired immunity (Chapter 5).

José Francisco Fernandes

ADDENDUM

SAMENVATTING

Koorts is een belangrijk symptoom van infectieziekten. Daarom zijn het begrip van koorts en de identificatie van de oorzakelijke pathogenen cruciale stappen voor efficiënte preventie en/ of behandeling van de meeste infectieuze koortsaandoeningen. Dit onderzoeksproject had tot doel de oorzaken van koorts te identificeren en bij te dragen aan de ontwikkeling van geneesmiddelen voor twee lokaal belangrijke infectieziekten: malaria en ebola-virusziekte. Het beschrijft ook de resultaten van wereldwijde strategieën die bedoeld zijn om koorts gerelateerde gezondheid aan te pakken met zowel nieuwe kandidaat-geneesmiddelen als vaccins. Dit werk is uitgevoerd in Lambaréné, in de Gabonese bevolking tussen 2013 en 2018. In een ziekenhuisonderzoek onder 600 kinderen van 0 tot 15 jaar oud, ziek genoeg om in het ziekenhuis te worden opgenomen met een lichaamstemperatuur gelijk aan of hoger dan 38 °C, waren malaria parasieten (*Plasmodium falciparum* met gelijktijdige infecties met *P. malariae*, *P. ovale wallikeri* en *P. ovale curtisi*) aanwezig bij 52% van de kinderen. Virale infecties waren heterogeen, met voornamelijk de luchtwegen en het maagdarmkanaal aangedaan, enerzijds met virussen zoals adenovirus, coronavirus, enterovirussen, influenzae en para-influenzavirus, rhinovirus, respiratoir virus en astrovirussen, norovirussen, rotavirussen, sapovirussen, en anderzijds systemische infecties veroorzaakt door *herpesviridae* (d.w.z. cytomegalovirus, Epstein Barr-virus en HHV6). Bacteriële ziekteverwekkers waren voornamelijk aantoonbaar in het bloed, met 3% van de sepsis, met een goede verdeling tussen gramnegatieve en grampositieve bacillen; een kwart was invasieve niet-tyfeuze salmonella (iNTS) -ziekte. In urine werden infecties echter voornamelijk veroorzaakt door gramnegatieve bacillen met *E. coli* en *K. Pneumoniae* het meest prevalent waren, waarvan sommige een uitgebreid spectrum aan bètalactamase (ESBL) bleken te produceren. Ten slotte werd een klein deel van de invasieve gramnegatieve bacillen in de ontlasting gezien. Bij 6% van de kinderen werden uiteindelijk geen ziekteverwekkers gevonden; dit aantal is waarschijnlijk hoger als de diagnostiek van oorzakelijke pathogenen *stricto sensu* wordt toegepast. Ondanks de diversiteit aan aangetroffen ziekteverwekkers, is malaria nog steeds verreweg de meest voorkomende oorzaak van koortsende ziekten die tot ziekenhuisopnames in Gabon leiden (hoofdstuk 2).

Gezien de grote ziektelast van malaria, is het absoluut noodzakelijk om de behandeling te blijven optimaliseren. Dit vooral in de context van de steeds groter wordende dreiging van resistentie tegen antimalaria middelen. Daarom hebben we een meta-analyse uitgevoerd over de klinische ontwikkeling van een mogelijk antimalariamiddel, fosmidomycine. Fosmidomycine, een natuurlijk antibacterieel middel, ook FR31564 gecodeerd (3- [formylhydroxy-amino] propylfosfonzuur mononatriumzout) is getest bij zowel kinderen als volwassenen in Afrika en Azië. Er zijn in totaal zes pediatrische onderzoeken uitgevoerd in Gabon en Mozambique bij kinderen van 0-14 jaar. Ook werden in Gabon en Thailand studies uitgevoerd bij volwassenen in leeftijdsgroepen van 15 tot 61 jaar oud. Zes klinische onderzoeken, waarin werd gekeken naar fosmidomycine tegen ongecompliceerde malaria bij Afrikaanse kinderen, lieten een algemeen genezingspercentage op dag 28 zien van 85% (95% BI: 71-98%); een "parasite clearance time" van 39 uur; en een "fever clearance time" van 30 uur. We zagen dat in de vier volwassen cohorten de overeenkomstige waarden respectievelijk 70% (95% BI: 40-100%), 49 en 42 uur waren. Fosmidomycine is veilig en wordt goed verdragen, ondanks enkele hematologische

