



Out of the  
Department of Infectious Diseases & Tropical Medicine, Medical Faculty, Ludwig-  
Maximilians University, Munich

**The effectiveness of intermittent screening and treatment with  
artemether-lumefantrine for malaria prevention in pregnancy in South  
East Nigeria**

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# ABSTRACT

## ***Background***

The spread of resistance to SP across Africa threatens the usefulness of intermittent preventive treatment of malaria in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) in West Africa. There is need for an alternative drug for IPTp or an alternative strategy for delivery of drug-based prevention of MiP. This trial, investigated, whether screening pregnant women for malaria using a rapid diagnostic test (RDT) at antenatal clinic and treating those with positive results with artemether-lumefantrine (Intermittent Screening and Treatment) is as effective and as safe as IPTp-SP.

## ***Methods***

Between October 2013 and November 2014, 460 pregnant women attending antenatal clinic in Calabar Southeast Nigeria were randomised to either IPTp-SP or intermittent screening and treatment with artemether-lumefantrine (ISTp-AL). All women received a long-lasting insecticide-treated net at enrolment. Study women had a maximum of four scheduled visits following enrolment. Haemoglobin concentration and peripheral parasitaemia were assessed in the third trimester (36-40 weeks gestation). Birth weights were measured at delivery or within six days for babies delivered at home. In addition, the prevalence of molecular markers of resistance to SP among pregnant women was established.

## ***Results***

In the third trimester, the overall prevalence of severe anaemia (Hb<8 g/dl) and moderate anaemia (8 -10.9 g/dl) was 0.8% and 27.7% respectively and was similar in both groups (p=0.204). The risk of severe anaemia did not differ significantly between both groups (risk difference -1.75% [95% CI; -4.16 to 0.66]. The risk of parasitaemia was considerably lower in the ISTp-AL arm (risk difference -3.96% [95% CI -7.76 to -0.16]). The risk of low birthweight was significantly lower in the ISTp-AL arm after controlling for maternal age, gravidity and baseline parasitaemia (risk difference -1.53% [95% CI; -1.54 to -1.51]). Complaints of fever were more frequent in the ISTp-AL arm (p=0.022). The prevalence of the triple *Pf dhfr* mutation and the A437G/A581G mutations were very high among malaria positive women.

## ***Conclusions***

The trial results suggest that in this area of high and perennial malaria transmission with moderate sulfadoxine-pyrimethamine resistance, ISTp with AL may be an effective strategy for controlling malaria in pregnancy.

**Keywords:** malaria, sulfadoxine-pyrimethamine resistance, gametocytes, QT-NASBA

## ABBREVIATIONS AND ACRONYMS

AC	Asymptomatic carrier
ACT	Artemisinin combination therapy
AE	Adverse event
AIDS	Acquired Immunodeficiency Syndrome
AIM	Action and Investment to Defeat Malaria
AL	Artemether-lumefantrine
ANC	Antenatal care
AS	Artesunate
ATP	According-to-protocol
AQ	Amodiaquine
BMGF	Bill and Melinda Gates Foundation
CD	Chlorproguanil-dapsone
cDNA	copy Deoxyribonucleic acid
CI	Confidence interval
CSA	Chondroitin Sulphate A
CQ	Chloroquine
DBS	Dried blood spot
DDT	Dichlorodiphenyltrichloroethane
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase enzyme
<i>Dhfr</i>	Dihydrofolate reductase gene
DHPS	Dihydropteroate synthase enzyme
<i>Dhps</i>	Dihydropteroate synthase gene
DNA	Deoxyribonucleic acid
ECL	Electrochemiluminescence
EIR	Entomological inoculation rate
EPI	Expanded Programme on Immunisation
FANC	Focused antenatal care
GDP	Gross Domestic Product
GTS	Global Technical Strategy for Malaria
GuSCN	Guanidiniumisothiocyanate

G6PD	Glucose-6-Phosphate Dehydrogenase
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HRP2	Histidine-Rich Protein II
HRP3	Histidine-Rich Protein III
IgG	Immunoglobulin G
IPT	Intermittent Preventive Treatment
IPTc	Intermittent preventive treatment in children
IPTi	Intermittent preventive treatment in infants
IPTp	Intermittent preventive treatment in pregnancy
IQR	Inter-quartile range
IRS	Indoor Residual Spraying
ISTp	Intermittent screening and treatment in pregnancy
ITN	Insecticide-treated net
IUGR	Intrauterine growth retardation
LAMP	Loop-mediated isothermal amplification
LBW	Low birth weight
LLIN	Long lasting insecticidal net
MDA	Mass drug administration
MDGs	Millennium Development Goals
MiP	Malaria in Pregnancy
mITT	Modified intention-to-treat
mRNA	Messenger RNA
MSAT	Mass Screening and Treatment
MQ	Mefloquine
NADPH	Nicotinamide adenine dinucleotide phosphate
NDHS	Nigeria Demographic and Health Survey
NMTP	National Malaria Treatment Policy
PABA	Para-aminobenzoic acid
PfEMP1	<i>Plasmodium falciparum</i> Erythrocyte Membrane Protein
PCR	Polymerase chain reaction
pLDH	Plasmodium lactate dehydrogenase
PQ	Piperaquine

Q-PCR	Quantitative Polymerase Chain Reaction
QT-NASBA	Quantitative nucleic acid sequence based amplification
RBM	Roll Back Malaria
RDT	Rapid diagnostic test
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
R&D	Research & Development
SAE	Serious adverse event
SDGs	Sustainable Development Goals
SERCaP	Single Encounter Radical Cure and Prophylaxis
SMC	Seasonal malaria chemoprevention
SNP	Single nucleotide polymorphism
SP	Sulfadoxine-pyrimethamine
sRNA	small Ribonucleic acid
T3	Test. Treat. Track.
VAR2CSA	Variant Surface antigen 2-CSA
VSA <sub>PAM</sub>	Variant Surface antigen-pregnancy associated malaria
WHO	The World Health Organization

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# 1 INTRODUCTION, JUSTIFICATION AND OBJECTIVES OF THE STUDY

## 1.1 INTRODUCTION

Malaria in pregnancy remains a global health problem, accounting for 15% of maternal deaths in some malaria endemic regions (Menendez et al., 2008). Malaria also contributes to low birth weight (LBW), either through intrauterine growth retardation (IUGR) or preterm delivery. Malaria also contributes to the occurrence of severe maternal anaemia (Desai et al., 2007). An estimated 125 million women living in malaria-endemic areas become pregnant each year (Dellicour et al., 2010), and over half live in high-transmission areas in Africa (World Health Organization, 2011).

The World Health Organization (WHO) recommends a package of interventions to prevent the adverse effects of malaria in pregnancy (MiP) in areas with stable transmission in sub-Saharan Africa. The package of interventions includes the use of insecticide-treated nets (ITN), intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) and effective case management of malaria and anaemia (World Health Organization, 2004). IPTp-SP is delivered to pregnant women primarily through antenatal care (ANC) clinics, and ITNs are delivered through ANC together with other systems and mechanisms. Since 2012, revised WHO guidelines now recommend a dose of IPTp-SP at each scheduled ANC visit, beginning as early in the second trimester, and with each dose at least a month apart with each woman receiving at least three doses of IPTp-SP during each pregnancy. This recommendation is for pregnant women living in areas of moderate-to-high malaria transmission (World Health Organization, 2012a).

IPTp-SP has been shown to be effective in preventing maternal and placental malaria as well as improving pregnancy outcomes among parturient women in Nigeria (Falade et al., 2007, Aziken et al., 2011, Agomo et al., 2011). SP has remained the drug of choice for IPTp because of its efficacy, convenient treatment dose, and high compliance rate. However, the emergence and increasing spread of *Plasmodium falciparum* parasites resistant to SP have raised concern over the long-term usefulness of SP as IPTp (Iriemenam et al., 2012, Mockenhaupt et al., 2008).

## **1.2 JUSTIFICATION OF THE STUDY**

There has been a global decline in the number of malaria cases globally including Africa but the deleterious effects of malaria in pregnancy remain a key public health problem in Nigeria. SP remains the drug of choice for IPTp despite growing resistance. It may be useful only to administer full malaria treatment doses to pregnant women only when parasitaemia is detected as a mechanism for reducing drug pressure and resistance. It has been argued that the continued use of SP for intermittent preventive treatment in pregnant women, may further increase the prevalence of SP-resistant parasites and/or lead to the selection of new mutations (Lucchi et al., 2015).

Studies have proposed that Intermittent Screening and Treatment (IST) - screening of pregnant mothers using rapid diagnostic test (RDT) for malaria infection at scheduled antenatal clinic visits may be a good alternative to IPTp-SP. With this approach, treatment with an effective antimalarial is given only to women with parasitologically confirmed malaria. (Tagbor et al., 2010, Tagbor et al., 2015). In Nigeria, artemether-lumefantrine (AL) is the first-line treatment for uncomplicated falciparum malaria in adults and children and is safe for use in pregnancy in the second and third trimesters (Federal Ministry of Health, 2011).

This study was designed to compare the effectiveness of intermittent screening and treatment with artemether-lumefantrine (ISTp-AL) to intermittent preventive treatment with SP (IPTp-SP) in pregnant women in Calabar, Cross River State, south-east Nigeria; an area of high perennial malaria transmission and widespread SP resistance in the general population (Federal Ministry of Health, 2002).

## **1.3 OBJECTIVES OF THE STUDY**

### **1.3.1 BROAD OBJECTIVE**

To show that the long-lasting insecticidal net (LLIN) plus parasitological screening followed by treatment with AL (ISTp-AL) is not inferior to LLIN plus IPTp-SP in reducing the burden of malaria during pregnancy.

### **1.3.2 SPECIFIC OBJECTIVES**

Primary objective

1. To compare the prevalence of severe anaemia (Hb < 8g/dl) at 36 to 40 weeks of gestation in the ISTp and IPTp arm.

#### Secondary objectives

2. To compare the prevalence of LBW (BW < 2500g) within 4 -6 days of birth in the ISTp and IPTp arm.
3. To compare the prevalence of anaemia (Hb < 11g/dl) at 36 to 40 weeks of gestation in the ISTp and IPTp arm.
4. To determine the prevalence of SP molecular resistance markers in *P. falciparum* isolates from pregnant women in Southeast, Nigeria
5. To compare prevalence and density of maternal parasitaemia at each visit in the ISTp and IPTp arm
6. To compare the incidence of spontaneous abortions, intrauterine deaths/stillbirths, neonatal and maternal mortality and developmental delays in the ISTp and IPTp arm.
7. To compare prevalence and density of gametocytaemia at each visit in the ISTp and IPTp arm.
8. To compare the prevalence of placental malaria at delivery in the ISTp and IPTp arm.

## 2 LITERATURE REVIEW

### 2.1 MALARIA BIOLOGY

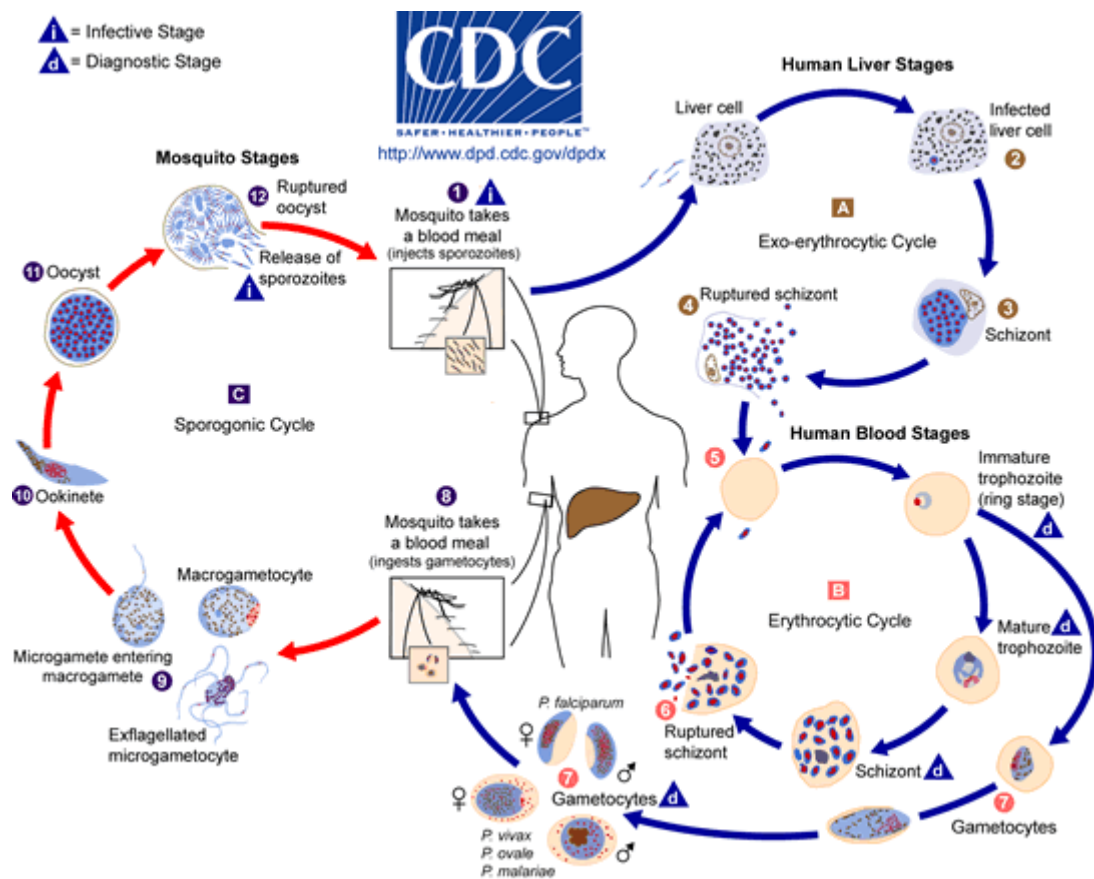
Malaria is a febrile illness caused by protozoan parasites of the genus *Plasmodium*. Infected female anopheline mosquitoes bite humans and transmit the parasite. Five species of *Plasmodium* cause malaria disease in humans: *P. falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and more recently *Plasmodium knowlesi*. *P. ovale* is made up of two subspecies *P. ovale curtisi* and *P. ovale wallikeri*. *Plasmodium falciparum* is the most common species globally, accounts for about 90% of malaria infections in Nigeria and is responsible for almost all of the severe disease and malaria deaths. The life cycle of *P. falciparum* consists of development in the human host and the mosquito vector (Figure 2.1).

#### 2.1.1 LIFE CYCLE OF THE MALARIA PARASITE – PLASMODIUM FALCIPARUM

The life cycle of *Plasmodium falciparum* begins with the bite of an infected female Anopheles mosquito. The mosquito while taking a blood meal, releases sporozoites into the bloodstream which travel to the liver and invade the liver cells within 30 minutes of the release. Inside the hepatic cells of the liver, the parasites rapidly differentiate and undergo asexual multiplication, resulting in the release of merozoites that invade other liver cells. The liver stage of *P. falciparum* life cycle lasts for 5 to 7 days and is not associated with any clinical manifestations. The liver stages of *P. vivax*, *P. ovale* and *P. malariae* last for 6-8 days, 9 days and 14-16 days respectively.

These merozoites from the hepatocytes burst into the host's bloodstream where they invade the erythrocytes. Further multiplication takes place inside the erythrocytes, enlarging into ring trophozoites that divide asexually producing schizonts. The schizonts divide further causing a release of merozoites when the erythrocytes are ruptured. The released merozoites in the blood stream are responsible for the clinical manifestation of malaria illness such as fever, joint and muscle pains and chills. This stage called the erythrocytic stage is the asexual life cycle of the parasite and usually lasts for 48 hours for *P. falciparum*.

Some of the schizonts divide into sexual forms resulting in male and female gametocytes. The female *Anopheles* mosquito takes up the gametocytes during another blood meal from a host. The male gametocytes undergo a rapid nuclear division inside the midgut of the mosquito resulting to microgametes, which fertilise the female macrogametes producing ookinete. The ookinete later crosses the gut wall as oocyst, which subsequently ruptures releasing sporozoites into the mosquito's body cavity from where they eventually migrate to the salivary gland of the mosquito. With another blood meal, the mosquito transmits the sporozoites from its salivary glands into the human blood stream. (Centers for Disease Control and Prevention).



**Figure 2.1:** Life cycle of malaria.

Source: (Centers for Disease Control and Prevention)

During malaria infection, both asexual and sexual forms of the parasite can circulate in the bloodstream. The proliferation of asexual forms in the host's red blood cells causes clinical disease. The gametocytes do not directly cause disease symptoms

but are essential for transmission of the disease and spread of parasites in the population (Shute and Maryon, 1951)

### **2.1.2 PLASMODIUM FALCIPARUM GAMETOCYTE DEVELOPMENT**

The developmental process of *P. falciparum* gametocytes to maturation involves five distinct stages (I-V) which occur over 10-12 days (Hawking et al., 1971). Although all five stages can be seen in *in vitro* parasite cultures, only stage V gametocytes can be viewed in the circulation because the immature stages (I-IV) sequester from the bloodstream. Stage I gametocytes cannot be distinguished from asexual trophozoites; because morphological changes begin from the Stage II and morphological differences between male and female gametocytes are clearly visible from Stage III gametocytes (Sinden, 1982).

In stage III, gametocytes lengthen further, and the ends become rounded. In females, mitochondria and Golgi bodies proliferate. Stage IV gametocytes continue to lengthen but now have pointed ends, and osmiophilic bodies and extensive rough endoplasmic reticulum develop in females. Gametocytes eventually assume their characteristically crescent shape in stage V. In this stage, male and female gametocytes can be readily differentiated, as the females are more elongated and curved than the thicker males (Dixon et al., 2012).

Stages I to III are susceptible to schizonticidal antimalarials. For Stages IV and V, which represent the last part of their maturation process, they become generally insensitive to most antimalarial drugs, except for primaquine and methylene blue (Adjalley et al., 2011, Smalley, 1977, Chutmongkonkul et al., 1992, Kumar and Zheng, 1990, Burgess and Bray, 1961). Mature stage V gametocytes are developmentally arrested, and current evidence suggests that they are minimally metabolically active (Sinden and Smalley, 1979). This reduced metabolism may account for their insensitivity to most schizonticidal drugs (Smalley, 1977, Chutmongkonkul et al., 1992).

Several stage-specific molecular and biochemical changes precede the morphological changes of the gametocyte (Schneider et al., 2004). Sexual stage development begins in the asexual cycle prior to morphological changes of the gametocytes with the expression of Pfs16 mRNA as the initial marker of parasite commitment to sexual stage development (Bruce et al., 1990, Niederwieser et al.,

2000). Schizonts that are committed to developing into sexual stage parasites then produce only one sex of gametocytes (all either male or female gametocytes) (Silvestrini et al., 2000, Smith et al., 2000).

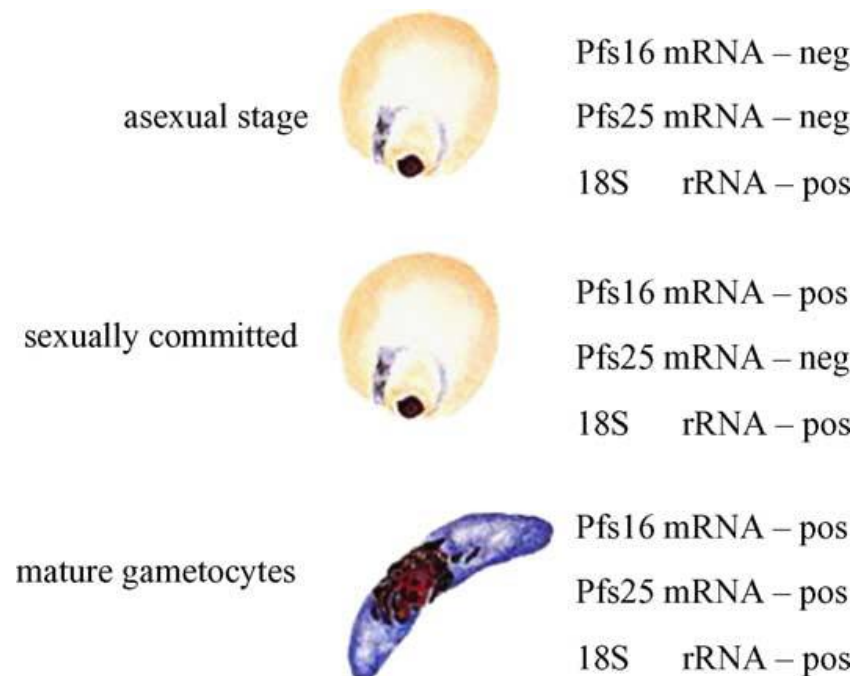
## **2.2 MOLECULAR DETECTION OF PLASMODIUM FALCIPARUM**

Molecular techniques for *P. falciparum* detection are based on DNA or RNA amplification and are more sensitive than light microscopy. However, these molecular techniques have their limitations. Problems are often encountered with either stage-specific detection or quantification. Polymerase chain reaction (PCR) can detect parasite DNA with a detection limit of about 20 parasites/ml of blood (Snounou et al., 1993, Rubio et al., 2002) however, it is semi-quantitative and thus does not accurately quantify the number of parasites present. Quantitative real-time PCR (Real-time Q-PCR) was developed for detection of *P. falciparum* 18S small subunit ribosomal RNA genes and can detect up to 20 parasites/ml of blood (Hermesen et al., 2001). The technique, however, requires relatively large amounts of venous blood (up to 0.5 ml), and white blood cells need to be filtered from the blood samples to prevent problems during processing, caused by otherwise gelatinous DNA preparation (Hermesen et al., 2001). Additionally, PCR techniques based on the amplification of DNA cannot detect several developmental stages of the parasites, including gametocytes. With reverse- transcriptase PCR (RT-PCR) detection of the various developmental stages in quantitative and sensitive assays based on stage-specific gene expression is possible (Babiker et al., 1999). However, because introns are absent in most *Plasmodium* genes, this method cannot definitively detect RNA without the complete removal of genomic DNA from the sample. Even a DNase treatment of the isolated RNA often does not ensure absolute removal of genomic DNA. This affects accurate quantification using RT-PCR (Schneider, 2006). One technique that may avoid the above-stated problems is quantitative nucleic acid sequence-based amplification (QT-NASBA).