bijwerkingen die moeten worden gecontroleerd (hoofdstuk 3). Naast het curatieve aspect, is er de afgelopen 40 jaar naar preventieve maatregelen in de vorm van vaccins gezocht als een belangrijk preventief instrument tegen malaria.

De RTS, S-vaccinkandidaat (een recombinant polypeptideconstruct van B-celepitopen en T-celepitopen van CSP gefuseerd met het N-terminale gebied van hepatitis B-virusoppervlakantigeen (S) waar de RTS en niet-gefuseerde S-polypeptiden (RTS, S) gezamenlijk tot expressie worden gebracht en transformeren in virusachtige deeltjes) is momenteel de meest geavanceerde in de pijplijn. Het leek ons daarom belangrijk om de chronologische geschiedenis van de klinische ontwikkeling ervan samen te vatten door een systematische review uit te voeren over alle fasen 1, 2 en 3 studies, met als belangrijkste eindpunt een analyse van de van werkzaamheid en immunogeniciteit van respectievelijke studies. Eveneens werden veiligheidsgegevens van een gepoolde analyse van RTS / AS fase 2-onderzoeken en RTS, S / AS01 fase 3-onderzoeken beoordeeld. In totaal zijn er 60 onderzoeken geïncludeerd, die een periode bestrijken van 1994 tot 2014. Fase 1 - 2 studies die bij verschillende bevolkingsgroepen over de hele wereld zijn uitgevoerd; onderzoek in volwassenen werd uitgevoerd bij malaria-naïeve patiënten (VS en Europa) of bij malaria-semi-immuunpopulaties (Gambia en Kenia). Pediatrische studies fase 1 - 2B werden eerst uitgevoerd bij kinderen ≥ 5 maanden ten tijde van de eerste dosis vaccinatie in drie Afrikaanse landen (Kenia, Mozambique en Tanzania), en later bij zuigelingen in vier Afrikaanse landen (Gabon, Ghana, Mozambique en Tanzania), met een algemeen bemoedigend veiligheids-, tolerantie- en immunogeniciteitsprofiel. Dit maakte uiteindelijk de voortgang mogelijk van de cruciale fase 3-studie, die van 2009 tot 2014 plaatsvond in 11 onderzoekscentra in zeven Afrikaanse landen (Burkina-Faso, Gabon, Ghana, Kenia, Malawi, Mozambique en Tanzania), waarbij een ongekend totaal van 8.922 kinderen en 6.537 zuigelingen van respectievelijk 5 tot 17 maanden en 6 tot 12 weken op het moment van de eerste injectie van het kandidaatvaccin werden gevaccineerd. Fase 2 werkzaamheids-onderzoeken bij zuigelingen, in de context van gelijktijdige toediening met de EPI-vaccinaties, toonden een verbeterde immuunrespons en bescherming na vaccinatie met RTS, S / AS01. Ook werd de hoogste concentratie anti-CSP-antilichamen geïnduceerd door RTS, S / AS01, en daarom werd deze combinatie geselecteerd voor de grote fase 3-studie. Analyse van de fase 3-studie liet zien dat hoewel RTS, S / AS01 een hogere VE induceerde bij toediening aan kinderen van 5-17 maanden in vergelijking met zuigelingen van 6-12 weken, het tegen klinische malaria beschermt tot 48 maanden na de primaire vaccinatie, met een verbeterde werkzaamheid door een boosterdosis die 18 maanden na de derde dosis wordt toegediend, vergelijkbaar bij kinderen en zuigelingen. Ten slotte, wat betreft veiligheid en verdraagbaarheid: meningitis kwam vaker voor bij RTS, S / AS01-ontvangende kinderen in vergelijking met controlegroepen, maar het oorzakelijk verband met RTS, S / AS01 blijft onzeker (hoofdstuk 4).