## **2.3 MOLECULAR QUANTIFICATION OF GAMETOCYTES**

Due to sequestration in the vasculature, only asexual ring stages, sexually committed ring stages, and mature gametocytes of *P. falciparum* can be found in the circulation (Schneider et al., 2004). The differential expression of the sexual stage-

specific genes *Pfs16* and *Pfs25* allows for separate quantification of these stages (Figure 2.2). *Pfs16* mRNA expression aids in the identification of circulating sexual stage parasites i.e. sexually-committed ring stages and fully mature gametocytes. However, *Pfs25* mRNA is expressed only in Stage V gametocytes, which means it can be used as a marker to quantify mature gametocytes specifically (Babiker et al., 1999). Recent findings suggest that it is possible to differentiate female from male gametocytes by RNA. It has been established that *Pfs25* and *Pfs230p* mRNA expression is specific to mature female and male gametocytes respectively (Schneider et al., 2015).



**Figure 2.2:** Differential expression of the sexual stage-specific genes *Pfs16* and *Pfs25*.

Neg: negative, no RNA expression; pos: positive, RNA expression.

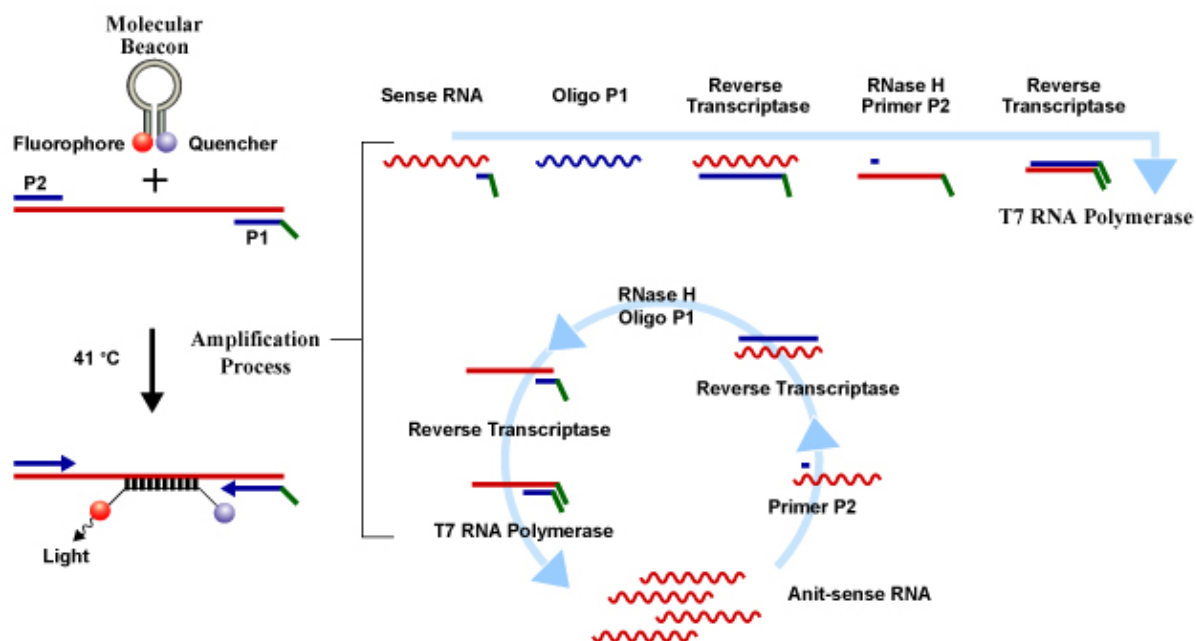
Source: (Schneider et al., 2004).

## 2.4 QUANTITATIVE NUCLEIC ACID SEQUENCE-BASED AMPLIFICATION (QT-NASBA)

QT-NASBA employs the activities of three enzymes (AMV-RT, RNase H, and T7 RNA polymerase). It also involves two target-specific primers, of which one includes

a T7 polymerase promoter, for continuous and direct amplification of RNA molecules in a single mixture at a temperature of 41°C (Compton, 1991, Van Gemen et al., 1993, Schneider et al., 2004). The low-temperature guarantees that primers only anneal to single-stranded target RNA and prevents the amplification of genomic DNA which may be present in the sample. This low-temperature allows for specific detection of living, metabolically active cells and organisms. Figure 2.3 shows a schema of the principle of QT-NASBA. Detection of the amplified nucleic acid during the amplification reaction results in the real-time detection of positive signal, thus minimising the hands-on time compared to detection methods that need post-amplification handling of the amplified nucleic acid. The various developmental stages of the same organism can be detected by QT-NASBA through the measurement of stage-specific gene expression. This can be performed on small finger-prick blood samples. The high accuracy, sensitivity and quick reaction times that allow for high throughput of samples, make QT-NASBA extremely suitable for large-scale epidemiological investigations.

QT-NASBA has proved to be more sensitive and specific than other available molecular techniques, especially for RNA targets (Deiman et al., 2002). QT-NASBA for the detection of *P. falciparum* parasite loads has a detection limit of about 10 parasites/ml of blood (Schoone et al., 2000, Smits et al., 1997). This technique is based on specific amplification of 18S rRNA of the parasites and detection using electrochemiluminescence (ECL). Due to the ability of QT-NASBA to accurately quantify RNA in the presence of DNA, it can be used to detect various parasite developmental stages based on their stage-specific differences in gene expression. For *P. falciparum*, this may enable separate quantification of gametocytes in the blood sample (Schneider et al., 2004).



**Figure 2.3:** Principle of nucleic acid sequence-based amplification.

Source: (Premier Biosoft, 2016)

This mechanism requires two short single-stranded DNA fragments, primers, and three enzymes. The viral RNA strands are represented as the sense strand present in the original samples. Primer P1 binds to the RNA and is elongated by reverse transcriptase (AMV-RT). The RNA strand of the yielded DNA: RNA hybrid is hydrolyzed by RNase H. After the binding of P1, primer P2 can also bind. AMV then elongates primer P2 – RT, yielding a double-stranded DNA molecule. Primer P1 is designed in such a manner that when it forms a double-stranded DNA, it codes for a T7 RNA polymerase Promoter site. This helps in generating antisense RNA copies using a DNA template. The new copies of DNA are generated using RNA. The process is same as followed for sense strand. Here, in this case, P2 will bind first. In a NASBA reaction, DNA is the final product formed. It has a promoter region. This allows T7 RNA polymerase to use it as a template. The reaction sequences starting from the sense and antisense RNA strands are different, but they are considered kinetically identical. They both are referred to as copy DNA (cDNA).

## 2.5 MALARIA TRANSMISSION AND ENDEMICITY

The pattern of malaria transmission underpins several aspects of malaria epidemiology. It affects the prevalence, age profile of infection, the incidence and types of syndromes as well as mortality. Knowledge of all these characteristics influences the choice of appropriate interventions to be used in malaria control and also affect the outcome of control efforts (Hay and Snow, 2006, Hay et al., 2008). The intensity of malaria transmission is dependent on factors related to the

environment, the vector, the parasite, and the human host. The level of malaria endemicity varies between different geographical areas and between and within countries.

Transmission is intense in places where the mosquitoes are anthropophilic and where favourable climatic conditions (temperature, humidity and rainfall patterns) prolongs the lifespan of the mosquitoes and increases abundance. This ensures parasites have ample time to complete their development inside the mosquito. These two attributes of the dominant African vector species-*Anopheles gambiae* is the principal reason why close to 90% of global malaria cases are in Africa (World Health Organization, 2015a).

In areas of stable transmission, there is often an increased incidence coinciding with increased mosquito breeding during the rainy season. In such areas, morbidity, and mortality from malaria are marked during early childhood, but by adulthood most malaria infections are asymptomatic (Dondorp et al., 2008).

In other settings, transmission is seasonal, with the peak during and immediately after the wet season. Malaria epidemics can occur when climatic factors and other conditions unexpectedly become favourable for transmission in areas where the population have little or no malaria immunity. They can also happen when people with low immunity move into areas with intense malaria transmission, for instance, to find work, or when displaced by conflict (White et al., 2014)

Human immunity is another important factor, especially among adults in areas of moderate or intense transmission conditions. Partial immunity develops over years of exposure, and while it never provides complete protection, it does reduce the risk of severe disease. Thus, most malaria deaths in Africa occur in young children under five years of age, whereas in areas with less malaria transmission and low immunity, all age groups are at risk (World Health Organization, 2014a).

## **2.6 MALARIA IN SUB-SAHARAN AFRICA**

Malaria continues to be a threat for the sub-Saharan Africa population. The past century has witnessed lots of research in malaria after the discovery of malaria parasites in human blood by Charles Laveran in 1880 (Raghavendra et al., 2011) and the establishment of the mosquito's role in the transmission of malaria by Sir

Ronald Ross in 1898. There were 214 million (95% confidence interval 149-303) cases of clinical malaria in 2015 of whom 88% or 188 million cases occurred in the African region. Approximately 438 000 malaria deaths occurred in 2015 of which 90% or 395 000 were in the African region; deaths in children less than five years old accounted for 74% of the estimated deaths (World Health Organization, 2015a). A concerted global effort, through renewed political will and social support, has been made in the last 15 years to reduce the high burden of malaria in the world and mainly in sub-Saharan Africa. Different approaches have been proposed to combat malaria. It has been postulated that malaria could only be surmounted through improved access to diagnosis and treatment, coupled with health system strengthening, human capacity building, environmental management, and economic development. Others favoured a more aggressive approach using large-scale formal campaigns of vector control or mass drug administration to quickly prevent and eradicate malaria from the world.

The latter approach achieved remarkable feats such as the interruption of malaria and yellow fever transmission during the construction of the Panama Canal and the elimination of the African vector *An. gambiae* in Brazil. However, sustainability seemed to require the solid public health foundations envisaged by the former approach (Nájera et al., 2011).

## **2.7 HISTORICAL MALARIA ERADICATION PROGRAMMES: SUCCESS AND FAILURE**

Eradication refers to the permanent reduction to zero of the global incidence of malaria as a result of deliberate efforts. Eradication would mean intervention measures are no longer needed. In the context of malaria, this would entail the extermination of the parasite, not the mosquitoes that transmit malaria (Roll Back Malaria, 2014).

Malaria was eliminated from most of Europe and the United States during the first half of the twentieth century as a result of changes in land use, agricultural practices and house construction and some targeted vector control (Greenwood and Mutabingwa, 2002). The discovery of the insecticidal activity of DichloroDiphenylTrichloroethane (DDT) in 1939 by Paul Müller initiated a global

programme for the elimination of malaria in the 1950s and sixties (Centers for Disease Control and Prevention, 2016).

DDT was used by many national malaria control programmes for indoor residual spraying (IRS) in the malaria eradication programme from 1957 to 1969. The World Health Organization (WHO) eradication efforts focused on house spraying with residual insecticides, mass drug administration (MDA), and surveillance. It was to be carried out in four successive steps: preparation, attack, consolidation, and maintenance (World Health Organization, 2006a).

MDA is the administration of a full therapeutic dose of an antimalarial regimen to an entire population or well-defined subpopulation at the same time. The strategy was recommended by the World Health Organization in the 1950's as an extra tool to replace failing strategies (von Seidlein et al., 2003). MDA works through the reduction of the prevalence of peripheral parasitaemia and the reduction of transmission by the inhibition of the liver or asexual intraerythrocytic stages of the parasite, the direct action on the gametocytes or a sporonticidal effect and the inhibition of the sporogonic cycle in the mosquitoes (Poirot et al., 2013).

As a result of IRS the total population at risk was halved by 1975, from 77% in 1900 and there was a dramatic decrease in the mortality rate from 19.4 per 10 000 in 1900 to 1.61 per 10 000 in 1975 (Enayati and Hemingway, 2010). Achievements included elimination in nations with temperate climates and seasonal malaria transmission. This strategy was initially very successful in eliminating malaria in countries with temperate climates and seasonal malaria transmission such as India, Sri Lanka and the former Soviet Union (Greenwood and Mutabingwa, 2002). However, this success was not sustained, as highlighted by substantial increases in the incidence of malaria, due to the high costs of the programme, the resistance of many communities to repeated spraying of their homes and the emergence of vector resistance to DDT (Greenwood and Mutabingwa, 2002). The failure of this global strategy coupled with the magnitude and intensity of malaria transmission meant that malaria eradication was never systematically attempted in sub-Saharan Africa. However, control interventions before 1960 resulted in some countries in Southern Africa (South Africa, Swaziland, Zimbabwe, and Mauritius) reaching consolidation phase (stage 2 of the WHO elimination plan). There was also a trend towards the

interruption of transmission in forest areas of Liberia and Cameroon and highland savanna areas in Madagascar and Uganda (Enayati and Hemingway, 2010).

Subsequently, pilot interventions were conducted in five eco-epidemiological zones of Africa to ascertain whether malaria transmission could be interrupted on a local scale (Molineaux et al., 1980). These pilot interventions recorded significant success in areas of Africa with tropical and temperate climates (semi-desert areas, remote islands, and Highland savannahs) where malaria was eliminated. There were also drastic reductions in transmission intensity in hyperendemic areas (Bradley, 1991, Delfini, 1969) with the disappearance of the vector *Anopheles funestus* and declined density of *An. gambiae* (Enayati and Hemingway, 2010). Despite their impressive health benefits, the African trials were considered failures because elimination was not achieved (Lines et al., 2007). Few years after the interventions stopped, entomological indices had returned to pre-intervention levels (Enayati and Hemingway, 2010).

MDA was used in conjunction with other control measures and proved to be successful in some settings. High propoxur IRS coverage was supplemented with MDA using sulfalene-pyrimethamine in the Garki project, Northern Nigeria (Molineaux et al., 1980). Notably, this combination of MDA and IRS also failed to interrupt transmission.

MDA was a component of several malaria elimination programmes during the eradication era, but it is not currently recommended due to concerns about efficacy, logistical feasibility, sustainability and the promotion of drug resistance (Watkins et al., 1987). Though not officially endorsed by WHO, MDA is being considered and evaluated as an approach to reduce the burden of malaria with the ultimate goal of elimination.

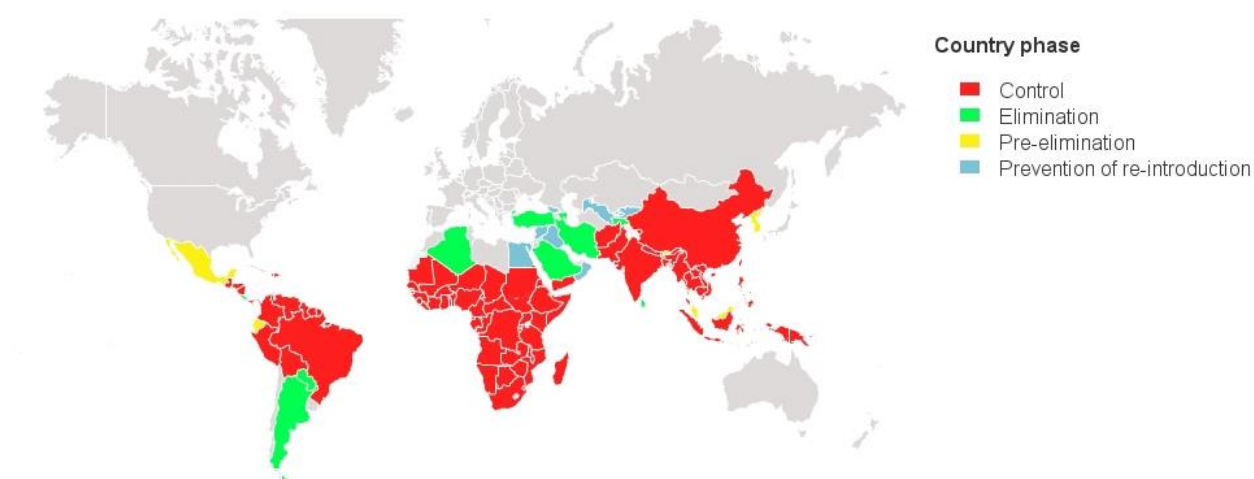
The development of intermittent preventive treatment was a way to build on limitations of MDA. With IPT, drug administration is repeated at intervals due to the short-lasting benefit of MDA (Zongo, 2014).

## **2.8 MODERN MALARIA ELIMINATION AND ERADICATION**

The Roll Back Malaria (RBM) programme was established in 1998 with the major aim of halving the malaria burden by 2010 (Roll Back Malaria, 2005). Subsequently,

44 Heads of State and government delegations in April 2000, adopted the Abuja Declaration on Roll Back Malaria. They agreed the objective was to ensure that 60% of pregnant women and children under five years of age, who were vulnerable and susceptible, had access to ITNs and prompt, effective treatment of malaria by the year 2005 (Roll Back Malaria and World Health Organization, 2000). Since then, countries have made efforts to support the international fight to control malaria through the Roll Back Malaria initiative. The Partnership has grown over time with a wide array of organisations from country level to regional and global level.

The goal of eradicating malaria was supported more ambitiously by the Bill & Melinda Gates Foundation (BMGF) in 2007 and supported by the WHO (Lines et al., 2007, Feachem and Sabot, 2008). They acknowledged that the current tools were insufficient to bring about eradication. They identified new priorities such as transmission-blocking, chemoprotection in vulnerable groups, (malERA Consultative Group on Drugs, 2011) and new target candidate profiles (Alonso et al., 2011). They called for a massive directed research effort to produce a malaria vaccine, and better drugs and insecticides, together with a clear strategy for delivery to provide access to these new technologies in the poorest of the populations in malaria-endemic countries with the highest burden of disease.



**Figure 2.4:** Classification of countries by stage of malaria elimination (December 2014).

Source: Global Malaria mapper (<http://www.worldmalariaeport.org/>).

Significant and widespread reductions in malaria prevalence and incidence have occurred across Africa since 2000, as a result of sustained financing and scale-up of malaria control interventions. Between 2005 and 2015, there has been a 50% reduction in childhood malaria. Mortality and cases have reduced by 50% and 25% respectively. In this period, four countries have been certified as malaria-free by WHO (World Health Organization, 2014b). Figure 2.4 shows global progress made in efforts at eliminating malaria. The progress made has prompted increased global dialogue on malaria elimination and eradication. However, the decline remains below internationally agreed targets for universal coverage (World Health Organization, 2015a). Insecticide-treated nets (ITNs) have had the greatest impact but have also been present for longer and at higher levels of coverage. Indoor residual spraying (IRS) and artemisinin combination therapy (ACTs) have both made substantial contributions to reducing prevalence and incidence where they have been implemented at scale (Bhatt et al., 2015a).

There is an arsenal of novel promising tools to detect, treat and prevent malaria which are at different stages of research and development (Hemingway et al., 2016). Experts suggest that by 2035, with sustained commitment, funding for the universal coverage of existing tools and rapid scale-up and uptake of these new tools, the economic benefits from preventable malaria deaths would surpass the costs invested in malaria control interventions by a factor of up to 20 (Jamison et al., 2013)

International development partners and public health experts seem to have embraced the goal of a “world free of malaria”. This is evident from the WHO’s Global Technical Strategy for Malaria (GTS), which was endorsed by the World Health Assembly in 2015, and the Action and Investment to defeat Malaria (AIM) document of the RBM Partnership which both allude to the need to achieve a “world free of malaria”. Both documents have put forward ambitious targets of reducing malaria case incidence and mortality rates globally by at least 90% by 2030 (Roll Back Malaria Action, 2015, World Health Organization, 2015b). However, challenges remain in achieving these targets. Vector and parasite resistance to insecticides and medicines respectively continues to increase. Resistance to ACTs has been identified in Cambodia, Lao DPR, Myanmar, Thailand and Vietnam all in Southeast Asia. The spread of these strains to the Indian subcontinent or Africa could be disastrous. Furthermore, resistance has been detected against two or more

insecticides in two-thirds of African malaria-endemic countries (Hemingway et al., 2016).

## **2.9 GLOBAL MALARIA CONTROL**

Global malaria control programmes have three main components; vector control, chemoprevention and case management. Vector control aims to prevent transmission of the parasite from humans to mosquitoes and back to humans. Chemoprevention prevents new cases and suppresses blood-stage infection from further development to a clinical case. Finally, case management through prompt diagnosis and treatment if infectious contact happens and results in a clinical case.

### **2.9.1 VECTOR CONTROL**

Vector control is an integral part of the strategies to prevent and reduce the malaria burden in endemic countries, by reducing transmission. It has been endorsed by WHO and the RBM partnership. ITNs or LLINs and indoor residual spraying (IRS) are currently the preferred methods of malaria vector control. In many malaria-endemic countries, both approaches are used simultaneously in the same households to suppress transmission in hyper- and holoendemic situations (Okumu et al., 2011).

#### **Insecticide-treated bed nets**

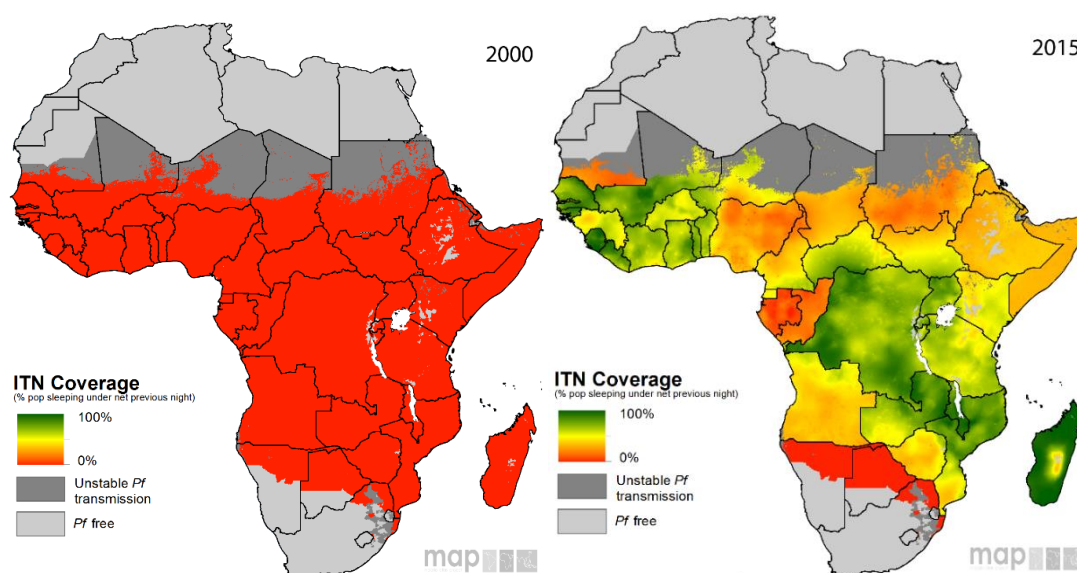
LLINs reduce the number of malaria cases and deaths and represent a cornerstone of the scale-up in malaria control (World Health Organization, 2015a). The effectiveness of ITNs at preventing malaria morbidity and mortality has been demonstrated from the 1990s (Lengeler, 2000). Their public health impact has been proven, especially for ITNs which, in areas with high coverage rates, can reduce malaria cases by 40–60% (Roll Back Malaria, 2008). In Sub-Saharan Africa, less than one-third (29%) of households had enough ITNs for all household members, and one-third of households did not own a single ITN (World Health Organization, 2014a).

The Roll Back Malaria Partnership set goal was to scale-up ITN use to all pregnant women by 2015 (Roll Back Malaria, 2011). This target, however, was not met. Despite their proven efficacy, the achievement of widespread use of ITNs and LLINs

has been difficult because of sociologic or financial barriers or insufficiency of the provision.

ITNs are bed nets that have been treated with safe, residual insecticide for the purpose of killing and repelling mosquitoes, which carry malaria (Curtis et al., 1996, Lines, 1996). LLINs are designed to remain effective for several years without retreatment. However, emerging resistance to pyrethroids, the only class of insecticides used in LLINs, is one of the key challenges for this tool. Also, replacing worn-out nets is crucial to achieving and maintaining universal coverage (Alonso and Tanner, 2013). Newer classes of insecticides which are longer-lasting are the current focus of research and development efforts.

Considerable effort has been made to make the LLINs available to those who need them. This effort has focused mainly on the free distribution of LLINs to pregnant women and children less than five years old during antenatal care and the Expanded Programme on Immunisation (EPI) vaccination respectively. There have also been massive campaigns of LLIN distribution to households free of charge by National Malaria Control Programmes (NMCPs). LLINs are also provided through the private sector and non-governmental organisation usually at a subsidised price. Figure 2.5 compares ITN coverage in 2000 to 2015 in sub-Saharan Africa.



**Figure 2.5:** Proportion of population sleeping under an ITN, sub-Saharan Africa, 2000 and 2015.

Source: (Bhatt et al., 2015b).

## **Indoor residual spraying**

IRS is the application of stable formulations of insecticides to the inside of houses to kill resting adult female mosquitoes (Enayati and Hemingway, 2010). The primary contributions of IRS in reducing malaria transmission are shortening the life span of female mosquitoes so that they no longer transmit malaria parasites, and reducing the vector density. Current IRS insecticides last from two to six months. IRS is used in most malaria endemic countries although it protects only a small proportion of the at-risk population. Although, as many as 88 countries (42 in the WHO African region) have adopted IRS for vector control (World Health Organization, 2014b), coverage of IRS is significantly lower than that of ITNs. In 2014, only 3.4% of the global population at risk was protected by IRS, decreasing from more than 5% in 2010 (World Health Organization, 2015a). Spraying at least 80% (ideally 100%) of houses, structures, and units in the target area in any round of spraying is recommended by WHO (World Health Organization, 2006b).

In addition to huge gaps in coverage, the effectiveness of vector control is threatened as malaria mosquitoes develop resistance to the classes of insecticides used in ITNs and IRS. In some areas, resistance to all four classes of insecticides used for public health has been detected (see Table 2.1). The Global plan for insecticide resistance management in malaria vectors (World Health Organization, 2012b) was launched by WHO to address this issue. It recommends strategies such as rotating insecticides and mosaic spreading to preserve the effectiveness of current compounds, and country implementation has started.

The threat of insecticide resistance represents one of the main challenges to future progress against malaria; new products and chemical compounds to fight resistance are therefore a top priority for research and development.

Other innovative vector control strategies include larval control measures and the development of transgenic mosquitoes.

**Table 2.1:** Insecticide resistance mechanisms of selected major malaria vector species.

Source: (World Health Organisation, 2012b).

Vector species	Pyrethroids		DDT		Organophosphates		Carbamates	
	Target-site	Metabolic	Target-site	Metabolic	Target-site	Metabolic	Target-site	Metabolic
<i>An. gambiae</i> s.s	x	x	x	x	x		x	x
<i>An. funestus</i> s.s				x				x
<i>An. arabiensis</i>	x		x	x	x		x	
<i>An. culicifacies</i> (C)	x		x					
<i>An. culicifacies</i> (B)	x		x	x		x		
<i>An. stephensi</i>	x	x	x	x		x		
<i>An. dirus</i>				x				
<i>An. sacharovi</i>				x	x	x	x	x
<i>An. albimanus</i>		x		x	x	x	x	

### 2.9.2 CASE MANAGEMENT

Successful malaria control depends greatly on the prompt diagnosis and treatment of patients with efficacious antimalarial drugs. The WHO Global Malaria Programme's initiative – T3 (Test. Treat. Track.) - supports malaria-endemic countries to achieve universal coverage with diagnostic testing and antimalarial treatment, as well as strengthen their malaria surveillance systems (World Health Organization, 2012c). Malaria-endemic countries are expected to ensure that all suspected malaria cases are tested and that every confirmed case is treated with quality-assured antimalarial medicine. Also, it is important that malaria disease is tracked through timely and accurate surveillance systems to guide policy and operational decisions (World Health Organization, 2012c).

For decades chloroquine (CQ) was the mainstay of malaria chemotherapy and prevention because it was easy to use and well accepted despite the bitter taste of the tablets. However, resistant strains which appeared spread quickly rendering CQ no longer effective to treat malaria (Ezedinachi, 1996, Sirima et al., 2003, Tinto et al., 2002).

Amodiaquine was considered as a replacement for CQ but was more poorly tolerated than CQ. Sulfadoxine-pyrimethamine replaced CQ as the treatment for uncomplicated falciparum malaria. However, routine use as monotherapy accelerated the spread of SP resistance. Today its use is restricted to chemoprevention in infants, children, and pregnant women. In the face of the failing drugs, the idea of combination therapy emerged and was advocated for by WHO (Attaran et al., 2004).

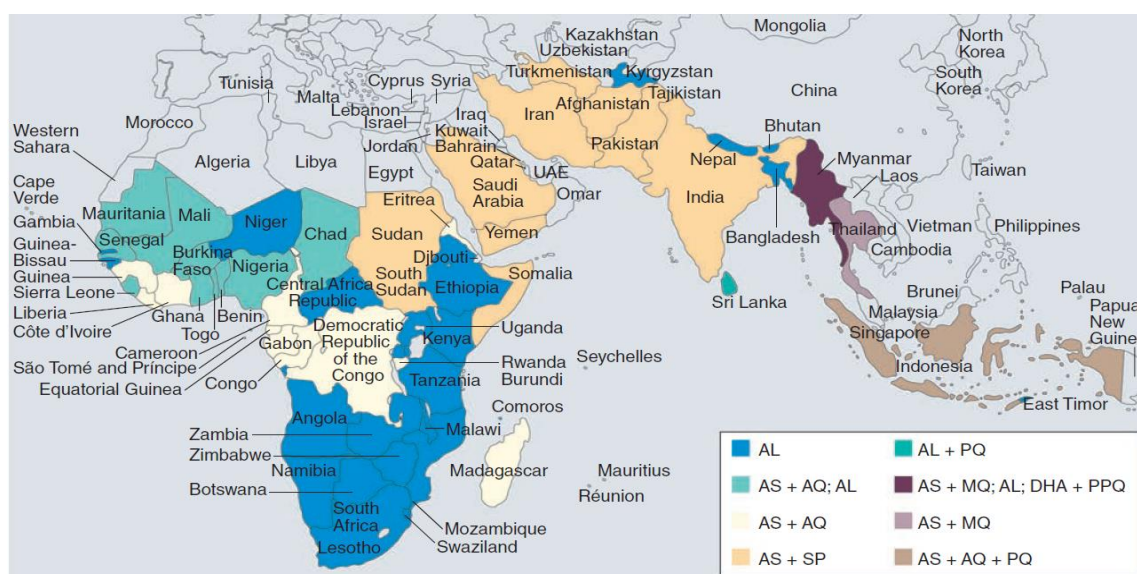
Today, countries have National Malaria Treatment Policies (NMTP), which specify drugs for the treatment of both uncomplicated and severe malaria, malaria in pregnancy as well as actions to be taken if first line treatment fails.

Currently, artemisinin-based combination therapies (ACTs) are used worldwide as the first line of treatment for uncomplicated *P. falciparum* malaria (Bosman and Mendis, 2007). The use of two or more drugs with different modes of action in combination is the current recommendation. This is to provide adequate cure rate and delay the development of resistance. Fast acting artemisinin-based compounds such as dihydroartemisinin (DHA), artesunate (AS) and artemether, are combined

with a drug from a different class. Partner drugs include lumefantrine, amodiaquine (AQ), mefloquine (MQ) sulfadoxine-pyrimethamine (SP), piperaquine (PQ) and chlorproguanil-dapsone (CD).

Currently, the management of clinical malaria is based on one of the five WHO recommended artemisinin combinations: Artemether-Lumefantrine, Artesunate-Amodiaquine, Artesunate-Mefloquine, Artesunate-Sulfadoxine-Pyrimethamine and more recently Dihydroartemisinin-Piperaquine (Figure 2.6). However, resistance to artemisinin and its partner drugs have been reported in Southeast Asia (Noedl et al., 2008, Dondorp et al., 2009, Arie et al., 2014, Straimer et al., 2015, Phyo et al., 2012).

As drug resistance develops to existing medicines, new ones need to be introduced. Several new compounds from different drug classes are at various stages of R&D. Experts have also agreed on the need for new chemical agents that can prevent relapse of *P. vivax* infection and protect vulnerable populations, especially in early pregnancy (Wells et al., 2015).



**Figure 2.6:** ACT used for the treatment of laboratory-confirmed uncomplicated *Plasmodium falciparum* malaria (February 2011).

AL: Artemether–lumefantrine; AQ: Amodiaquine; AS: Artesunate; DHA: Dihydroartemisinin-piperaquine; MQ: Mefloquine; PPQ: Piperaquine; PQ: Primaquine; SP: Sulfadoxine/pyrimethamine.

Source: (Mutabingwa and Adam, 2013)

Single Encounter Radical Cure and Prophylaxis (SERCaP) is being proposed as a silver bullet for the eradication of malaria. SERCaP should be capable of radical cure, be suitable for mass administration and be able to have a prophylactic effect. Radical cure implies the elimination of all parasites (including the long-lived hypnozoites of *P. vivax* or *P. ovale* in the liver). The drug should be suitable for mass administration and have an excellent safety profile as it will be administered to healthy subjects. Any candidate for use as SERCaP should provide prophylaxis for at least one month following treatment, to outlast the development period of Plasmodia parasites in anopheline mosquitoes. The ideal scenario for malaria eradication would be a drug that is suitable for mass administration because it provides a prophylactic effect and acts against *P. vivax* as well as *P. falciparum*. It is clear that a drug with this profile may take a long time to develop, but the development of new medicines that meet some of these essential requirements could dramatically improve chances of elimination and eradication (malERA Consultative Group on Drugs, 2011).

### **2.9.3 CHEMOPREVENTION OF MALARIA**

Chemoprevention was originally used with the aim of interrupting transmission. Although this failed, the administration of antimalarial drugs has repeatedly resulted in marked reductions in the prevalence of malaria and the incidence of clinical attacks (Greenwood, 2004). The WHO currently recommends three chemoprevention strategies involving intermittent preventive treatment (IPT) among pregnant women (IPTp), infants (IPTi) and more recently, seasonal malaria chemoprevention (SMC). These involve the administration of full treatment course of an antimalarial medicine given to prevent the consequences of malaria infections by maintaining therapeutic drug levels in the blood throughout the period of greatest malarial risk. The treatment course is given irrespective of whether the individual is infected with malaria. The aim is to prevent malarial illness.

IPTp is to be given to pregnant women at routine antenatal visits in areas with moderate to high malaria transmission in sub-Saharan Africa. IPTi should be delivered to infants through immunisation services in regions with moderate to high malaria transmission in sub-Saharan Africa while SMC is recommended to be administered to children less than five years of age during the rainy season which is

associated with higher malaria transmission in the Sahel sub-region of Africa (World Health Organization 2012e)

These interventions target specific population groups in specific transmission settings in Africa and research has demonstrated that they are effective, cost-effective and safe for preventing malaria in the target populations (World Health Organization, 2012a, World Health Organization, 2010a). IPTp has been discussed in greater detail in Section 2.11.1.

#### **2.9.4 VACCINATION**

Lots of time, effort and money have been spent on the development of malaria vaccines. Developing a vaccine against this pathogen has been a tremendous challenge because even after multiple infections, adults in malaria-endemic regions naturally acquire only partial immunity against the disease (Doolan et al., 2009). Despite considerable efforts over the past 40 years, there is currently no approved malaria vaccine.

Malaria is a protozoan disease, with an active antigenic variation that distinguishes it from the viruses and bacterial toxins that have been successful vaccine targets (Rappuoli and Aderem, 2011).

The most advanced malaria vaccine, Mosquirix (the peptide RTS, S and the proprietary AS01 adjuvant, which contains monophosphoryl lipid A and QS 21), was co-developed by GlaxoSmithKline, the PATH Malaria Vaccine Initiative, the Walter Reed Army Institute of Research, USA, and the Bill and Melinda Gates Foundation. It targets the circumsporozoite protein of *P. falciparum* and is boosted with the potent ASO adjuvant (Wells et al., 2015). Mosquirix provides efficacy against all episodes of malaria with 55% protection in children but only 33% protection in infants (RTS, 2014). The WHO has recently recommended pilot implementation be done to understand how to use the vaccine best.

#### **2.10 MALARIA IN PREGNANCY**

MiP is a significant public health problem with deleterious adverse effects to the mother and foetus. An estimated 125 million living in malaria-endemic areas become pregnant each year (Dellicour et al., 2010), and pregnancy increases the risk of

malaria infection and the severity of the disease compared to non-pregnant women of the same age (Desai et al., 2007). This increased vulnerability can be attributed to the immunological changes and hormonal factors induced by pregnancy (Rogerson et al., 2007b). Also, the higher attractiveness of pregnant women to mosquitoes and the behavioural and physiological changes associated with pregnancy are believed to contribute (Lindsay et al., 2000, Ansell et al., 2002). *P. falciparum* infection in pregnancy is associated with increased risks of maternal and foetal complications including maternal anaemia, stillbirth, premature delivery, low birth weight (LBW), perinatal and neonatal morbidity & mortality (Breman et al., 2004).

### **2.10.1 RISK FACTORS**

Maternal factors associated with the risk of MiP include age, parity, and gestational age. Younger maternal age is known to increase the risk of malaria infection (Hamer et al., 2009, Ayoola et al., 2012), independent of parity (Hamer et al., 2009). The effect of MiP is parity-specific with the risk decreasing with increased gravidity (Ayoola et al., 2012, Hamer et al., 2009, Kalilani et al., 2010, Valea et al., 2012). The peak of malaria prevalence seems to occur during the second trimester (Agbor-Enoh et al., 2003) although a study has reported higher malaria prevalence in the first trimester compared to the second and third trimesters (Coulibaly et al., 2007).

### **2.10.2 ADVERSE EFFECTS OF MALARIA INFECTION**

The effect of malaria infection during pregnancy depends on the amount of acquired immunity, which is dependent on the intensity of transmission. Pregnant women resident in areas of low and unstable *P. falciparum* malaria transmission, have little or no immunity and the adverse effects of MiP include stillbirth, miscarriage or maternal death. However, among pregnant women resident in stable transmission areas with considerable acquired immunity, the predominant adverse effects are maternal anaemia, placental malaria and low birth weight (World Health Organization, 2008) and asymptomatic infections are common. Also in these areas, the risk of low birth weight is higher when women have placental malaria especially during first pregnancies (Desai et al., 2007, White et al., 2014). Maternal mortality associated with malaria is probably under-reported. However, it has been reported as an important cause of death in several studies (Romagosa et al., 2007, Anya, 2004, Ali et al., 2012, Somigliana et al., 2011).

In high transmission settings, malaria increases the risk of low birth weight. The mechanism is mainly through intrauterine growth retardation (IUGR) rather than pre-term delivery, as most infections in these settings are asymptomatic (Takem and D'Alessandro, 2013). A meta-analysis of cross-sectional data from 32 African countries, showed malaria prevention during pregnancy was associated with 21% (95% CI; 14 to 27) relative risk reduction in LBW (Eisele et al., 2012).

Congenital malaria may occur in the neonatal period and can thus contribute to infant morbidity and mortality (Bardaji et al., 2011). Placental malaria, especially active infection, has also been linked to neonatal and infant mortality (Bardaji et al., 2011). Malaria infection during pregnancy increases the risk of infant and perinatal mortality, by causing LBW. Although the long-term effects of MiP have not been studied, malaria causes IUGR which results in LBW. LBW may be related to diseases occurring during adulthood, including some cancers and the metabolic syndrome (Christensen et al., 2011).

### **2.10.3 PATHOPHYSIOLOGY**

A unique characteristic of *P. falciparum* is that infected erythrocytes in pregnant women adhere to chondroitin sulphate A (CSA) molecules, and sequester along the endothelial and syncytiotrophoblast cells of the placenta (Fairhurst and Wellem, 2006, Cserti and Dzik, 2007, Rogerson et al., 2007a). For this reason, screening of peripheral blood may fail to detect infection of the placenta (Gosling et al., 2010). Chronic infection is often associated with IUGR whilst acute infection is more often the cause of miscarriage or stillbirth (Brabin et al., 2004). During pregnancy, the parasite antigens expressed on infected erythrocytes are known as variant surface antigen-pregnancy associated malaria (VSA<sub>PAM</sub>). They are different from the antigens normally expressed in non-pregnant individuals. In stable transmission settings, these VSA<sub>PAM</sub> are not recognised by the immune system, and this is responsible for primigravidae being at higher risk (Staalsoe et al., 2004). The binding of the variant surface antigen (VAR2CSA) with chondroitin sulphate A is connected to the pathology of falciparum malaria in pregnancy (Salanti et al., 2004, Ndam et al., 2005). The VAR2CSA belongs to the family of the erythrocyte membrane protein (PfEMP1). It is encoded by the var2csa gene, and it is expressed in pregnant women with falciparum malaria (Sander et al., 2011). Levels of anti-VAR2CSA specific

immunoglobulin G (IgG) increase with parity, are only found in women and are associated with a favourable pregnancy outcome (Sander et al., 2011, Ndam et al., 2005).

Increased cortisol levels have also been associated with increased risk of MiP (Bouyou-Akotet et al., 2005). Rosetting, commonly observed in non-pregnant women is very rare in pregnant women with falciparum malaria (Rogerson et al., 2000). LBW as a result of IUGR is associated with maternal anaemia (Tako et al., 2005) and elevated levels of cytokines (Thévenon et al., 2010). This appears to be due to chronic infections that cause reduced foetal circulation and placental insufficiency (Takem and D'Alessandro, 2013).

#### **2.10.4 CLINICAL PRESENTATION**

The clinical presentation of malaria disease ranges from severe and complicated, to mild and uncomplicated, to asymptomatic malaria. In low transmission settings, severe MiP is usually associated with hypoglycaemia, pulmonary oedema, and severe anaemia. Mortality in pregnant women with severe malaria has been reported to be between 9% and 12% (Dondorp et al., 2005). In stable transmission settings, the clinical signs of MiP are usually non-specific and suspected malaria cases should be confirmed by parasitological diagnosis (World Health Organization, 2015c).

Routine laboratory diagnosis of malaria is mostly based on microscopic detection of *Plasmodium* parasites in Giemsa-stained blood slides. Microscopy enables speciation, detection of gametocytes, and parasite density estimation. However, this technique is relatively laborious and the detection limit ~20 parasites/μL (Beurskens et al., 2009, Takem and D'Alessandro, 2013). Therefore, a considerable proportion of infected pregnant women would not be detected because of extremely low parasite densities or parasites sequestered in the placenta.

RDTs to detect *Plasmodium*-specific antigens (proteins) in whole blood of infected people have emerged as an attractive alternative to microscopy. However, the sensitivity of RDTs is lower than that of microscopy for diagnosing MiP. However, diagnosis can be made quicker than with microscopy and the time and amount of training required for their use are minimal. However, even though RDTs can detect malaria antigens, they cannot estimate the parasite density. The currently available RDTs come in various formats (dipstick, cassette or hybrids) and contain antibodies

bound to specific antigens, such as histidine-rich protein II (HRP2) unique to *P. falciparum*, pan-specific and species-specific plasmodium lactate dehydrogenase (pLDH) or aldolase specific to all the major *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*) (World Health Organization, 2015d). A limitation is that PfHRP2-based tests might remain positive for weeks after acute infection, which limits their usefulness in stable transmission settings (White et al., 2014).

The sensitivity of RDT on peripheral blood infections confirmed by microscopy/or PRC as a reference test among pregnant women ranged from 49% to 91% across West Africa. The study also found RDT sensitivity to be lower at the time of delivery relative to earlier time points in pregnancy (Williams et al., 2016).

PCR, which detects parasite DNA, can also be used for the diagnosis of malaria infection. However, it is not readily available in health facilities because it is expensive to setup. In stable transmission settings, the sensitivity of PCR was >80% when using microscopy as the reference (Kattenberg et al., 2011).

## **2.11 PREVENTION OF MALARIA IN PREGNANCY**

The use of ITNs, including LLINs in addition to intermittent preventive treatment in pregnancy (IPTp) with SP, are the most widely used interventions to prevent MiP.