Ook was er op het gebied van vaccins voor de preventie van ernstige / dodelijke met koortsende infectieziekten, de ontwikkeling van een kandidaat-vaccin tegen ebola. Tijdens de grootste uitbraak ooit in West-Afrika (Guinee, Liberia en Serra Leone), die in december 2013 begon en in augustus 2014 door de Wereldgezondheidsorganisatie (WHO) als een noodsituatie op het gebied van de volksgezondheid van internationaal belang werd bestempeld, werd de wetenschappelijke gemeenschap aangespoord tot het ontwikkelen van een effectief

vaccin dat kan worden ingezet in crisisgebieden om de epidemie een halt toe te roepen. Daartoe diende ons werk in het Centre de Recherches Médicales de Lambaréné (CERMEL) in het kader van het VEBCON-consortium (VSV-Ebola CONsortium); om in een fase 1 gerandomiseerde studie de veiligheid en immunogeniciteit van het rVSV-ZEBOV-GP ebola vaccin te evalueren bij volwassenen en kinderen in Lambaréné. Onze resultaten en andere bevindingen tonen aan dat dit vaccin veilig en immunogeen is bij een dosis van 2 x 107 PFU bij volwassenen, en ondersteunen dat de lagere doses mogelijk nodig zijn bij pediatrische populaties en voor versterking ("booster") na primaire vaccinatie of natuurlijk verworven immuniteit (Hoofdstuk 5).

CURRICULUM VITAE

PERSONAL PROFIL

Name: José Francisco Fernandes
Date & Place of Birth: 04 August 1980, Strasbourg (FRA)
Nationality: Bissau Guinean

I am a medical doctor and researcher with extensive experience in the management of vaccine trials. I have co-led a phase III GSK sponsored vaccine trials (Mosquirix™ vaccine) and have recently been involved as a lead clinician in a Phase I vaccine trial on EBOLA (rVSV G-ZEBOV - Ervebo™ vaccine, of MSD Vaccines). My area of expertise includes vaccine development, infectious diseases, and epidemiology. I am proficient in project management and have successfully led various multidisciplinary and multicultural research teams in matrix environments. Throughout my 12 years of work experience, I have developed substantial leadership skills. I am proactive, flexible and effective working to tight deadlines, independently or as a team.

EDUCATION

Discipline: Tropical Medicine
Institution: University of Amsterdam (co-supervision with the Institute of Tropical Medicine of Tubingen, Germany)
Degree: Ph.D. (Thesis completed, awaiting for its defense scheduled this year).

Discipline: Epidemiology
Institution: ISPED-Université Victor Segalen, (Bordeaux 2) France
Degree: Postgraduate Diploma in Epidemiology (Distance learning)

Discipline: Tropical and Parasitic Diseases
Institution: UPMC, (Paris 6), France
Degree: Postgraduate Diploma in Tropical and Parasitic Diseases (Distance learning)

Discipline: Medecine
Institution: Université des Sciences de la Santé (USS) d'Owendo, Gabon
Degree: Doctor of Medicine

WORK EXPERIENCE

2016 – 2020 (Concomitantly) **Research scientist** Institute of Tropical Medicine – Universitätsklinikum Tübingen

2015 - 2016 **Principal investigator** of a hospital-based survey "**Assessment of fever without sources** (FWS)" in Lambaréne Gabon