### **2.11.1 INTERMITTENT PREVENTIVE TREATMENT**

Following the spread of resistance to CQ (Sirima et al., 2003) and poor compliance (Kaseje et al., 1987, Helitzer-Allen et al., 1993) with weekly CQ chemoprophylaxis in pregnant women, IPTp with SP was investigated as an alternative. SP was cheap to implement, had an excellent safety profile, and was easier to administer being a single dose treatment. Thus, it was quickly recommended by the WHO as policy for areas of high malaria transmission. Initially, a full course of SP was to be given twice during pregnancy, beginning in the second trimester after quickening and provided partial protection (World Health Organization, 2004). Currently, a minimum of three doses of SP is recommended to provide continuous preventive effects. The success of IPTp led to the adaptation of this approach in infants and children.

Currently, 36 sub-Saharan African countries have adopted an IPTp policy even though coverage among pregnant women who actually received at least two doses

of SP remains low, particularly in Nigeria and the Democratic Republic of Congo (DRC) (World Health Organization, 2015a). SP resistance spread emerged in Southeast Asia much faster than was the case with CQ and is increasing in many parts of Africa (White, 1992). This has led to the evaluation of alternative drugs. Studies of amodiaquine and mefloquine have not found definite evidence of superior benefit to SP. Also, these drugs were found to have more adverse side effects than SP (Clerk et al., 2008, Gonzalez et al., 2014).

More recently, dihydroartemisinin-piperaquine has been evaluated for use in IPTp and showed promising results (Desai et al., 2015, Kakuru et al., 2016). A potential alternative strategy for preventing malaria in pregnancy being evaluated is intermittent screening and treatment.

The percentage of pregnant women receiving at least three doses of intermittent preventive treatment in pregnancy (IPTp) has increased since revisions to the WHO recommendation in 2012 (World Health Organization, 2015a). According to the 2015 World Malaria Report, in 2014 an estimated 52% of eligible pregnant women received at least one dose of IPTp, 40% received two or more doses, and 17% received three or more doses of SP.

However, there are huge gaps between the proportion of women attending antenatal care (ANC) clinics and the proportion receiving one or more doses of IPTp. These represent missed opportunities to deliver IPTp at these clinics. In sub-Saharan Africa, the proportion of women receiving IPTp varies across the continent, with 10 countries reporting more than 60% of pregnant women receiving at least one dose, and another nine countries reporting more than 80% receiving one or more doses (World Health Organization, 2015a).

Some important barriers to the provision of IPTp have been identified as unclear policy and guidance on IPTp; general health care system issues, such as stockouts and user fees; poor organisation in health facilities, leading to poor quality of care; poor healthcare provider performance, including misunderstanding over the timing of each IPTp dose. In some settings, women's poor antenatal attendance affects IPTp uptake. Key determinants of IPTp coverage have been identified to be education, knowledge about malaria/IPTp, parity, socioeconomic status, and the number and timing of antenatal clinic visits (Hill et al., 2013).

### 2.11.2 INTERMITTENT SCREENING AND TREATMENT

Recent success in malaria control efforts has prompted renewed discussions about malaria elimination. This has led to a shift in attention from targeting clinical malaria to also identifying and treating asymptomatic malaria parasitaemia. The rationale being that, as endemic countries achieve sustained results in malaria control, the persistence of malaria would rely mainly on asymptomatic carriers (AC) who harbour the *P. falciparum* asexual forms, with or without gametocytes but do not present clinical symptoms of the disease (Kern et al., 2011).

The transmission of *P. falciparum* parasites from the host (humans) to the vector (mosquitoes) requires the presence of infectious gametocytes in the host's peripheral circulation. Thus, ACs who would not usually seek treatment, then become a reservoir of parasites for the inoculation of newly-hatched mosquitoes and this contributes to the transmission of the disease (Kern et al., 2011). Also, these individuals with asymptomatic parasitaemia are at risk of developing anaemia and may progress to clinical malaria (Tiono et al., 2013).

The detection and treatment of ACs of *P. falciparum* as a novel strategy for malaria control has been previously considered (Ogutu et al., 2010, El-Sayed et al., 2007, Dunyo et al., 2006a). The WHO has noted that infectivity-reducing drug regimens will play a useful role in maintaining the reductions in disease transmission achieved through integrated malaria control programmes (World Health Organization, 2015c). Among pregnant women in malaria endemic countries, a significant proportion of malaria infections are asymptomatic. Therefore eliminating this reservoir of infection will be crucial in attaining malaria elimination and then eradication.

The potential of IST as an intervention for malaria prevention was first highlighted by modelling studies (Griffin et al., 2010, Kern et al., 2011). Griffin and colleagues demonstrated the potential of mass screening and treatment (MSAT) combined with scale up in coverage of LLINs and use of ACTs to reduce transmission levels from 60% to about 10% in the whole population (Griffin et al., 2010).

The comparative efficacy of IST compared to IPT in pregnant women has been investigated in West Africa. The first trial in Ghana (Tagbor et al., 2010) randomised 3,333 women into three intervention groups; standard IPTp-SP, ISTp with SP or ISTp with amodiaquine-artesunate (AQ+AS). This trial found ISTp with AQ+AS or SP

to be non-inferior to IPTp-SP in reducing the risk of low birth weight and third-trimester severe anaemia.

The subsequent multicentre trial was conducted in four West African countries (Ghana, The Gambia, Burkina Faso, Mali) enrolling 5,354 primi- or secundigravidae women. ISTp-AL was shown to be non-inferior to IPTp-SP in preventing low birth weight, anaemia and placental malaria (Tagbor et al., 2015).

There has been general acceptability of this new intervention by pregnant women (Pell et al., 2014, Smith et al., 2010b) and health providers (Smith Paintain et al., 2011). However, qualitative studies have identified some concerns amongst pregnant women. Some of these include fears about covert HIV testing and pain from finger pricks. Also, questions remain about adherence to a multiple dose antimalarial regimen during pregnancy. Community engagement is also required to educate communities on the consequences of asymptomatic malaria (Shuford et al., 2016).

## **2.12 TREATMENT OF MALARIA IN PREGNANCY**

For the management of malaria in pregnancy, it is recommended that pregnant women are treated only after parasitological confirmation of the diagnosis, to reduce unnecessary exposure to antimalarials of both the mother and the foetus.

Women in the first trimester of pregnancy are usually excluded from clinical trials on the safety and efficacy of antimalarials in pregnancy so that the evidence is mostly from observational studies (Moore et al., 2016, Manyando et al., 2010, McGready et al., 2012, McGready et al., 2002). WHO recommends ACTs only in the second and third trimester. They may only be used if there are no suitable alternatives and are aimed at saving life. For uncomplicated malaria in the first trimester, the recommendation is 7 days' oral quinine alone or combined with clindamycin (World Health Organization, 2015c)

Nevertheless, in low-income countries, many pregnant women are exposed to ACTs in the first trimester from prescriptions early in pregnancy when women are not aware they are pregnant and from self-medication which is widespread (Mutabingwa and Adam, 2013). Evidence suggests artemisinin derivatives are relatively safe in the first trimester of pregnancy. Recent studies have found artemisinin exposure

during the first trimester was not associated with increased risk of miscarriage (Dellicour et al., 2015), perinatal mortality, malformations, or developmental impairment (Manyando et al., 2010).

In the second trimester, effective ACTs in the area, or 7 days artesunate plus clindamycin, or 7 days quinine plus clindamycin are recommended for uncomplicated malaria. (World Health Organization, 2015c). In the case of severe malaria, parenteral artesunate and quinine are considered options.

There is more experience on the use of artemisinin derivatives in the second and third trimesters of pregnancy, and several artemisinin compounds have been evaluated for the treatment of malaria in pregnancy.

Clinical trials conducted in Tanzania and Ghana have shown amodiaquine (AQ) is efficacious in pregnant women with falciparum malaria (Tagbor et al., 2006, Mutabingwa et al., 2009). It was relatively safe and well tolerated but associated with some minor side effects like nausea, weakness, and dizziness. However, AQ is not recommended by WHO as monotherapy for malaria even in pregnancy. Also, cross resistance between CQ and AQ due to the accumulation of mutations in the *Pfcr* gene has been established (Sa and Twu, 2010).

There are fewer studies on the efficacy and safety of mefloquine (MQ) for the treatment of MiP. However, high cure rates have been reported in Thailand, for the combination of MQ and AS- cure rate of 98.2% at day 63 (McGready et al., 2000). One study (Briand et al., 2009) reported minor side effects with mefloquine. However, concerns about still births and neuropsychiatric disorders remain (Takem and D'Alessandro, 2013).

DHAPQ is used in the Western Pacific for treating malaria in pregnant women (Poespoprodjo et al., 2011). A multicentre, randomised, open-label trial of four treatments (AL, AQ-AS, MQ-AS, DHAP) for malaria in pregnant women in four African countries found cure rates between 94% and 98%. However, drug-related adverse events such as poor appetite, asthenia, dizziness, nausea, and vomiting occurred significantly more frequently in the mefloquine–artesunate group (50.6%) and the amodiaquine– artesunate group (48.5%) than in the dihydroartemisinin– piperazine group (20.6%) and the artemether–lumefantrine group (11.5%) (PREGACT Study Group, 2016).

There is certainly more evidence available on the use of ACTs for malaria in pregnancy. However, the currently available evidence may not be sufficient for an immediate revision of the treatment guidelines as it relates to malaria in pregnancy.

## **2.13 INVESTIGATIONAL PRODUCTS**

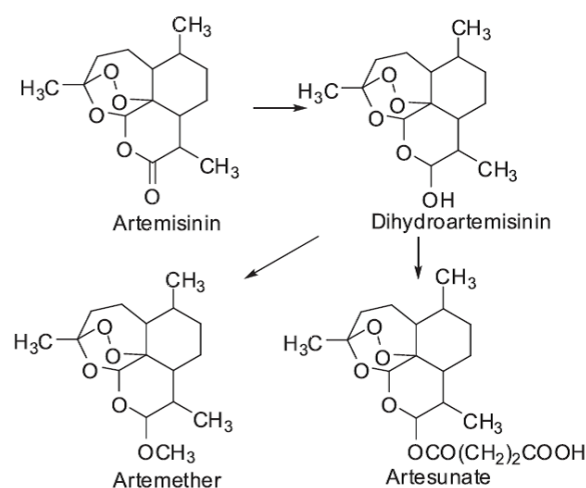
### **2.13.1 ARTEMISININ AND ITS DERIVATIVES**

Artemisinin and its derivatives are currently the mainstays of the treatment of malaria.

Artemisinin is the active component developed from the leaves of the old Chinese herbal medicine *Artemisia annua*-sweet wormwood or qinghao which has been used for several centuries for the treatment of fever. The commonly used derivatives of artemisinin are artesunate, artemether, and dihydroartemisinin (Figure 2.7). Every form of artemisinin is converted to the active metabolite dihydroartemisinin (DHA).

Artemisinins are very effective at rapidly clearing parasites by killing young, circulating ring-stage parasites and preventing further maturation and sequestration of these parasites (White, 2008). However, this is short-lived when used as a monotherapy due to its very short half-life and results in high rates of recrudescence. Thus, for effective treatment of malaria, artemisinin is combined with a long-acting blood schizonticide which will clear remaining parasites, thereby reducing the risk of developing resistance. Some drugs commonly used in combination with artemisinins are lumefantrine, mefloquine, piperaquine, SP, and amodiaquine.

Unfortunately, fake or substandard antimalarials are widespread in many Asian and African countries, which compromises effectiveness, selects for antimalarial drug resistance, and reduces confidence in the health sector (White et al., 2014).



**Figure 2.7:** Chemical structures of artemisinins and semi-synthesized derivatives.

### 2.13.2 ARTEMETHER-LUMEFANTRINE

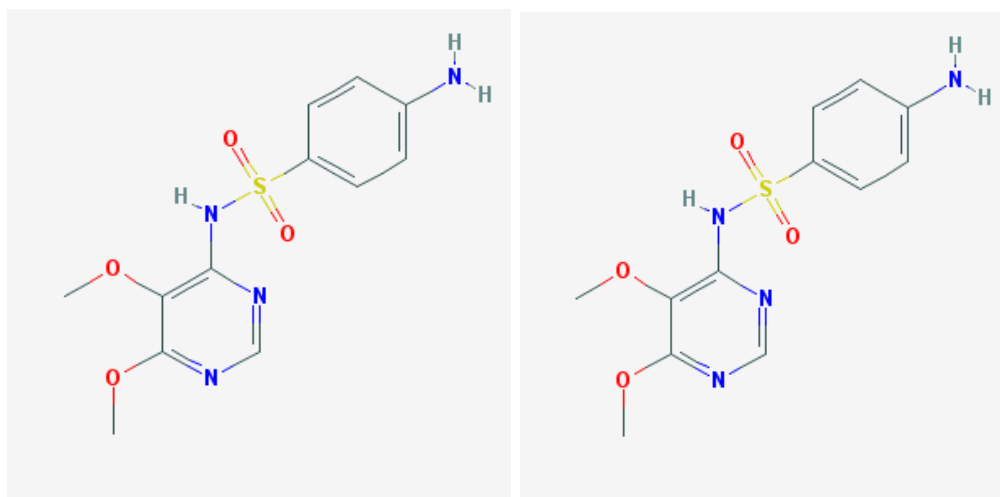
Artemether-lumefantrine was introduced in Nigeria in 2004 as the first line antimalarial drug for the treatment of uncomplicated malaria (World Health Organization, 2014b). The drug artemether-lumefantrine is a fixed combination of artemether-lumefantrine in the ratio 1(20mg, artemether): 6 (120mg, lumefantrine). It is indicated for the treatment of acute, uncomplicated infections due to *Plasmodium falciparum* or mixed infection including *P. falciparum* and strains from multi-drug resistant areas for all age groups. Artemether is a sesquiterpene lactone derived from the naturally occurring compound artemisinin (Tringali, 2011). It is the methyl ether of dihydroartemisinin while lumefantrine belongs to the aryl-amino-alcohol family and is a racemic mixture of a synthetic fluorene derivative. Both components act in the food vacuole of the malaria parasite. Lumefantrine interferes with the polymerization process that converts toxic haem, to non-toxic haemozoin while artemether may generate toxic, reactive metabolites as a result of the interaction between its endoperoxide bridge and haem iron (Aweeka and German, 2008, Chijioke-Nwauche, 2014). Artemether and lumefantrine have a secondary action involving the inhibition of nucleic acid and protein synthesis (Novartis, 2012).

Lumefantrine has a similar mechanism of action to halofantrine perhaps due to similarities in structure and pharmacokinetic properties. However, lumefantrine is very safe as it does not prolong the electrocardiographic QT interval (Van Vugt et al., 1999).

### 2.13.3 SULFADOXINE-PYRIMETHAMINE

Sulfadoxine-pyrimethamine (SP) was introduced following the development of widespread resistance to CQ. SP is a combination of sulfadoxine and pyrimethamine. Sulfadoxine is a broad-spectrum sulphanilamide antimicrobial which acts by inhibiting the synthesis of folic acid by bacteria and protozoa. Sulfadoxine competes with para-aminobenzoic acid (PABA) for the bacterial enzyme dihydropteroate synthase (DHPS), thereby preventing the addition of PABA into dihydrofolic acid, the immediate precursor of folic acid. This leads to an inhibition of parasitic folic acid synthesis and *de novo* synthesis of pyrimidines and purines and subsequently cell growth arrest and cell death.

Pyrimethamine is a synthetic derivative of ethyl-pyrimidine with potent antimalarial properties. Pyrimethamine is a blood schizontocide, which competitively inhibits dihydrofolate reductase (DHFR). DHFR is a key enzyme in the redox cycle for production of tetrahydrofolate, a cofactor that is required for the synthesis of DNA and proteins. Figure 2.8 shows the chemical structures of sulfadoxine and pyrimethamine.



**Figure 2.8:** Chemical structures of Sulfadoxine and Pyrimethamine.

The synergistic effect of sulfadoxine with pyrimethamine has been effective as a combined antimalarial monotherapy against asexual erythrocytic forms of malaria parasites (trophozoites and schizonts). However, SP is ineffective against mature gametocytes within the sexual developmental phase.

SP is readily absorbed from the gastrointestinal tract after oral administration. Sulfadoxine and pyrimethamine have relatively long half-lives of 4.1 – 10.9 days and 2.5 – 18.8 days respectively (World Health Organization, 2015c). SP has a good safety profile in pregnancy. Compliance is high with SP as it can be delivered as a single dose treatment under observation by health workers. These attributes have led to high levels of IPT acceptance by pregnant women.

Both sulfadoxine and pyrimethamine are generally considered safe in the second and third trimesters of pregnancy. However, nausea, vomiting, urticarial, rashes, headache and insomnia are documented side effects of SP. A rare adverse effect that could occur in persons with sensitivity to sulfa drugs is Stevens Johnson Syndrome.

High doses (5mg daily) of folic acid adversely affects the antimalarial effects of SP. For this reason, a low dose (0.4mg daily) of folic acid is recommended in the ANC period.

## **2.14 ANTIMALARIAL RESISTANCE**

Antimalarial drug resistance is defined as the ability of a parasite strain to survive or multiply despite the proper administration and absorption of an antimalarial drug at the normally recommended dose (World Health Organization, 2015c). It is a shift to the right of the dose-response curve and implies higher drug concentrations are required to achieve the same parasite clearance. In many cases even the higher concentration results in treatment failure (White, 2004). Parasite resistance to antimalarial drugs has been recognised in three of the five malaria species that are known to affect humans: *P. falciparum*, *P. vivax*, and *P. malariae*. Antimalarial resistance to CQ and SP has been well established. Unfortunately, *P. falciparum* resistance to artemisinin has been detected in five countries of the Greater Mekong sub-region: Cambodia, Myanmar, the Lao People's Democratic Republic, Thailand and Viet Nam. In many areas along the Cambodia–Thailand border, *P. falciparum*

has become resistant to most available antimalarial medicines (World Health Organization, 2014c).

Cross-resistance further compounds the problem of antimalarial drug resistance. Cross-resistance implies that resistance to one drug results in resistance to other drugs in the same chemical family or with similar modes of action. During the past decades, several antimalarials had to be removed from markets after the spread of parasite resistance (World Health Organization, 2014c).

It is important to distinguish between a failure to clear malaria parasitaemia or resolve clinical disease following treatment with an antimalarial drug and true antimalarial drug resistance. While drug resistance can cause treatment failure, not all treatment failure is due to drug resistance. The success of any antimalarial drug depends on the varying response of the parasites. Also, the various stages of the parasite life cycle differ in their susceptibility to different medicines.

Medicines for the treatment of uncomplicated malaria ought to effectively eradicate parasites or reduce the parasite burden to the point that the humoral response can cope with the infection (White, 2002, White, 1998). Thus, an effective antimalarial drug treatment should cure malaria and reduce morbidity associated with treatment failures (World Health Organization, 2015c). Antimalarial drug resistance is often associated more with treatment failure than with prophylactic failure (Miller et al., 2005).

Antimalarial treatments are designed to sustain blood concentrations which are sufficient to restrict parasite multiplication until the last viable parasite has been eliminated from the body (White, 1997). To confirm the development of antimalarial drug resistance, the treatment regime must be proven to be inefficient despite being in the body in sufficient amounts and time periods to target a minimum of four asexual parasite life cycles (approximately six days for *P. falciparum*) (White, 1998).

Sub-optimal drug concentrations increase the probability for drug-resistant parasites to emerge. These sub-optimal drug concentrations happen mainly as a result of improper dosing, poor pharmacokinetic properties, and fake drugs. Within the context of chemoprevention and chemotherapy, malaria infections acquired during the drug elimination phase of a prior antimalarial treatment can also expose parasites to sub-optimal drug concentrations (Petersen et al., 2011). The factors

influencing antimalarial drug resistance have also be summarised in three categories which include the nature of the parasite, human immune responses and pharmacological properties of the drug (Travassos and Laufer, 2009).

Repeated exposure to *P. falciparum* leads to the development of slowly acquired immunity in communities (White, 2004). Individuals with this acquired immunity tend to have better treatment outcomes when given antimalarial drugs compared to those without any background immunity (Mayxay et al., 2001).

Individuals with lower immunity such as children and pregnant women or persons with opportunistic infections such as HIV are more vulnerable to antimalarial drug resistance.

## **2.15 EMERGENCE AND SPREAD OF DRUG RESISTANCE**

The evolution of antimalarial drug resistance has been characterised by three events: the appearance of mutations, the establishment of mutations and the spread of mutations (Smith et al., 2010a). Firstly, the emergence of resistance is characterised by infections with *de novo* mutations whereby the sensitive parasites are initially the majority strain until treatment is administered and the proportion of resistant strains eventually outnumber the sensitive forms (Smith et al., 2010a). However, the genetic events that lead to drug resistance are spontaneous and rare (either mutations or change in the copy number of genes encoding for a parasite target) and are thought to be independent of the drug used (White, 2004). The second phase is when resistance becomes established after its initial appearance and as mutant parasites persist and spread within the population (Smith et al., 2010a). This establishment phase depends on the fitness cost of mutant parasite strains which in turn depends on its biological cost versus the benefits conferred by drug pressure (Smith et al., 2010a). Parasites carrying mutations depend on sustained drug pressure and once this drug pressure is reduced, the mutant strains will eventually be outnumbered by sensitive strains. The third phase, 'spread' involves displacement of sensitive parasite strains with resistance strains within the population and this is affected by the malaria transmission intensity in communities.

The emergence of resistance in *Plasmodium* depends on several factors, including the mutation rate of the parasite, the fitness costs associated with the resistance

mutations, the overall parasite load, the strength of drug selection, and the treatment compliance (Petersen et al., 2011).

*P. falciparum* parasites from Southeast Asia seem to have an 'accelerated resistance to multiple drugs' phenotype which gives them an increased propensity to develop drug resistance (Rathod et al., 1997). Also, the emergence of drug-resistant parasites can also be accelerated by strong drug selection pressure, which decreases the prevalence of competing sensitive wild type parasites (Petersen et al., 2011).