2014 - 2016	**Lead clinician** - **Phase I**, Randomized, Open-Label, Dose-Escalation Study to Evaluate the Safety and Immunogenicity of the BPSC1001 (VSV G-ZEBOV) **Ebola Virus Vaccine Candidate** in Healthy Adult Volunteers in Lambaréné, Gabon
2009 - 2014	**Co-lead clinician** - **Phase III trial** "Efficacy of **GSK Biologicals' Candidate Malaria Vaccine** against Malaria Disease Caused by P. Falciparum Infection in Infants and Children in Africa" in Gabon
2008 - 2009	**Investigator** - **Phase II trial**: "Safety and Immunogenicity Study of **GSK Biological's** Investigational Vaccination Regimen **Malaria Vaccine**, When Incorporated Into an Expanded Program on Immunization (EPI)" in Gabon (ClinicalTrials.gov Id: NCT00436007)

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PHD PORTFOLIO

Name PhD student:	José F. Fernandes
PhD period:	2012 - 2021
Name PhD supervisors:	Prof. Dr. Martin Grobusch, Prof. Dr. Peter Kremsner, and Prof. Dr. Benjamin Mordmüller

	Year	Workload (ECTS)
1. PhD training		
General courses		
ICH Good Clinical Practice of the Global Health Network	2016	0.2
Reanimationstraining "Basic Life Support nach den aktuelle Empfehlungen des European Resuscitation Councils (ERC)"	2014	0.2
ICH Good Clinical Practice of the Global Health Network	2014	0.2
Specific courses		
Selected chapters in human parasitology, tropical medicine, and vaccinology (Summer term), University of Tübingen	2019	1
Methods in Tropical Medicine IV (Summer term), University of Tübingen	2019	1
Selected chapters in human parasitology, tropical medicine, and vaccinology (Winter term), University of Tübingen	2018	1
Methods in Tropical Medicine IV (Winter term), University of Tübingen	2018	1
Selected chapters in human parasitology, tropical medicine, and vaccinology (Summer term), University of Tübingen	2018	1
Methods in Tropical Medicine IV (Summer term), University of Tübingen	2018	1
Selected chapters in human parasitology, tropical medicine, and vaccinology (Winter term), University of Tübingen	2017	1
Methods in Tropical Medicine IV (Winter term), University of Tübingen	2017	1
Selected chapters in human parasitology, tropical medicine, and vaccinology (Summer term), University of Tübingen	2017	1
Methods in Tropical Medicine IV (Summer term), University of Tübingen	2017	1
Scientific Writing in English for Publication (PhD course program) Graduate School for Medical Sciences, AMC of the University of Amsterdam	2016	1.5
Clinical Data Management (PhD course program) Graduate School for Medical Sciences, AMC of the University of Amsterdam	2016	0.9
Good Clinical Epidemiology: Systematic Reviews (PhD course program) Graduate School for Medical Sciences, AMC of the University of Amsterdam	2016	0.7
Infectiological literature seminar, University of Tübingen	2016	1
Selected chapters in human parasitology, tropical medicine, and vaccinology, University of Tübingen	2016	2
Methods in Tropical Medicine IV, University of Tübingen	2016	1
Methods in Tropical Medicine for doctoral candidates, University of Tübingen	2016	1
Seminars, workshops and masterclasses		
Short Course on Abdominal Ultrasound in Infectious Diseases and Tropical Medicine of the European Accreditation Council for Continuing Medical Education.	2014	2

PhD Portfolio (continued)