In high-transmission regions, infections acquired after treatment from a previous malaria episode are common and result in the exposure of parasites to sub-therapeutic drug concentrations. This selects for drug-tolerant parasites, which may be a precursor to full resistance (Stepniewska and White, 2008). The period during which sub-therapeutic concentrations are present within the patient is prolonged in antimalarials that possess a long half-life (Hastings and Watkins, 2006) and increase the probability of resistance developing. The intensity of transmission also plays an important part in determining if parasites are effectively transmitted during a mosquito blood meal.

If mutations conferring drug resistance are associated with a significant fitness cost, they are more likely to be outcompeted by sensitive parasites and not transmitted efficiently. The spread of resistant parasites is also affected by the impact of antimalarials on the gametocytes, which are the transmissible stages of the parasite.

Artemisinins have been shown to decrease the number of gametocytes carried by a patient, thereby reducing transmission (Okell et al., 2008). However, both SP and CQ elevate the gametocytaemia (White, 2008). In addition, drug resistance may enhance transmission if drug selection pressure diminishes the viability of sensitive gametocytes in a polyclonal infection, increasing the propensity for transmitting drug-resistant parasites (Hastings, 2006).

## **2.16 SP DRUG RESISTANCE MECHANISM**

The antifolate class of drugs consists of compounds that bind enzymes essential for folate biosynthesis in the parasite. The most widely used anti-malarial drug within this class is SP. SP is a blood schizonticide that inhibits folate metabolism which is

an essential biochemical pathway for *P. falciparum* survival. While vertebrates use preformed folate from their diet, microorganisms and plants synthesise folates *de novo* from guanosine triphosphate, p-amino benzoic acid (PABA) and glutamate, using a chain of enzymes (Warhurst, 2002). Sulphadoxine mimics para-aminobenzoic acid (PABA) in competing for the active site on the dihydropteroate synthase (DHPS) enzyme. The PABA interaction with DHPS would normally lead to folic acid production in the parasite, but it is interrupted in the presence of sulphadoxine (Olliaro, 2001). Pyrimethamine inhibits plasmodial dihydrofolate reductase (DHFR) and thereby prevents the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate to tetrahydrofolate (Olliaro, 2001).

Mutations in the *dhfr* and *dhps* genes of *P. falciparum* parasites have been associated with decreased parasite sensitivity to the antifolate drugs. The appearance and accumulation of mutant SNPs in *dhfr* and *dhps* genes have been associated with tolerance to SP treatment (Kublin et al., 2002, Vinayak et al., 2010).

Assessment of molecular markers of resistance present in a geographical area is one of the methods for assessing antimalarial drug resistance. Molecular markers of SP resistance are presented below.

## **2.17 MOLECULAR MARKERS OF SP DRUG RESISTANCE**

Since the late 1980s and 1990s specific point mutations of the *dhfr* and *dhps* gene encoding DHFR and DHPS were implicated in SP resistance (Cowman et al., 1988, Peterson et al., 1988, Brooks et al., 1994, Triglia and Cowman, 1994). Mutations at seven *dhfr* codons (16, 50, 51, 59, 108, 140 and 164) and six *dhps* codons (431, 436, 437, 540, 581 and 613) have since then been isolated as molecular markers for SP resistance. The mutation at codon I431V is novel and is the substitution of valine for isoleucine at codon 431. The significance of this I431V *dhps* mutation to antifolate resistance remains unclear. However it has been detected in occurrence with 437G, 581G and 613S from Nigerian isolates (Sutherland et al., 2009). Mutations at codons 581 and 613 have long been associated with SP resistance (Plowe et al., 1997). The new *dhps* I431V mutation was recently detected, at a prevalence of 9.8% in Cameroon and was associated with other *dhfr/dhps* alleles to form an

octuple N51I, C59R, S108N/I431V, S436A, A437G, A581G, A613S mutant (Chauvin et al., 2015).

There appears to be a relationship between identified molecular markers and clinical failure of antifolates. *Pfdhfr* and *Pfdhps* mutations have been associated with an enhanced transmission of gametocytes to mosquitoes despite low levels of *in vivo* treatment failure (Hallett et al., 2006, Méndez et al., 2002).

Genotypes consisting of multiple mutations in the *dhfr* gene have evolved across the world and are most often associated with higher levels of resistance than the single mutant genotypes. The triple *dhfr* mutant genotype consisting of N51I, C59R, and S108N has demonstrated a strong association with *in vivo* SP treatment failure (Kublin et al., 2002, Happi et al., 2005). The addition of the N51I, C59R, and I164L mutations in the presence of S108N confers high levels of resistance to both cycloguanil and pyrimethamine and cycloguanil (Foote et al., 1990, Sirawaraporn et al., 1997).

Data from several malaria endemic areas suggest an asymmetric selection of resistant genotypes beginning with mutations in *dhfr* and then by those in *dhps* (Sibley et al., 2001). The *dhps* mutations A437G, K540E, and A581G are particularly associated with established sulfadoxine-pyrimethamine failure in vivo (Dunyo et al., 2006b, Alker et al., 2008, Happi et al., 2005). However, the quintuple mutant genotype consisting of the double *Pfdhps* mutant genotype (A437G, K540E) in combination with the *Pfdhfr* triple mutant genotype (S108N, N51I, C59R) is a better predictor of clinical failure than either multiple mutant genotype alone (Mugittu et al., 2004). A Nigerian study (Happi et al., 2005) established the quintuple mutant genotype was strongly associated with SP treatment failure in children less than five years.

Table 2.2 and Table 2.3 show the amino acid substitutions and the underlying sequence changes in the three letter code for each codon.

**Table 2.2:** Molecular markers of *dhfr* resistance.

<b><i>dhfr</i> Codon</b>	<b>Sensitive</b>	<b>Resistant</b>	
<b>16</b>	GCA	Ser(S) TCA	Val(V) GTA
<b>50</b>	Cys(C) TGT	Arg(R) CGT	
<b>51</b>	Asn(N) AAT	Ile(I) ATT	
<b>59</b>	Cys(C) TGT	Arg(R) CGT	
<b>108</b>	Ser(S) AGC	Asn(N) AAC	Thr(T) ACC
<b>140</b>	Val(V) GTT	Leu(L) CTT	
<b>164</b>	Ile(I) ATA	Leu(L) TTA	

**Table 2.3:** Molecular markers for *dhps* resistance.

<b><i>dhps</i> Codon</b>	<b>Sensitive</b>	<b>Resistant</b>		
<b>431</b>	Val (V)	Ile (I)		
<b>436</b>	Ser(S) TCT	Ala(A) GCT	Cys(C) TGT	Phe(F) TTT
<b>437</b>	Ala(A) GCT	Gly(G) GGT		
<b>540</b>	Lys(K) AAA	Gly(E) GAA		
<b>581</b>	Ala(A) GCG	Gly(G) GGG		
<b>613</b>	Ala(A) GCC	Ser(S) TCC	Thr(T) ACC	

In East Africa SP clinical failure has been established and is now widespread (Iriemenam et al., 2012, Karema et al., 2010, Eriksen et al., 2008) with a high frequency of the quintuple mutations in *Pf dhfr* (51I/59R/108N) and the double mutation in *Pf dhps* (S437G/K540E). There has also been the emergence of A581G mutation (Gesase et al., 2009, Iriemenam et al., 2012, Sendagire et al., 2005).

Consequently, continued monitoring of *Pf dhfr* and *Pf dhps* mutations in West Africa is essential due to the use of sulfadoxine-pyrimethamine for Intermittent Preventive Treatment of malaria in pregnant women and infants in Africa.

## **2.18 NIGERIA AND THE STUDY SITE**

### **2.18.1 GEOGRAPHY**

Nigeria is on the West coast of Africa and has a surface area of 923,708 square kilometres lying between Latitudes 4° and 14°N and Longitudes 2° and 15°E.

Nigeria is bounded by Cameroon on the East, Benin on the West, Chad to the North-East, the Niger Republic to the North and on the south by the Atlantic Ocean. The topography of its landmass is diverse with its terrain consisting of lowlands in the South, plateaus, and hills towards the Centre, mountains in the South East and plains in the North. The highest elevation is Chappal Waddi at 2,419m in Taraba State in the North-Eastern region of the country. The Rivers Niger and Benue run from the North-Western and North-Eastern parts of the country respectively with their confluence in Lokoja from which it runs to the Delta region in the South where it interconnects with the Atlantic Ocean. The capital city is Abuja.

### **2.18.2 DEMOGRAPHY**

Nigeria is the most populous country in Africa. The latest estimates of the population of Nigeria is reported to be 181.6 million inhabitants in 2015 (United States Census Bureau, 2015). About 44% of the population is less than 15 years of age with only 5% aged above 60 years and the median population age is 18 years. The proportion of the population pregnant during one year is put at 5%. The country has a total fertility rate of 6.6 however, one in eight children die before their fifth birthday. Life

expectancy at birth in Nigeria is 55 and 54 years in females and males respectively (World Health Organization, 2015e).

The bulk of the people are farmers living in rural areas where there is a shortage of access to infrastructure and health facilities. There is an ever growing rural -urban drift resulting in pressure on existing facilities in the towns and cities. Maternal mortality ratio in 2014 was estimated to be 820/ 100,000 live births (World Health Organization, 2015f). Table 2.4 shows some other key socioeconomic and demographic data for Nigeria.

**Table 2.4:** Key Socio-Economic and Demographic information

Variable/Indicator	Value	Source
Population (2016)	181.6 million	US Census Bureau, 2015
Crude Birth Rate, 2013	41	UNICEF, 2015
Crude Death Rate per 1000, 2013	13	UNICEF, 2015
Total Fertility Rate 2013	6.6	UNICEF, 2015
Under-5 mortality rate (U5MR), 2013	117	UNICEF, 2015
Infant mortality rate (under 1), 2013	74	UNICEF, 2015
Neonatal mortality rate 2009	37	UNICEF, 2015
Maternal Mortality Ratio 2009-2013	550	UNICEF, 2015
Antenatal Attendance (%) At least Once	61	UNICEF, 2015
GDP (US\$) 2014	568.5 billion	World Bank, 2014
GDP per capita (US\$) 2014	1,091	World Bank, 2014
Life expectancy at birth (years) 2015	53	UNICEF, 2015
Total adult literacy rate (%) 2009-2013	51	UNICEF, 2015
Primary school net enrolment ratio (%) 2009-2013	66	UNICEF, 2015

### 2.18.3 ECONOMY

Agriculture is the primary source of livelihood for most of the population who are subsistence farmers. The nation's principal source of income is crude oil exports. Other sources of revenue include mining and export of cash crops. Nigeria is currently classified as a lower middle-income country by the World Bank. In 2014, the Gross Domestic Product (GDP) was 568.5 billion USD; while GDP per capita was 1,091 USD, with a GDP growth rate of 6.3% (World Bank, 2014). Most health expenses are borne by families and individuals as "out of pocket" expenses while limited health insurance services are available, especially to civil servants and some rural communities.

#### **2.18.4 CLIMATE**

The climate is arid in the North, with an annual rainfall of 600 - 1,000 mm lasting for 3-4 months, and predominantly humid in the South with a yearly average of 1,300-1,800 mm but in some coastal areas may be up to 2,500 mm) lasting for 9-12 months (Federal Ministry of Health, 2015). Rainfall is highest in the Northern parts of the country between the months of June and September and from March to November in the Southern areas, which usually coincides with the peak incidence of malaria. The country's vegetation characterised by Sahel Savannah in the far North, Guinea Savannah in the Middle Belt, and rain forest in the South with mangrove forest in the coastal areas. As a result of increases in rainfall in Nigeria and neighbouring countries, flooding has now become a frequent occurrence in all parts of the country, most especially in riverine communities and the Niger Delta Region.

#### **2.18.5 EPIDEMIOLOGY OF MALARIA**

Malaria is holoendemic in Nigeria with transmission all year round. Nigeria has two main seasons in the year, the dry season (October to March) and the rainy season (April to September) with a peak of the rains between May and July when malaria transmission is very intense. The rainfall pattern in Nigeria varies with the South having more rains than the North. Annual rainfall increases southward from 500-750 millimetres in the north to about 2,000 millimetres in the coastal zone with an average of more than 3,550 millimetres in the Niger Delta region.

Malaria is transmitted all through Nigeria, with the entire population at risk (World Health Organization, 2014b). According to the National Malaria Elimination Programme Strategic Plan 2014-2020, malaria accounts for about 60% and 30% of outpatient visits and hospitalizations in Nigeria respectively. It is a leading cause of mortality in children under five years of age, and is responsible for an estimated 300,000 deaths yearly. It also contributes to an estimated 11% of maternal mortality, 25% of infant mortality, and 20% of under-five mortality (Federal Ministry of Health, 2015). Malaria is also a foremost cause of mortality in children under five and contributes to an estimated 25% of infant mortality and 11% of maternal deaths. Treatment of malaria illnesses accounted for 40% of the curative health care cost incurred by households. Intensity and seasonality of transmission vary by region.

The duration of the transmission season ranges from year-round transmission in the south to three months or less in the north. *P. falciparum* is the predominant malaria species.

Thus, malaria remains one of the leading causes of morbidity and mortality in Nigeria. Nigeria and the Democratic Republic of Congo account for 38% of the global estimate of malaria deaths (World Health Organization, 2015a).

The direct financial loss to the Nigerian economy due to malaria is estimated to be about 132 billion Naira (about 8.8million US dollars) in the form of treatment costs, prevention and loss of person-hours annually (National Population Commission, 2012).

#### **2.18.6 MALARIA TRANSMISSION**

Recently, modelling studies based on age-corrected malaria point prevalence data in children 2- 10 years and the effects of temperature, rainfall, distance to major rivers and urbanization have been used to predict malaria risk across Nigeria (Snow et al., 2013). It was found that as at 2010, 85% of Nigerians lived in areas supporting mesoendemic transmission, 15% used to live under conditions of hyper-holoendemicity and areas within the north (Abuja, Adamawa, and Borno State) support hypoendemicity.

#### **2.18.7 MALARIA SPECIES AND VECTORS**

The dominant species of malaria parasites in Nigeria is *P. falciparum* (95%). There are also mixed infections with other *Plasmodium* species. The other non-falciparum species in Nigeria are *P. malariae* (9.8%) and *P. ovale* (5.8%) and mixed infections (10.4%) (World Health Organization, 2012e).

In Nigeria, the major malaria vectors include the *An. gambiae* complex (*An. gambiae* s.s. and *An. arabiensis*) and the *Anopheles funestus* group. These three species are widely distributed across the country, from the mangrove and coastal areas of the south to the Sahel savannah of the far north. *An. gambiae* and *An. arabiensis* typically breeds in sunlit, shallow, temporary bodies of fresh water such as ground depressions, puddles, pools and hoof prints. *An. funestus* breeds in permanent or semi -permanent body of fresh water with emergent vegetation; such as swamps, large ponds which sustain malaria transmission during the dry season. *An. melas*,

another member of the *An. gambiae* complex breeds in the mangrove and coastal areas of the south-south and southwestern zones (Federal Ministry of Health, 2015).

The molecular forms (M and S) of *An. gambiae* s.s. have been found in Nigeria (Awolola et al., 2005). *An. gambiae* s.s. feeds primarily on humans (anthropophagic) and rest indoors (endophilic) while *An. arabiensis* feeds on humans and other animals and mainly rests outdoors (exophilic). *An. gambiae* s.s. also tend to have higher sporozoite rates compared to *An. arabiensis*. *An. funestus* feed on both humans and other animals predominate in the forest, rest indoors. Where the major malaria vectors co-exist, infection rates have been reported to be higher in *An. gambiae* s.s. followed by *An. funestus* and *An. arabiensis* (Federal Ministry of Health, 2015).

With the scaling up of vector control interventions, a decline in vector densities and transmission is expected. However, as LLIN and IRS coverage increases, there remains the possibility of behavioural adaptation of malaria vectors.

#### **2.18.8 MALARIA CONTROL IN NIGERIA**

Nigeria is in the malaria control phase. Early diagnosis and prompt treatment of malaria with effective antimalarial drugs is the main strategy for malaria control. Malaria diagnosis and ACTs are provided at no cost for all age groups in public sector facilities since 2009. Since 2004, artemether-lumefantrine and artesunate-amodiaquine over 3 days have been the first-line treatments for uncomplicated malaria and *P. falciparum* malaria; severe cases are treated with either parenteral artesunate, amodiaquine or quinine. Treatment failures are treated with quinine. Other malaria control strategies are the free distribution of ITNs/LLINs to all age groups since 2009 (World Health Organization, 2015a). 58 million LLINs were distributed between 2009 and 2013 through mass distribution campaigns, antenatal clinics, immunisation clinics and other channels. The national average for household ownership of at least one ITN is 42% with wide within-country variations. IRS has been recommended since 2007 however, the use of DDT is not authorised. In 2010, IRS was implemented in 7 States (21 LGAs) with support from the World Bank Malaria Booster Programme (Federal Ministry of Health, 2015). Larval source management (LSM) has been incorporated as a component of integrated vector management within the last two years. There is no systematic coordination of

insecticide resistance monitoring at the national level. There is reported resistance to all four classes of insecticide in *An. coluzzii* and *An. gambiae s.l.* (World Health Organization, 2015a).

Nigeria adopted Focused Antenatal Care (FANC) since 2004 with a view to drastically reduce the burden of malaria. FANC provides the most practical platform for the delivery of these interventions. The key interventions provided at the ANC for the prevention of malaria in pregnancy include administration of SP for intermittent preventive treatment (IPT) under the direct supervision of skilled service providers, distribution of LLINs, and appropriate case management through prompt diagnosis and effective treatment with recommended medicines. The implementation of FANC in Nigeria recommends that pregnant women should make at least, four visits; within 16 weeks or when the woman first thinks she is pregnant, at 20-24 weeks or at least once in the second trimester, at 28-32 weeks and at 36 weeks or later. FANC encourages expectant mothers to make unscheduled visits whenever they experienced danger/warning signs in the course of pregnancy.

Though the provision of malaria preventive services in pregnancy have been free in all public facilities, across the country, the utilisation has remained low due to the consistently low antenatal attendance, especially in some states in northern Nigeria where ANC attendance is still less than 30% (NDHS, 2014). The 2008 NDHS showed an average of 43.7% and 76.9% ANC attendance by rural and urban dwellers respectively. This improved slightly to 46.5% and 86.0% in 2013. This makes the administration of the intervention difficult in higher prevalence rural areas as it is primarily facility based at present and requires supervision by skilled health care providers. However, community-based strategies are being developed (Federal Ministry of Health, 2015).

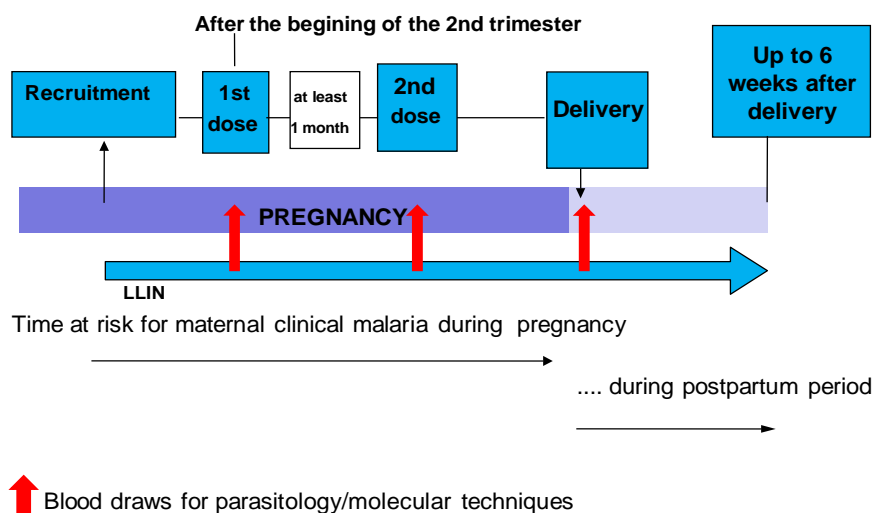
### 3 METHODS

#### 3.1 OVERALL DESIGN

An individually randomised, two-arm, open, parallel group, non-inferiority pragmatic trial was undertaken. The trial investigated whether screening for malaria with a rapid diagnostic test and treating women with that test positive for malaria with artemether-lumefantrine was not inferior to intermittent preventive treatment with sulfadoxine-pyrimethamine in the prevention of anaemia in pregnancy. Eligible and consenting pregnant women were randomly assigned (1:1) to one of the two study arms:

- i) presumptive treatment with sulfadoxine-pyrimethamine (SP)-IPTp plus LLIN or
- ii) malaria screening and treatment with artemether-lumefantrine (AL) plus LLIN .

Figure 3.1 shows the study plan for the trial including blood draws and timing of visits



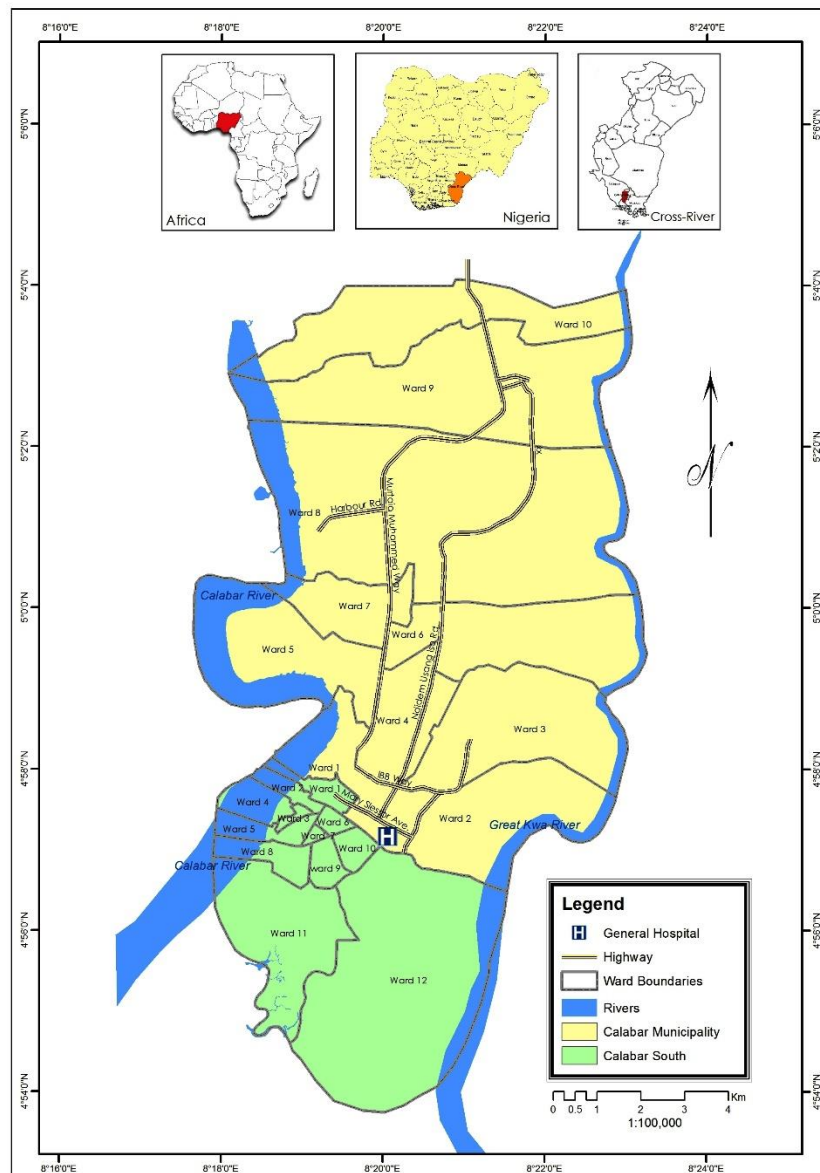
**Figure 3.1:** Study plan.