	Year	Workload (ECTS)
Presentations		
Oral communication / Conference on Tropical Medicine and Global Health (CTM2019) By the German Society for Tropical Medicine and International Health (DTG), in Munich (Germany)	2019	0.5
Poster / Seventh Multilateral Initiative on Malaria Pan-African Malaria Conference, in Dakar (Sénégal)	2018	0.5
Poster / Tenth European Congress on Tropical Medicine and International Health (ECTMIH) 2017, in Antwerp (Belgium)	2017	0.5
Oral communication / The German Center for Infection Research (DZIF) Meeting 2016, in Cologne (Germany)	2016	0.5
Oral communication / Eighth European and Developing Countries Trials Partnership (EDCTP) Forum 2016, in Lusaka (Zambia)	2016	0.5
Oral communication / World Malaria Day 2015 By the National Malaria Control Program – Gabon, in Libreville (Gabon)	2015	0.5
Poster / International Summer school 'Microbes, Host and Infection' Interfakultäres Institut für Mikrobiologie und Infektionsmedizin in Tübingen (Germany)	2014	0.5
Oral communication / Seventh European and Developing Countries Trials Partnership (EDCTP) Forum Theme: "The Partnership journey: New horizon for better health", in Berlin (Germany)	2014	0.5
Poster / Biannual meeting of the German Society for Tropical Medicine and International Health (DTG): 14 – 15 March 2014 in Düsseldorf (Germany)	2014	0.5
Oral communication / World Malaria Day 2013 By the National Malaria Control Program-Gabon, in Libreville (Gabon)	2013	0.5
(Inter)national conferences		
The annual conference on Tropical Medicine and Global Health (CTM2019) of the German Society for Tropical Medicine and International Health (DTG) (Germany)	2019	0.5
The Seventh Multilateral Initiative on Malaria Pan-African Malaria Conference (Sénégal)	2018	0.5
The tenth European Congress on Tropical Medicine and International Health (Belgium)	2017	0.5
The annual meeting (2016) of the German Center for Infection Research (Germany)	2016	0.5
The Eighth European and Developing Countries Trials Partnership Forum (Zambia)	2016	0.5
The World Malaria Day meeting by the Gabonese Malaria Control Program (Gabon)	2015	0.5
The Seventh European and Developing Countries Trials Partnership Forum Theme: "The Partnership journey: New horizon for better health" (Germany)	2014	0.5

PhD Portfolio (continued)

	Year	Workload (ECTS)
The biannual meeting of the German Society for Tropical Medicine and International Health (Germany)	2014	0.5
The World Malaria Day by the Gabonese Malaria Control Program-Gabon, in Libreville (Gabon)	2013	0.5
Other		
Journal Club at the Institute for Tropical Medicine University of Tübingen (Germany)	2013 -2014	0.25
Journal Club at the Centre de Recherches Médicales de Lambaréné, Lambaréné (Gabon)	2015	0.2
Journal Club at the Institute for Tropical Medicine University of Tübingen (Germany)	2016-19	0.7
2. Teaching		
Lecturing		
German Academic Exchange Servie (DAAD) course of infectious diseases, 12 – 17 Oct. 2015, in Lambaréné (Gabon)	2015	0.6
Tutoring, Mentoring		
Mr. David Weber bachelor's project: "Prevalence of Mansonella sp. "DEUX" in Fougamou, Gabon"	2017-18	2
Mr. David Weber master's project: "Typing of Human herpesvirus-6 subtypes in a paediatric population by real-time qPCR"	Ongoing	2
Supervising		
Clinical and data management teams of Fever Without Source (FWS) study	2015-16	1
Other		
Training on Good clinical practice, study protocols, and specific study procedures for both Ebola vaccine phase 1 trial & FWS study staff.	2014-16	1.5
3. Parameters of Esteem		
Awards and Prizes		
Awards		
Travel award for the 7th Multilateral Initiative on Malaria Pan-African Malaria Conference	2018	
Baden-Württember Stipendium	2017 - 2018	
Travel award for the tenth European Congress on Tropical Medicine and International Health	2017	
Travel award for the Eighth European and Developing Countries Trials Partnership Forum Lusaka	2016	

PhD Portfolio (continued)

	Year	Workload (ECTS)
Travel award for the Seventh European and Developing Countries Trials Partnership Forum Berlin	2014	
European and Developing Countries Trials Partnership scholarship	2010 – 2014	
Prize		
1st place poster presentation (Poster#5: "Effect of antihelminthic treatment on immune responses to seasonal influenza vaccine in geohelminth-infected individuals") at the Biannual meeting (March 2014) of the German Society for Tropical Medicine and International Health (DTG) in Düsseldorf (Germany).	2014	
4. Publications		
Peer reviewed		
Fernandes JF, Laubscher F, Held J, Eckerle I, Docquier M, Grobusch MP, et al. UNBIASED METAGENOMIC NEXT-GENERATION SEQUENCING OF BLOOD FROM HOSPITALIZED FEBRILE CHILDREN IN GABON. Emerging Microbes & Infections. 2020 Jan 1;9(1):1242–4.	2020	
Fernandes JF, Held J, Dorn M, Lalremruata A, Schaumburg F, Alabi A, Agbanrin MD, Kokou C, Ben Adande A, Esen M, Eibach D, Adegnika AA, Agnandji ST, Lell B, Eckerle I, Henrichfreise B, Hogan B, May J, Kremsner PG, Grobusch MP, Mordmüller B. CAUSES OF FEVER IN GABONESE CHILDREN: A CROSS-SECTIONAL HOSPITAL-BASED STUDY. Sci Rep. 2020 Feb 7;10(1):2080. doi: 10.1038/s41598-020-58204-2.	2020	
Dejon-Agobe JC, Ateba-Ngoa U, Lalremruata A, Homoet A, Engelhorn J, Paterne Nouatin O, Edoa JR, **Fernandes JF**, Esen M, Mouwenda YD, Betouke Ongwe EM, Massinga-Loembe M, Hoffman SL, Sim BKL, Theisen M, Kremsner PG, Adegnika AA, Lell B, Mordmüller B. CONTROLLED HUMAN MALARIA INFECTION OF HEALTHY LIFELONG MALARIA-EXPOSED ADULTS TO ASSESS SAFETY, IMMUNOGENICITY AND EFFICACY OF THE ASEXUAL BLOOD STAGE MALARIA VACCINE CANDIDATE GMZ2. Clin Infect Dis. 2018 Dec 18. doi: 10.1093/cid/ciy1087	2018	
Huttner A, Agnandji ST, Combescure C, **Fernandes JF**, Bache EB, Kabwende L, Ndungu FM, Brosnahan J, Monath TP, Lemaître B, Grillet S, Botto M, Engler O, Portmann J, Siegrist D, Bejon P, Silvera P, Kremsner P, Siegrist CA; VEBCON; VSV-EBOVAC; VSV-EBOPLUS Consortia. DETERMINANTS OF ANTIBODY PERSISTENCE ACROSS DOSES AND CONTINENTS AFTER SINGLE-DOSE RVSV-ZEBOV VACCINATION FOR EBOLA VIRUS DISEASE: AN OBSERVATIONAL COHORT STUDY. Lancet Infect Dis. 2018 Apr 4. pii: S1473-3099(18)30165-8. doi: 10.1016/S1473-3099(18)30165-8.	2018	

PhD Portfolio (continued)

	Year	Workload (ECTS)
Lim JK, Carabali M, Lee JS, Lee KS, Namkung S, Lim SK, Ridde V, **Fernandes J**, Lell B, Matendechero SH, Esen M, Andia E, Oyembo N, Barro A, Bonnet E, Njenga SM, Agnandji ST, Yaro S, Alexander N, Yoon IK. EVALUATING DENGUE BURDEN IN AFRICA IN PASSIVE FEVER SURVEILLANCE AND SEROPREVALENCE STUDIES: PROTOCOL OF FIELD STUDIES OF THE DENGUE VACCINE INITIATIVE. BMJ Open. 2018 Jan 21;8(1):e017673. doi: 10.1136/bmjopen-2017-017673.	2018	
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