## 3.2 RANDOMIZED CLINICAL TRIAL IN NIGERIA

### 3.2.1 STUDY SITE

The study was conducted in Calabar, Cross River State in South-East Nigeria (Figure 3.2) In the 2006 Population and Housing Census, Cross River state's population was 2,868,966 made up of 1,492,465 males and 1,396,501 females. Using a growth rate of 3.4% the population of Cross River State in 2015 was 3,908,692. The climate in Calabar is tropical-humid with wet and dry seasons, with average temperatures ranging between 15-30°C and the annual rainfall between 1300-3000 mm. The vegetation in Calabar is mangrove swamp forest. Malaria transmission in this area is intense and perennial with a peak in the rainy season. *P. falciparum* is the predominant malaria-causing species (National Population Commission, 2012, World Health Organization, 2015a).

Previous in vivo therapeutic efficacy studies have reported resistance to chloroquine and sulfadoxine/pyrimethamine in this area to be in the range of 30% to 80% (Ezedinachi et al., 1992, Federal Ministry of Health, 2002, Federal Ministry of Health, 2005). *An. gambiae* is the predominant vector species. There is no information available on entomological inoculation rate (EIR) in the study area, but an EIR of about 259 infectious bites per person-year has been reported from Odukpani, a neighbouring area (Alaribe, 1999). National HIV prevalence is reported to be about 3.4% with a prevalence of 5.5% in the region where the trial was conducted (Federal Ministry of Health, 2012).



**Figure 3.2:** Map of Calabar showing the study site with insert maps of Africa, Nigeria, and Cross River State

The trial was conducted at the antenatal clinics of the General Hospital in Calabar, Nigeria. The General Hospital is the largest government-owned secondary health facility in the city and caters to the health needs of the majority of the inhabitants. Since August 2009, pregnant women and children under five years of age have received free medical care as part of a state-funded welfare program by the Government of Cross River State. The average annual antenatal clinic attendance at the hospital between 2013 and 2015 was 16,550; with an average of 3,100 births in the hospital annually (Ekpo A. personal communication). In the study area, the proportion of births attended by unskilled personnel has been estimated to be about

85% (Etuk et al., 2000, Etuk and Etuk, 2001). Additionally, only about 15% of births take place in health facilities in a health demographic and surveillance site 15 km from the study site (Enang, et al., 2013).

### **3.2.2 RECRUITMENT OF PARTICIPANTS**

The study population included pregnant women of all parities who presented at the antenatal clinics at their first booking. Women were screened for eligibility and invited to participate in the study if they met all of the inclusion criteria.

**Inclusion criteria:** pregnant with a gestational age of 16 to 24 weeks at their first booking; willing to have a supervised delivery; and permanent residence within Calabar and its environs.

**Exclusion criteria:** a prior dose of IPTp-SP, a haemoglobin concentration < 6 g/dl, a history of sensitivity to SP, lumefantrine or an artemisinin. Other exclusion criteria were any illness requiring hospital admission (including severe malaria as defined by WHO), known HIV infection, known G6PD deficiency, past obstetric and medical history that would adversely affect the evaluation of outcomes and an unwillingness to participate in the study.

### **3.2.3 RANDOMIZATION AND BASELINE EVALUATION**

After written informed consent had been obtained, eligible women were randomised to one of the two treatment groups. An allocation sequence was generated with STATA by a statistician who did not participate in the study. Women were randomly allocated to either of the two treatment groups in permuted blocks of ten. This randomly generated assignment to trial arms was printed and cut into slips which were sealed in opaque envelopes according to their blocks of 10. During enrolment, an eligible pregnant woman was asked by a designated study nurse to pick a slip from the sealed envelope. The treatment group printed on each slip indicated the treatment arm to which the women belonged. Another opaque envelope was opened by the designated nurse only when the contents of the previous one had been exhausted. The study arm women belonged to was not identifiable by the study identification numbers given to them. Also, the Principal Investigator and outcome assessors (midwives and microscopist) were blinded to the randomisation process and treatment allocation to prevent bias in outcome assessment. Women who

declined to participate in the trial were treated with the routine standard of care, IPTp-SP according to the national guidelines. At the enrolment visit, a finger prick blood sample was obtained for measurement of haemoglobin concentration, preparation of blood films (thick and thin smears) for malaria parasite counts and preparation of dried blood spots (DBS) on filter paper.

### 3.3 DESCRIPTION OF THE INTERVENTION

#### 3.3.1 INTERMITTENT SCREENING AND TREATMENT WITH AL

Pregnant women were screened for malaria infection with malaria RDT (SD Bioline Malaria Ag P.f/Pan RDT kit, a Histidine-Rich Protein-2 (HRP-II) antigen and pLDH (Pan) antigen test, Standard Diagnostics, Inc.) at scheduled ANC visits. The test kits were purchased from the licensed local distributors of the product (Codix Pharma Limited Nigeria). If the study women tested positive by RDT, they were treated with AL (20 mg artemether/ 120 mg lumefantrine) given as a 6-dose course, administered twice daily for three days). Study women were advised on the time and mode of administration for the three days treatment taken at home unobserved.

#### 3.3.2 INTERMITTENT PREVENTIVE TREATMENT WITH SP

Women in the IPTp-SP arm received a single dose (3 tablets of 500mg sulfadoxine and 25mg pyrimethamine each) at enrolment (Visit 1) and the following visit (Visit 2) at least one month apart. Table 3.1 shows a summary of the drugs used for the intervention.

**Table 3.1:** Summary of the intervention drugs.

Study drug	Manufacturer	Drug formulation	Dosing schedule
Sulfadoxine-pyrimethamine (Laridox®)	IPCA Laboratories Ltd, India	Tablet 1500 mg + 75 mg	Single dose
Artemether-lumefantrine (Coartem®)	Novartis Pharma, Switzerland	20 mg + 120 mg	Daily for 3 days

#### 3.3.3 ADDITIONAL INTERVENTIONS

All study women received a long-lasting insecticidal net (LLIN) which they were encouraged to use throughout the pregnancy. Also, they received the routine

supplement of folic acid (4mg) and ferrous sulphate (200 mg) tablets usually provided at ANC in Nigeria based on national guidelines. HIV screening was offered to all study women in the context of the routine antenatal services recommended in Nigeria with an option for treatment, but the results of HIV screening were not available to the study team at the time of enrolment.

### **3.3.4 FOLLOW-UP**

Study participants were asked to return to the clinic for follow-up antenatal care at which either IPTp-SP or screening with RDTs was given at 24, 32 and 36 weeks of gestation. At the next two follow-up visits (24 and 32 weeks of gestation), women in the IPTp-SP treatment group received SP while women in the ISTp-AL group were screened with the RDT and, if positive, treated with AL. As part of adverse event monitoring, all women were followed-up on the phone by a trained trial team member to record any complaints that the women might have a week after each scheduled antenatal visit. Study women were also provided with a mobile phone number which they could call to arrange unscheduled visits. A team of nurses and lab scientists were dedicated to follow-up of women who did not present for delivery at the General Hospital within 2-3 weeks of the estimated delivery date to establish the outcome of the pregnancy.

## **3.4 MALARIA DIAGNOSTICS**

### **3.4.1 MALARIA MICROSCOPY**

Microscopy blood smears were obtained at enrolment, the second follow-up visit, whenever a malaria infection was suspected, before delivery (36 to 40 weeks gestation) and at the postpartum visit (6 weeks post-delivery). At each of these time points, two thick and one thin film was prepared. Thick and thin smears were prepared for the estimation of asexual parasite density and to identify parasite species, respectively. Clean frosted microscope slides were used to prepare blood smear, and these were stained with Giemsa (pH 7.2) using WHO procedures (World Health Organization, 2010b). Thick smear slides were rapidly stained for 15 minutes with 10% Giemsa stain. Second slides taken from study subjects were stained for about 30-45 minutes with 3% Giemsa stain. Slides were placed on wooden slide racks to dry and transferred to plastic slide boxes for safe handling and storage. An

experienced microscopist, blinded to trial group assignment, read all the blood films and calculated parasite density against 200 leucocytes in thick blood smears. A thick blood film was declared negative only after examination of 100 high power fields (HPF).

### **3.4.2 RAPID DIAGNOSTIC TESTS**

Histidine-Rich Protein-2 (HRP-II) antigen and pLDH (Pan) antigen RDT kit (SD BIOLINE Malaria Ag P.f/Pan test, Standard Diagnostics, Inc.) was used for diagnostic confirmation of malaria in all patients in the ISTp-AL arm. The SD BIOLINE Malaria Ag P.f/Pan test is a qualitative and differential test for the detection of HRP-II antigen of *P. falciparum* and plasmodium lactate dehydrogenase (pLDH) of Plasmodium species in human whole blood. About 5µl of whole blood was added to the well of the RDT kit, and two drops of the assay buffer were added to the well. The result was interpreted within 20 minutes based on the indication of the bands presented. The test kits were stored according to manufacturer's instructions, and the viability of the kits was tested monthly using positive test controls obtained from the manufacturer. The tests were performed and interpreted by the trial team following the manufacturer's instructions.

### **3.5 EVALUATION OF HAEMOGLOBIN CONCENTRATION**

At enrolment and subsequent second and third follow-up visits, maternal haemoglobin concentration (Hb) was determined in finger prick blood using a HemoCue analyser (HemoCue Hb 301, HemoCue Ltd, United Kingdom) at the General Hospital Calabar. One microcuvette preparation was analysed per patient from a single blood specimen. Laboratory personnel collecting and analysing blood samples were trained for one day on machine operation before the actual data collection. Microcuvettes were kept free of moisture while microcuvette containers were stored at room temperature and kept away from heat or direct sunlight. The microcuvette holder was cleaned at the end of each day's work with an alcohol swab. The machine was always checked before use according to manufacturer's instructions.

### **3.6 DETERMINATION OF BIRTHWEIGHT AND PREGNANCY-RELATED OUTCOMES**

For women who delivered at the hospital, birth weight was recorded by a midwife who was blinded to the treatment group of the woman whom she was attending. Women who delivered at home were traced by a follow-up team (made up of a lab scientist, trained community health worker and study nurse) within 6 days of delivery and the infant's weight was measured. The occurrence of miscarriages, stillbirths, neonatal deaths and the presence of congenital abnormalities were recorded by midwives. WHO definitions of miscarriage, stillbirth, and neonatal deaths were used for this trial. A miscarriage was defined as the premature loss of a foetus up to 23 weeks of pregnancy and weighing up to 500 g while a stillbirth was defined as a baby born with no sign of life at or after 28 weeks' gestation. Neonatal death was defined as a death occurring during the first 28 days of life (0-27 days).

### **3.7 PLACENTAL MALARIA**

Immediately after delivery, the paracentric side of the maternal placenta was cleaned with sterile cotton wool and incised with a scalpel blade as previously described (Sowunmi et al., 1996). Two drops of blood collected by aspiration through a Pasteur pipette were used to prepare a thick blood smear and a blood spot on commercially available filter paper (Whatman 3MM, GE Healthcare Life Sciences, USA). The thick smears and blood spots were air-dried and stained with 4% Giemsa. An experienced microscopist immediately examined the stained smears by light microscopy to detect asexual forms of *P. falciparum* malaria parasites.

### **3.8 DRIED BLOOD SPOT SAMPLES**

Three drops of peripheral blood (measured with 25µl pipette) were collected by finger prick and transferred onto commercially available filter paper (Whatman 3MM, GE Healthcare Life Sciences, USA). A sample was collected at each scheduled visit (V1, V2, and V3) before delivery. One blood spot was for DNA extraction, the second for RNA extraction and the third was a spare one. These filter papers were air dried for 3 hours and packed into small plastic bags with desiccant (silica gel). Dried blood spot (DBS) samples on filter paper were shipped to the Tropical Institute, Munich.

For pregnant women with malaria parasitaemia at any of these three visits, genomic DNA was subsequently extracted from the DBS on the filter paper using QIAamp® DNA Blood Mini kit (Qiagen, Krefeld, Germany), following manufacturer's protocol. Species differentiation was then undertaken through the amplification of *Plasmodium* small subunit ribosomal RNA using nested PCR methods (Snounou et al., 1993, Schoone et al., 2000).

### **3.9 SUB-STUDIES: LABORATORY PROCEDURES**

#### **3.9.1 GENOTYPING PROCEDURES AND ANALYSIS OF *dhfr* AND *dhps* GENES**

Molecular genotyping was performed only on dried blood spot filter paper samples from pregnant women with a positive microscopy blood slide. *P. falciparum* parasites were genotyped for mutations in the dihydrofolate reductase (*dhfr*) and dihydrofolate synthase (*dhps*) genes by PCR-restriction fragment length polymorphism (PCR-RFLP). Mutations were investigated at codons 51, 59, 108 and 164 of the *Pfdhfr* gene and codons 436, 437, 540, 581 and 613 of the *Pfdhps* gene as previously described (Duraisingh et al., 1998). Analysis of mutation at codons 51, 59, 108, 164, 436, 437, 540, 581 and 613 was performed using the following enzymes: Tsp509I, XmnI, AluI, DraPI, MspA1I, AvaI, FokI, BstUI, and RsaI respectively. They were subjected to 2.0% agarose gel electrophoresis and visualised under UV light. Amplified and digested products were compared with those of reference isolates. Isolates with mixed *dhfr/dhps* alleles comprising both wild-type and mutated alleles were categorised as mutants.

#### **3.9.2 MOLECULAR QUANTIFICATION OF SUB-MICROSCOPIC GAMETOCYTAEMIA (QT-NASBA)**

Several antimalarials including SP have been shown to lead to the proliferation of gametocytes. This sub-study sought to quantify sub-microscopic gametocytaemia using real-time quantitative nucleic acid sequence-based amplification. This section describes the methods employed in the molecular quantification of gametocytaemia. The methods used for the QT-NASBA were as previously described (Pritsch et al., 2012).

## **Specimen collection**

Finger prick DBS filter paper and thick film samples of study women who were positive for malaria by microscopy were chosen for the analysis. The samples were collected between March and August 2014 at the General Hospital. The finger prick DBS were of a sample volume of 25µl measured with a pipette and 25µl pipette tips. After air-drying, all samples were individually sealed in labelled plastic bags and stored with desiccant (silica gel). The samples had been stored for 1-3 weeks at room temperature and then shipped to Munich, Germany and subsequently stored at -20°C until ribonucleic acid (RNA) extraction and then quantitative nucleic acid sequence based amplification (QT-NASBA) was performed.

## **RNA extraction**

For analysis, the samples containing 25 µL of finger-prick blood were cut out of the filter paper with heat sterilised scissors maintaining a distance of approximately 3 mm to the border of the sample. The spots were subsequently soaked in 2 ml NucliSens® easyMAG® (bioMérieux, Lyon, France) lysis buffer containing guanidium isothiocyanate (GuSCN) and rocked at 150 rotations per minute (rpm) for 30 minutes at room temperature. The solution was then centrifuged at 1,500 g for 5 minutes, and the filter paper removed. 50 µL of silica particle solution (bioMérieux, Lyon, France) was added and then the RNA extraction method originally described by Boom et al. (Boom et al., 1990) was performed. Subsequently, nucleic acids were eluted from the silica with 30 µL elution buffer provided by the manufacturer.

For each extraction, a negative control of Plasmodium-negative full blood spotted on filter paper and a positive control of Plasmodium-spiked (NF54 strain) full blood spotted on filter paper was used. After extraction, the samples were either stored at -80°C for a maximum of 24 hours or immediately analysed by real-time QT-NASBA technology using a previously described primer and beacon mix (Pritsch et al., 2012).

## **Real-time quantitative nucleic acid sequence-based amplification (QT-NASBA)**

Mature *P. falciparum* gametocytes were quantified using *Pfs25* mRNA QT-NASBA (Schneider et al. 2006) as it is expressed only in stage V gametocytes. For *Pfs25*

mRNA, the forward primer was: 5'-GAC TGTAAG TAA ACC ATG TGG AGA-3'; the reverse primer was: 5'-AAT TCT AAT ACG ACT CAC TAT AGG GAG AAG GCA TTT ACC GTT ACC ACA AGT TA-3'. *Pfs25* molecular beacon probe was 5'-TexasRed-CGA TCG CCG TTT CAT ACG CTT GTA ACG ATC G-DABSYL-3. Briefly, real-time QT-NASBA for *Pfs25* mRNA was performed using Nuclisens® EasyQ® Basic kit (bioMérieux, Lyon, France) according to the manufacturer's manual at a KCl concentration of 80nM. The reaction mixture including reverse and forward primers and RNA eluate was incubated at 65°C for five minutes and subsequently at 41°C for 5 minutes.

Before isothermal amplification at 41°C, 5 µl of Nuclisens enzyme mixture (AMV-RT, T7 RNA polymerase, and RNase H) was added to a total reaction volume of 20 µl and real-time amplification allowed for 90 minutes.

Samples were also analysed in duplicate and considered positive when the time-point of amplification at which the fluorescence detecting target amplicons (time to positivity = TTP) exceeded the mean fluorescence of three negative controls plus 20 standard deviations (SD) as previously described (Schneider et al., 2004).

### **3.10 DEFINITION OF OUTCOMES**

#### **3.10.1 ANAEMIA**

Severe and moderate anaemia in this study was defined as pregnant women with a haemoglobin level less than 8g/dL and less than 11g/dL respectively.

#### **3.10.2 LOW BIRTH WEIGHT**

Low birth weight (LBW) was defined as a birth weight of a liveborn infant of less than 2,500 g.

#### **3.10.3 ASYMPTOMATIC MALARIA PARASITAEMIA**

Asymptomatic malaria parasitaemia was defined as malaria infection confirmed by light microscopy without any clinical symptoms.

#### **3.10.4 CLINICAL MALARIA**

Clinical malaria was defined as the presence of clinical symptoms in addition to malaria parasitaemia confirmed by light microscopy.

### **3.10.5 PERINATAL DEATH**

Perinatal death was defined as the number of stillbirths and deaths in the first week of life. The perinatal period begins at 22 completed weeks of gestation and ends seven completed days after birth.

### **3.10.6 ADVERSE EFFECT**

Any untoward medical incident in a patient or clinical investigation subject, temporarily associated with the use of a medicinal product, whether or not considered related to the medicinal product. This also includes failure to produce expected benefits, abuse or misuse.

### **3.10.7 SEVERE ADVERSE EVENT**

A serious adverse event is any untoward medical occurrence that, at any dose:

- a. Results in death.
- b. Can be considered life threatening (i.e. an event in which the subject was at risk of death at the time of the event).
- c. May requires hospitalisation or prolongation of existing hospitalisation.
- d. Results in disability/ incapacity.
- e. Results in congenital anomaly/birth defect.
- f. Additionally, a haemoglobin value of  $\leq 5$  g/dl in any pregnant woman was considered a serious adverse event.

### **3.11 PARTICIPANT WITHDRAWAL**

Patients were withdrawn if they withdrew their informed consent or if they left the study area permanently.

### **3.12 ETHICAL CONSIDERATIONS**

Participation in the study was voluntary; subjects were free to withdraw from the study at any time, and this did not affect their access to quality care. Information obtained from all subjects was treated as confidential. The trial was conducted under the provisions of the Declaration of Helsinki and in accordance with Good Clinical Practices guidelines set up by the WHO and by the International Conference on Harmonization.

The purpose of the study, the procedures to be followed, and the risks and benefits of participation were explained to all participants. Information sheets and consent forms were provided to them for their review. Each participant was asked to sign an informed consent to participate in the research study.

The study proposal and informed consent forms were reviewed and approved by two ethics committees: the Cross River Health Research Ethics Committee, Calabar, Nigeria and the Ethics Board of the Medical Center of LMU, Munich, Germany. The trial was registered prior to the enrolment of participants in the Pan African Clinical Trials Registry (PACTR201308000543272).

### **3.13 SAMPLE SIZE**

The sample size was calculated based on the assumption that the prevalence of severe anaemia in the third trimester of pregnancy in the IPTp-SP arm of the study would be at least 3% based on findings from a previous study undertaken in Ghana (Tagbor et al., 2010). Due to widespread resistance to SP, it was hypothesised that the prevalence of severe anaemia would be significantly lower in the LLIN plus intermittent malaria screening with AL (ISTp-AL arm) compared to the LLIN plus intermittent preventive treatment with SP (IPTp-SP). To establish that AL-IST was not inferior to IPTp-SP it was necessary to show that the differences in the proportion of women with severe anaemia between the ISTp-AL and IPTp-SP arms would not be more than 5%, differences which would be of clinical and public health importance.

Thus, if there were truly no difference between the prevalence of severe anaemia in the conventional IPTp-SP and ISTp-AL arm, then 366 participants (183 per arm) were required to detect a 5% difference between the two study arms at 95% significance with 80% power. This meant that if there was truly no difference between IPTp-SP and ISTp-AL, then 366 patients were required to be 80% sure that the upper limit of a 95% two-sided confidence interval would exclude a difference in favour of the IPTp-SP group of more than 5%. With 20% adjustment for attrition, a sample size of 460 pregnant women (230 per arm) was adequate. Thus a sample size of 460 (230 per arm) was sufficient to detect any differences in the prevalence of severe anaemia between the two arms. 460 pregnant women (230 IPTp-SP: 230

ISTp-AL) were randomised, allowing for a 20% withdrawal/loss to follow-up and a non-inferiority margin of 5%, to yield 366 evaluable pregnant women for the primary outcome (183 IPTp-SP: 183 ISTp-AL).

### **3.14 STATISTICAL METHODS**

Stata version 12 (StataCorp, College Station, Texas) was used for data analyses. The primary objective of the study was to demonstrate that the risk of third-trimester severe anaemia ( $Hb \leq 8$  g/dl) in the ISTp-AL group was no more than 5% greater than in the IPTp-SP group. The secondary objectives were to demonstrate that the risks of low birth weight, neonatal and maternal mortality, spontaneous abortions, intrauterine deaths and stillbirths, were not significantly higher in women in the ISTp-AL group than in women who received IPTp-SP. The main analysis of primary and secondary outcomes was according-to-protocol (ATP). However, a modified intention-to-treat (mITT) analysis was also undertaken. In the ATP analysis, only data from women who remained within their randomization group and had a record for the primary outcome were included. This implied that study women had to have received two courses of SP (IPTp-SP arm) or been screened twice using an RDT at scheduled visits (ISTp-AL arm) and in addition, have a measurement for the primary outcome (haemoglobin level at 36-40 weeks gestation) to be eligible for inclusion into the ATP analysis.

In the mITT analysis, data from women who received an initial treatment of IPTp or had an initial screening test done and had a record for the primary outcome or the outcome of interest recorded. This implied that study women had to have received one course of SP (IPTp-SP arm) or been screened once using an RDT at scheduled visits (ISTp-AL arm) and in addition, have a measurement for the primary outcome or outcome of interest.

The proportion of ATP and mITT populations experiencing the primary and secondary outcomes in both trial arms, and the associated 2-sided 95% CI for the difference, was estimated using the generalised linear model. To declare non-inferiority, the upper boundary of the 2-sided 95% CI for the estimated treatment effect (i.e. risk difference) had to be below the pre-defined non-inferiority margin ( $\Delta$ )

of 5% at a significance level of 0.05. We controlled for gravidity, gestational age at enrolment, baseline anaemia and parasitaemia using binomial regression.

Only birth weights from singleton pregnancies of live births  $\geq 22$  weeks' gestation with no congenital abnormality and measured within six days of delivery were included in birth weight analyses.

## **4 RESULTS**

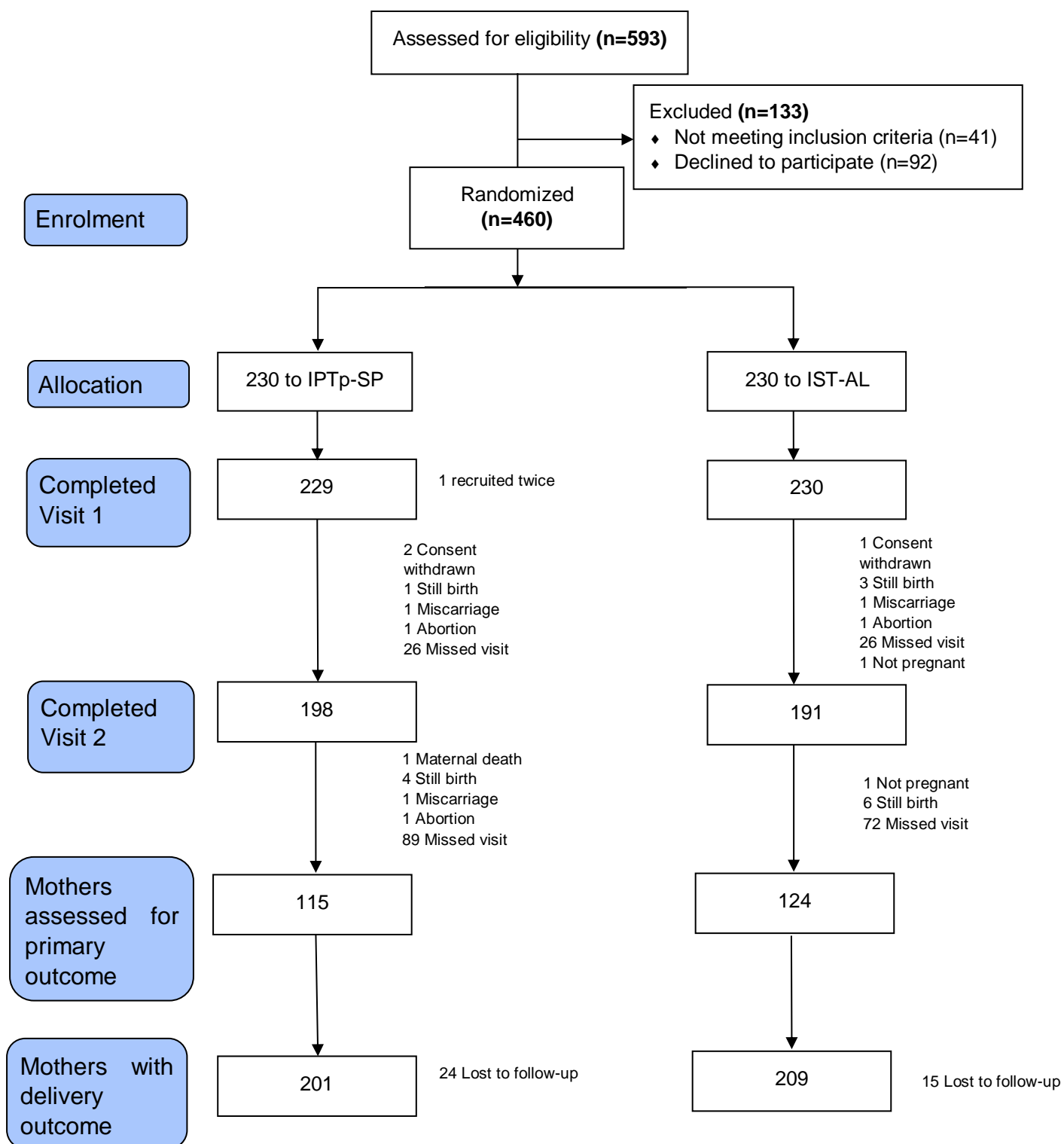
### **4.1 OVERVIEW**

The preceding chapter 3 described the methods used in the clinical trial and the sulfadoxine-pyrimethamine molecular markers study in Nigeria which led to key findings presented in this chapter: the trial profile, the impact of ISTp on malaria-related morbidity, and safety. The prevalence of molecular markers of SP resistance and gametocytes determined by QT-NASBA are also presented in this chapter.

### **4.2 TRIAL PROFILE**

A total of 593 potentially eligible women were screened starting in October 2013 and 460 women were included in the trial while 133 women were not included because they did not meet the inclusion criteria (41) or declined to participate in the study (92).

The 460 pregnant eligible women were randomised, 230 to IPTp-SP and 230 to ISTp-AL. One allocation error occurred, a woman previously enrolled in the trial was randomised a second time. Thus leaving 459 women who completed the first visit (i.e. received an initial treatment of IPTp with SP or had had an initial screening test done); 229 in the IPTp-SP group and 230 in the ISTp-AL group. At the end of follow-up, 239 (52.1 %) evaluable records for third-trimester haemoglobin concentration and 329 (71.7 %) records for birth weight were available. Three women withdrew from the study (two in the IPTp-SP group and one in the ISTp-AL group). The distribution of the randomised pregnant women is summarised in Figure 4.1.



**Figure 4.1:** Trial profile showing enrolment and follow-up status of study women.

### 4.3 BASELINE DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Baseline characteristics of study women in both treatment groups were very similar at enrolment and are shown in Table 4.1. About three-quarters of the study women in both arms were aged between 13 and 30 years with an overall mean age of 28.2 years. All study women had some level of formal education, and over 90% of them had been educated up to at least secondary school level.

The majority of the study women were students in tertiary institutions. Only 22% of the study women were salary workers comprised mainly of civil servants and teachers. 57% of the study women's households already owned a bed net at enrolment. However self-reported bed net use the night preceding enrolment was about 15%. Forty-seven percent of study women were primigravidae, and 29% were multigravidae.

Overall, 1.1% (5/459) of women had baseline severe anaemia (i.e. a haemoglobin (Hb) concentration below 8 g/dl). The overall mean baseline haemoglobin concentration was 11.5 g/dl. Asymptomatic parasitaemia as determined by microscopy was present in 7% (32/459) of study women overall with parasite densities less than 500/mL in about 37.5% (12/32). The prevalence of malaria infection on presentation at the antenatal clinic in women in the ISTp-AL arm as determined by the SD Bioline® rapid diagnostic test was 5.7% (RDTs were not done in women in the IPTp-SP arm). Baseline anaemia was significantly associated with asymptomatic malaria parasitaemia ( $p < 0.0001$ ). *P. falciparum* was the prevalent malaria species. One mixed infection of *P. malariae* and *P. falciparum* was observed. The overall prevalence of HIV in pregnant women who were enrolled during the period of the trial was 1.3%.

**Table 4.1:** Comparison of demographic and baseline characteristics of study women

	Control IPTp-SP (N=229)		Intervention ISTp-AL (N=230)		All participants (N=459)	
	N	(%)	n	(%)	n	(%)
<b>Age (years)</b>						
≤ 25	58	25.3	76	33	135	29.4
26-30	103	45	86	37.4	189	41.0
≥ 31	68	29.7	68	29.6	136	29.6
Mean (SD)	28.4	4.6	27.9	5.4	28.2	5.0
Median (IQR)	28	6	28	7	28	6
<b>Educational attainment</b>						
Primary	7	3.1	6	2.6	13	2.8
Secondary	96	41.9	99	43	195	42.5
Tertiary	126	55	125	54.4	251	54.7
<b>Occupation</b>						
None	10	4.4	21	9.1	31	6.8
Student	49	21.4	46	20	95	20.6
Housewife	26	11.4	24	10.4	50	10.9
Small business owner	92	40.1	90	39.1	182	39.7
Salary worker	52	22.7	49	21.3	101	22
<b>Gravidity</b>						
Primigravidae	107	46.7	109	47.4	216	47.1
Secundigravidae	68	29.7	69	30.0	137	29.8
Multigravidae	54	23.6	52	22.6	106	23.1
<b>Ownership of bed net</b>						
No	99	43.2	108	47	207	45.1
Yes	130	56.8	122	53	252	54.9
<b>Slept under bed net (previous night)</b>						
No	197	86.0	195	84.8	392	85.4
Yes	32	14.0	35	15.2	67	14.6
<b>Baseline Parasitaemia</b>						
No	212	92.6	215	93.5	427	93.0
Yes	17	7.4	15	6.5	32	7.0
<b>Parasite density</b>						
Geometric mean [95% CI]	954.8	[490.4 -1859.3]	951	[452.5 - 1998.5]	953	[597.3 - 1520.6]
<b>Haemoglobin (g/dl)</b>						
11 and above	135	59	165	71.7	300	65.4
8-10.9	92	40.2	62	27.0	154	33.5
<8	2	0.8	3	1.3	5	1.1
Mean (SD)	11.4	1.4	11.6	1.3	11.5	1.4
Median (IQR)	11.3	1.8	11.6	1.4	11.5	1.6

## 4.4 STUDY OUTCOMES-ACCORDING TO PROTOCOL (ATP) ANALYSIS

### 4.4.1 ANAEMIA

At 36–40 weeks of gestation (third trimester before delivery) the overall prevalence of the primary outcome, severe anaemia (Hb<8 g/dl) was 0.84% and similar in both treatment groups (p=0.145). The prevalence of moderate anaemia (Hb 8 -10.9 g/dl) was 27.7% (Table 4.2) and was similar in both treatment groups (p=0.204).

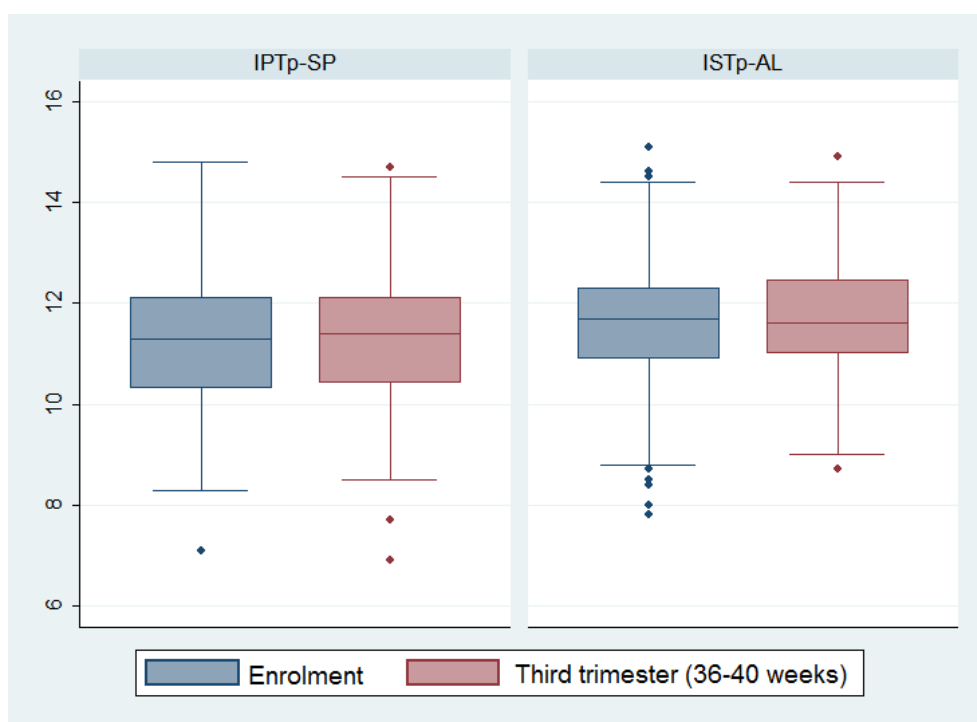
The mean haemoglobin concentration (Hb) of all women between 36 and 40 weeks of gestation was 11.5 g/dl. The mean Hb concentration in the ISTp-AL and IPTp-SP groups were 11.7g/dl and 11.4 g/dl respectively. However, this difference was not statistically significant (p= 0.073). There was a non-significant increase in mean haemoglobin concentration of 0.05 g/dl at 36-40 weeks of gestation over the baseline haemoglobin concentration (Figure 4.2). There was no significant difference in haemoglobin concentration during the course of pregnancy between the two trial arms.

**Table 4.2:** Comparison of the main outcomes in women enrolled in IPTp-SP and ISTp-AL arms (ATP analysis).

Outcome	All participants	Control (IPTp-SP)	Intervention (ISTp-AL)	p-value
<b>Anaemia</b>				
Severe anaemia (Hb< 8g/dl)	2/238 (0.84)	2/114 (1.7)	0/124 (0)	<b>0.145</b>
Moderate anaemia (Hb 8-10.9 g/dl)	66/238 (27.7)	36/114 (31.6)	30/124 (24.2)	<b>0.203</b>
Hb ≥ 11 g/dl	170/238 (71.4)	76/114 (66.7)	94/124 (75.8)	<b>0.121</b>
Mean haemoglobin g/dl (SD)		11.4 (1.3)	11.7 (1.2)	<b>0.073</b>
<b>Birth weight</b>				
Normal (≥ 2.5kg)	269/286 (94.1)	130/139 (93.5)	139/147 ( 94.6)	
Low (< 2.5kg)	17/286 (5.9)	9/139 ( 6.5)	8/147 (5.4 )	<b>0.694</b>
Mean (SD)	3.19 (0.5)	3.21 (0.51)	3.18 (0.54)	<b>0.630</b>
Median (IQR)	3.20 (2.5)	3.20 (0.6)	3.20 (0.6)	
<b>Parasitaemia</b>				
Peripheral blood (light microscopy)				
All infections	4/208 (1.9)	4/96 (4.2)	0/112 (0)	<b>0.029</b>

SD: standard deviation IQR: inter-quartile range

The risk of third-trimester severe anaemia (Hb <8g/dl) did not differ significantly between both treatment groups (RD -1.75% [95% CI; -4.16 to 0.66]. The upper boundary of the 2-sided 95% CI for the risk difference estimated between the ISTp-AL and IPTp-SP groups was below the non-inferiority margin of 5% set for third-trimester severe anaemia.



**Figure 4.2:** Variation of haemoglobin concentration between enrolment and third trimester (36 – 40 weeks gestation).

The risk of anaemia (severe or moderate) did not differ significantly between both treatment groups (Table 4.2). Primigravidae women were significantly associated with a lower risk of anaemia compared to secundi- and multigravidae in univariate analysis. In a multivariate analysis, which adjusted for baseline anaemia, treatment group, maternal age, and baseline parasitaemia, primigravidae remained significantly associated with lower risk of anaemia in the third trimester (Table 4.3).

The risk of moderate anaemia (<11g/dl) was not higher in the ISTp-AL group than in women who received IPTp-SP; (RD= -6.1% [95% CI; -17.9 to 5.8]. In the adjusted analysis, the risk difference was -5.4% [95% CI; -17.0 to 6.2]. All malaria infections were mild, and thus no study woman was admitted to hospital.

**Table 4.3:** Factors associated with third-trimester anaemia (<11g/dl) in study women (ATP analysis).

	Unadjusted Risk Ratio	(95%CI) <sup>a</sup>	p- value <sup>b</sup>	Adjusted Risk Ratio	(95%CI)	p-value
<b>Treatment group</b>						
ISTp-AL	0.83	0.57 to 1.20	<b>0.318</b>	0.81	0.57 to 1.17	<b>0.259</b>
IPTp-SP	1			1		
<b>Age category</b>						
≤ 25	1			1		
26-30	1.07	0.67 to 1.72	<b>0.766</b>	0.95	0.59 to 1.52	<b>0.824</b>
≥ 31	1.31	0.81 to 2.12	<b>0.279</b>	1.03	0.61 to 1.75	<b>0.899</b>
<b>Baseline parasitaemia</b>						
Yes	1.49	0.90 to 2.45	<b>0.119</b>	1.46	0.89 to 2.41	<b>0.138</b>
No	1			1		
<b>Gravidity</b>						
Primigravidae	0.57	0.38 to 0.87	<b>0.008</b>	0.59	0.37 to 0.93	<b>0.024</b>
Secundigravidae	0.67	0.42 to 1.08	<b>0.104</b>	0.66	0.41 to 1.06	<b>0.087</b>
Multigravidae	1			1		

CI: confidence interval

<sup>a</sup> p ≤0.05 means observed differences between comparison groups is statistically significant or not significant if p>0.05.

<sup>b</sup> Risk ratios were modelled using binomial regression. Treatment group, age category, baseline parasitaemia and gravidity were included in the final model.

#### 4.4.2 BIRTH WEIGHT

The overall prevalence of low birth weight was 5.9% in the study women and did not differ significantly between the two groups (p=0.694). However, the trial was not powered to investigate non-inferiority for this outcome (Table 4.2). The risk difference for low birthweight between the ISTp-AL and IPTp-SP arms was (RD - 1.03% [95% CI; -6.53 to 4.46]. In an adjusted analysis (Table 4.4) which controlled for maternal age, gravidity baseline anaemia and baseline parasitaemia, the risk difference for low birthweight was (RD -1.53% [95% CI; -1.54 to -1.15] and significantly lower in the ISTp-AL arm (p<0.0001).

The risk of low birth weight did not differ significantly between the various age groups (Table 4.4). Baseline parasitaemia and gravidity were not significantly associated with low birth weight babies (Table 4.4).

None of the study women with low birthweight babies had severe anaemia at enrollment.

**Table 4.4:** Factors associated with low birth weight of babies delivered by study women (ATP analysis).

	Unadjusted Risk Ratio	(95%CI)	p-value <sup>a</sup>	Adjusted Risk Ratio <sup>b</sup>	(95%CI)	p-value
<b>Treatment group</b>						
ISTp-AL	0.84	0.33 to 2.12	<b>0.712</b>	0.84	0.33 to 2.17	<b>0.72</b>
IPTp-SP	1			1		
<b>Age category (years)</b>						
≤ 25	1			1		
26-30	1.17	0.37 to 3.77	<b>0.787</b>	1.43	0.44 to 4.59	<b>0.548</b>
≥ 31	1.08	0.30 to 3.86	<b>0.911</b>	2.22	0.58 to 8.42	<b>0.242</b>
<b>Baseline parasitaemia</b>						
Yes	1.46	0.35 to 5.99	<b>0.603</b>	1.62	0.39 to 6.63	<b>0.505</b>
No	1					
<b>Baseline mild anaemia</b>						
Yes						
No	1.32	0.52 to 3.37	<b>0.558</b>	1.41	0.53 to 3.70	<b>0.489</b>
	1					
<b>Gravidity</b>						
Primigravidae	3.18	0.73 to 13.81	<b>0.123</b>	4.54	0.94 to 21.84	<b>0.059</b>
Secundigravidae	1.31	0.23 to 7.65	<b>0.761</b>	1.44	0.24 to 8.69	<b>0.694</b>
Multigravidae	1					

CI: confidence interval

<sup>a</sup> p ≤0.05 means observed differences between comparison groups is statistically significant or not significant if p>0.05.

<sup>b</sup> Risk ratios were modelled using binomial regression. Treatment group, age category, baseline parasitaemia and mild anaemia, and gravidity were included in the final model.

#### 4.4.3 PERIPHERAL AND PLACENTAL MALARIA

The prevalence of peripheral malaria (asymptomatic parasitaemia) among pregnant women at any time during the study was 9.2% in study women overall and was very similar in both treatment groups (p=0.80).

The risk of malaria parasitaemia at 36 - 40 weeks was significantly higher in the IPTp-SP arm than in the ISTp-AL arm as shown in Table 4.2 (4.2% versus 0%, p=0.029). The risk difference for malaria parasitaemia between the ISTp-AL and IPTp-SP groups was RD -3.96% [95% CI -7.76 to -0.16]. One episode of illness associated with malaria parasitaemia was recorded in the IPTp-SP arm, during the course of the trial. In all, 533 RDT tests were done in women in the ISTp-AL treatment group of which 40 (7.5%) were positive and led to treatment.

The prevalence of placental malaria at delivery as determined by microscopy was low with just one low-density infection identified from 46 (10%) samples collected from study women.

Between March and August 2014, 125 samples (106 enrollment samples and 19 follow-up samples) were collected and analysed for *Pfs25mRNA* by QT-NASBA. Sample collection and analysis in Munich had to be stopped due to the Ebola crisis. Of these samples, 51 (40.8%) were from women in the IPTp-SP group while 74 (59.2%) were from women in the ISTp-AL group (Table 4.5). Enrolment gametocyte prevalence was 0% (0/106) by microscopy and 2.8% (3/106) by QT-NASBA. The enrolment gametocyte prevalence by QT-NASBA in the IPTp-SP and ISTp-AL groups were 2.2% (1/45) and 3.3% (2/61) respectively but did not differ significantly between treatment groups ( $p=0.7358$ ). No gametocytes were detected by microscopy or QT-NASBA in the follow-up samples.

**Table 4.5:** Gametocyte prevalence of *Plasmodium falciparum* by microscopy and QT-NASBA

	Total	IPTp-SP	ISTp-AL	p-value
<b>Enrolment</b> n (%)				
Microscopy	0/106 (0)	0/45 (0)	0/61 (0)	-
QT-NASBA	3/106 (2.8)	1/45 (2.2)	2/61 (3.3)	0.7358
<b>Follow-up</b> n (%)				
Microscopy	0/19 (0)	0/6 (0)	0/13 (0)	-
QT-NASBA	0/19 (0)	0/6 (0)	0/13 (0)	-
Total	125			

#### 4.4.4 HAEMOGLOBIN LEVELS AND BIRTH WEIGHT OUTCOMES IN WOMEN WHO WERE RDT NEGATIVE THROUGHOUT THE TRIAL

The risk of third-trimester anaemia (36-40 weeks gestation) and low birth weight in pregnant women who were RDT negative throughout pregnancy (received no antimalarial treatment) was not higher than in the IPTp-SP arm (Table 4.6)

**Table 4.6:** Anaemia before delivery (36-40 weeks) and birth weight by treatment group in RDT negative women

Outcome	According to Protocol Analysis		
	Control (IPTp-SP)	Intervention (ISTp-AL)	p-value
<b>Anaemia</b>			
Severe anaemia (Hb< 8g/dl)	2/114 (1.7)	0/84 (0)	<b>0.23</b>
Moderate anaemia (Hb 8-10.9 g/dl)	36/114 (31.6)	20/84 (23.8)	<b>0.229</b>
Hb ≥ 11 g/dl	76/114 (66.7)	64/84 (76.2)	<b>0.147</b>
Mean haemoglobin g/dl (SD)	11.4 (1.3)	11.7 (1.17)	<b>0.096</b>
<b>Birth weight</b>			
Normal (≥ 2.5kg)	130/139 (93.5)	62/65 (95.4)	<b>0.591</b>
Low (< 2.5kg)	9/139 (6.5)	3/65 (4.6)	
Mean (SD)	3.21 (0.51)	3.23 (0.43)	<b>0.753</b>
Median (IQR)	3.2 (0.6)	3.2 (0.5)	

## 4.5 STUDY OUTCOMES – MODIFIED INTENTION TO TREAT (mITT) ANALYSIS

All the primary and secondary outcomes were also analysed using modified intention to treat (mITT) analysis with similar results to those of the ATP analysis.

### 4.5.1 ANAEMIA

The overall prevalence of third trimester (36-40 weeks) severe anaemia was 0.84% and not significantly different between both study groups ( $p=0.14$ ). Similarly, there was no significant difference between study groups regarding the prevalence of moderate anaemia (Table 4.7). The overall mean haemoglobin concentration in the third trimester (36-40 weeks gestation) for study women was 11.5 g/dl. The average haemoglobin concentration in the ISTp-AL and IPTp-SP groups were 11.7g/dl and 11.4 g/dl respectively. However, this difference was not statistically significant ( $p=0.076$ ).

The risk of third-trimester severe anaemia (Hb <8g/dl) did not differ significantly between both treatment groups (RD -1.74% [95% CI; -4.1 to 0.65]. The upper boundary of the 2-sided 95% CI for the risk difference estimated between the ISTp-AL and IPTp-SP groups was below the non-inferiority margin of 5% set for third-trimester severe anaemia as was the case in the ATP analysis.

**Table 4.7:** Comparison of the main outcomes in women enrolled in IPTp-SP and ISTp-AL arms (mITT analysis)

Outcome	mITT analysis			
Anaemia	Total	IPTp-SP	ISTp-AL	p-value
Severe anaemia (Hb< 8g/dl)	2/239 (0.84)	2/115 (1.7)	0/124 (0)	0.14
Moderate anaemia (Hb 8-10.9 g/dl)	66/239 (27.6)	36/115 (31.3)	30/124 (24.2)	
Hb ≥ 11 g/dl	171/239 (71.5)	77/115 (67)	94/124 (75.8)	
Mean haemoglobin g/dl (SD)	11.5 (1.3)	11.4 (1.4)	11.7 (1.2)	0.076
Birth weight				
Normal (≥ 2.5kg)	305/325 (93.8)	148/158 (93.7)	157/167 (94)	0.898
Low (< 2.5kg)	20/325 (6.2)	10/158 (6.3)	10/167 (6.0)	
Mean (SD)	3.2 (0.5)	3.21 (0.53)	3.17 (0.53)	0.497
Median (IQR)	3.2 (0.4)	3.2 (0.6)	3.2 (0.6)	
Parasitaemia				
Peripheral blood (light microscopy)				
All infections	4/213 (1.9)	4/101 (4.0)	0/112 (0)	0.034

Also, primigravidae women were significantly associated with a lower risk of anaemia compared to secundi- and multigravidae in univariate analysis (RR 0.57, 95% CI 0.38 to 0.87). In a multivariate analysis, which adjusted for baseline anaemia, treatment group, maternal age, and baseline parasitaemia, primigravidae women were still associated with a significantly lower risk of anaemia in the third trimester (Table 4.8)

**Table 4.8: Factors associated with third-trimester anaemia (<11g/dl) in study women (mITT analyses)**

	Unadjusted Risk Ratio	(95%CI) <sup>a</sup>	p- value <sup>b</sup>	Adjusted Risk Ratio	(95%CI)	p-value
<b>Treatment group</b>						
ISTp-AL	0.83	0.58 to 1.21	<b>0.341</b>	0.82	0.57 to 1.17	<b>0.272</b>
IPTp-SP	1			1		
<b>Age category</b>						
≤ 25	1			1		
26-30	1.06	0.66 to 1.71	<b>0.798</b>	0.94	0.58 to 1.05	<b>0.785</b>
≥ 31	1.31	0.81 to 2.12	<b>0.279</b>	1.03	0.61 to 1.75	<b>0.904</b>
<b>Baseline parasitaemia</b>						
Yes	1.49	0.91 to 2.46	<b>0.115</b>	1.45	0.89 to 2.43	<b>0.128</b>
No	1			1		
<b>Gravidity</b>						
Primigravidae	0.57	0.38 to 0.87	<b>0.008</b>	0.59	0.37 to 0.93	<b>0.024</b>
Secundigravidae	0.66	0.41 to 1.07	<b>0.092</b>	0.65	0.40 to 1.05	<b>0.076</b>
Multigravidae	1			1		

CI: confidence interval

<sup>a</sup> p ≤0.05 means observed differences between comparison groups is statistically significant or not significant if p>0.05.

<sup>b</sup> Risk ratios were modelled using binomial regression. Treatment group, age category, baseline parasitaemia and gravidity were included in the final model.

The risk of moderate anaemia (<11g/dl) was no higher in the ISTp-AL group than in women who received IPTp-SP; (RD= -5.8% [95% CI; -17.6 to 6.1]. In the adjusted analysis, the risk difference was -5.5% [95% CI; -17.1 to 6.1]. Again these results are similar to those obtained in the ATP analyses.

#### **4.5.2 BIRTHWEIGHT**

The overall prevalence of low birth weight was 6.2% in the study women and did not differ significantly between the two groups (p=0.898, Table 4.7). However, the trial was not powered to investigate non-inferiority for this outcome. The risk of low birth weight in the ISTp-AL group was not significantly different from that in the IPTp-SP group (RR 0.95 [95% CI; 0.40 to 2.21], p=0.898. In an adjusted analysis (Table 4.8) which controlled for maternal age, gravidity baseline parasitaemia and baseline anaemia the risk of low birth weight in the ISTp-AL group was not significantly different from that in the IPTp-SP group (RR 0.94 [95% CI; 0.40 to 2.25], p=0.896.

### 4.5.3 PERIPHERAL MALARIA

The prevalence of peripheral malaria parasitaemia at 36-40 weeks gestation was 1.9% in study women overall (Table 4.7). There was also significantly higher peripheral malaria parasitaemia in the IPTp-SP arm compared to the ISTp-AL arm (4% versus 0%,  $p=0.034$ ).

## 4.6 CLINICAL TOLERABILITY OF THE INTERVENTIONS

### 4.6.1 MILD ADVERSE EVENTS

All women were followed up for side effects after the enrolment visit. The most commonly reported mild adverse events were a headache, cough, catarrh, vomiting, and dizziness. These symptoms were not significantly different between both treatment groups. However, women in the ISTp-AL arm complained of fever since their last antenatal clinic visit more frequently than in the IPTp-SP arm ( $p= 0.022$ ) (Table 4.9). All malaria infections were mild, and thus no study woman was admitted to hospital.

**Table 4.9:** Comparison of the number of women who experienced adverse events.

	Total	IPTp-SP	ISTp-AL	
	n (%)	n (%)	n (%)	p-value
Headache	69 (15)	35 (15.3)	34 (14.8)	<b>0.929</b>
Cough	40 (8.7)	23 (10)	17 (7.4)	<b>0.323</b>
Catarrh	30 (6.5)	15 (6.6)	15 (6.5)	<b>0.966</b>
Vomiting	16 (3.5)	7 (3.1)	9 (3.9)	<b>0.641</b>
Dizziness	16 (3.5)	6 (2.6)	10 (4.3)	<b>0.319</b>
Fever	12 (2.6)	2 (0.9)	10 (4.3)	<b>0.022</b>
Body pain	9 (2.0)	5 (2.2)	4 (1.7)	<b>0.699</b>
Itching	5 (1.1)	4 (1.7)	1 (0.4)	<b>0.172</b>
Nausea	1 (0.2)	1 (0.4)	-	<b>0.337</b>
Rash	1 (0.2)	-	1 (0.4)	<b>0.338</b>
Sleepiness	1 (0.2)	-	1 (0.4)	<b>0.338</b>

### 4.6.2 SERIOUS ADVERSE EVENTS

There was one maternal death in the IPTp-SP arm. No drug-related serious adverse events occurred during the trial. The prevalence of perinatal deaths was 6.2% and did not differ significantly between both trial groups (Table 4.10). Also, there were no statistically

significant differences in the risk of preterm deliveries and abortions between both study arms (Table 4.10).

**Table 4.10:** Comparison of delivery outcomes for singleton births

	Total n (%)	IPTp-SP n (%)	ISTp-AL n (%)	p-value
Term deliveries	389 (92.6)	191 (93.2)	198 (92.1)	
Preterm deliveries	1 (0.24)	1 (0.48)	-	0.306
Abortions	4 (0.95)	2 (0.96)	2 (0.93)	0.962
Perinatal deaths	26 (6.2)	11 (5.36)	15 (6.97)	0.493

\*Outcome of pregnancy not determined for 39 study women who were lost to follow-up

## 4.7 SULFADOXINE-PYRIMETHAMINE MOLECULAR MARKERS OF RESISTANCE

To determine the prevalence of SP molecular resistance markers in *P. falciparum* isolates from pregnant women in Southeast Nigeria, DBS filter paper of women with positive peripheral malaria microscopy slide samples were analysed for *Pfdhfr* and *Pfdhps* genes.

A total of 43 samples from 39 study women infected with *P. falciparum* based on microscopic examination of blood smear and nested PCR were included in the analysis of *Pfdhfr* mutations at codons 51, 59, 108, and 164, and *Pfdhps* mutations at codons 436, 437, 540, 581 and 613. The majority of study women (74.4%) were aged  $\leq 30$  years old, and 76.9% (30/39) of them did not use insecticide-treated bed nets the night before enrolment. 97.4% (38/39) of the patients had no fever. The mean Hb concentration was  $10.1 \pm 1.5$  g/dl. The median parasite density was 776 asexual parasite/ $\mu$ l with an interquartile range of 237–3248 asexual parasite/ $\mu$ l.

Mutant alleles are presented in Table 4.11. *Pfdhfr* mutations were detected in 93% (40/43) of *P. falciparum* isolates for codons 51 (**51I**) and 59 (**59R**). All isolates had mutant alleles for codon 108(**108N**). No mutation was identified at codon 164. A single mutation in codon 108 (**108N**) in the *Pfdhfr* gene was detected in 7% (3/43) isolates.

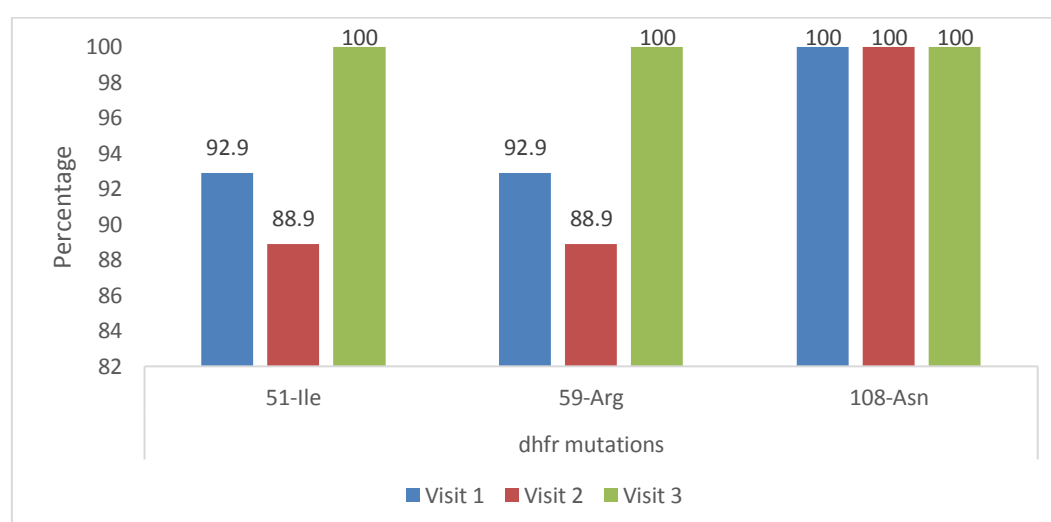
*Pfdhps* mutations were detected in 79% (34/43), and 95% (41/43) of the *P. falciparum* isolates for codons 436 and 437 respectively. No mutation was identified at codon 540. The prevalence of mutations at codons 581 and 613 was 69.8% each.

A single mutation in codon 437 in the *Pfdhps* gene was detected in 16.3% (7/43) isolates. There was no significant difference in the distribution of the mutant alleles between the IPTp-SP and ISTp-AL groups in the *Pfdhfr* and *Pfdhps* genes (Table 4.11).

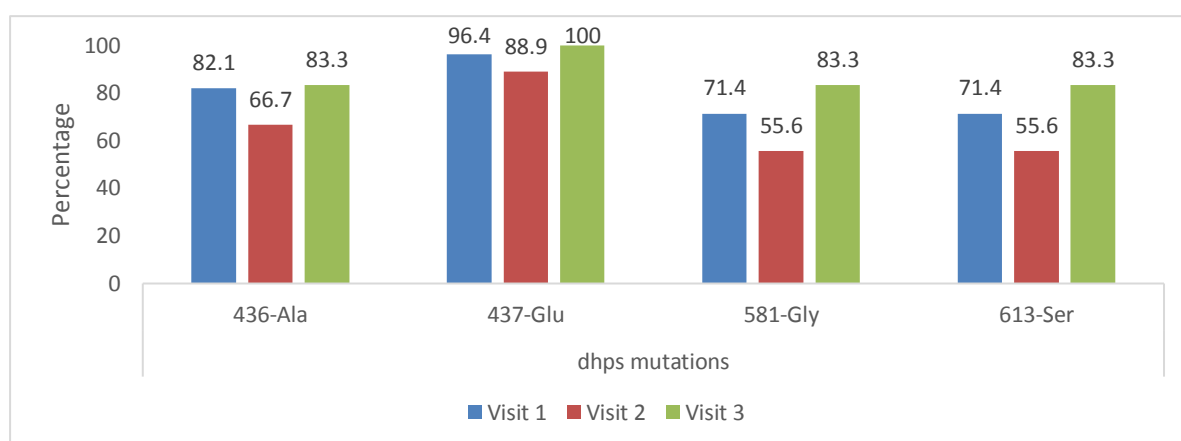
**Table 4.11:** Proportion of SP molecular markers of resistance in both trial arms

		IPTp-SP	ISTp-AL	Total	
(PCR positive)		23	20	43	
		Proportion (%) carrying the mutant alleles			p-value
<b><i>Pfdhfr</i></b>	51-Ile	22 (95.7)	18 (90)	40 (93)	0.464
	59-Arg	22 (95.7)	18 (90)	40 (93)	0.464
	108-Asn	23 (100)	20 (100)	43 (100)	-
	164-Leu	0 (0)	0 (0)	0 (0)	-
<b><i>Pfdhps</i></b>	436-Ala	18 (78.3)	16 (80)	34 (79.1)	0.891
	437-Glu	23 (100)	18 (90)	41 (95.3)	0.120
	540-Gly	0 (0)	0 (0)	0 (0)	-
	581-Gly	18 (78.3)	12 (60)	30 (69.8)	0.192
	613-Ser	18 (78.3)	12 (60)	30 (69.8)	0.192

The prevalence of mutations in the *Pfdhfr* and *Pfdhps* genes was high at all visits as shown in Figure 4.3 and Figure 4.4. Even the baseline prevalence of *Pfdhfr* and *Pfdhps* resistance markers was above 70% in all *P. falciparum* isolates identified.



**Figure 4.3:** Prevalence of *Pfdhfr* resistance markers by trial visit.



**Figure 4.4:** Prevalence of *Pfdhps* resistance markers by trial visit.

Genotyping analysis was conducted based on sequences for *Pfdhfr*, *Pfdhps*, and combined *Pfdhfr*–*Pfdhps* genes. Single (NCNI) and triple (IRNI) mutant genotypes of *Pfdhfr* were detected in 4.7 % (2/43) isolates and 79.1 % (34/43), respectively.

For *Pfdhps*, no single mutant genotype was detected in the isolates. Genotyping of *P. falciparum* isolates for the combined *Pfdhfr*–*Pfdhps* genes showed that one (2.3 %), six (14 %), 22 (51.2 %), and 30 (69.8%) isolates had two, four, five and six mutant genotypes, respectively. The quintuple mutant genotype (108/59/51/436/437) was significantly associated with women in the IPTp-SP arm (Table 4.12).

**Table 4.12:** Proportion of combined mutated genotypes in both trial arms.

Mutated codons	IPTp-SP arm N= 23		ISTp-AL arm N=20		Total	p-value
	n	%	n	%	n (%)	
<i>Pfdhfr</i> -108	0	0	2	10	2 (4.7)	0.120
<i>Pfdhfr</i> -108/59/51	18	78.3	16	80	34 (79.1)	0.891
<b>Combined <i>Pfdhfr</i>-<i>Pfdhps</i> genotypes</b>						
108/437	1	4.3	0	0	1 (2.3)	0.348
108/59/51/437	4	17.4	2	10	6 (14)	0.485
108/59/51/436/437	18	78.3	4	20	22 (51.2)	0.0001
108/59/51/436/437/581/613	18	78.3	12	60	30 (69.8)	0.192

## 5 DISCUSSION

The research conducted in this thesis aimed to evaluate the effectiveness of intermittent screening and treatment with AL (ISTp-AL) for preventing malaria in pregnancy. The thesis also evaluated the baseline prevalence of parasite molecular markers of resistance to SP. This concluding chapter contains a summary and discussion of the major findings. It highlights the main recommendations and limitations of this work, considers possibilities for future work and outlines its implications for the broader context of malaria control.

### 5.1 KEY FINDINGS

This trial has shown that intermittent screening and treatment using artemether-lumefantrine was not inferior to intermittent preventive treatment with sulfadoxine-pyrimethamine in preventing severe maternal anaemia, the primary trial outcome. The trial was conducted in Calabar, South East Nigeria, a setting with perennial malaria transmission where *P. falciparum* is still sensitive to SP albeit at reduced levels and IPTp-SP is still likely to confer some protection against malaria in pregnancy.

The overall prevalence of low birth weight was lower than the national average of 15% (UNICEF, 2015). Babies born to women in the ISTp-AL treatment group were at a lower risk of being underweight at birth. Peripheral malaria parasitaemia at any time during pregnancy did not differ significantly between both treatment groups. However, there was a significantly higher risk of peripheral malaria parasitaemia in the IPTp-SP treatment group in the third trimester (36-40 weeks). Gametocytaemia detected by QT-NASBA was also very low, and no gametocytaemia was detected by microscopy. This is attributable to the low density of the malaria infections among the study participants.

Among a subset of women in the ISTp-AL group who were RDT negative throughout pregnancy and did not receive any antimalarial treatment, the prevalence of third trimester severe anaemia and low birth weight was not significantly different from

women in the IPTp-SP treatment group. The incidence of preterm deliveries, abortions, and perinatal deaths was similar in both treatment groups.

There was a high proportion of *Pfdhfr* and *Pfdhps* mutations among *P. falciparum* isolates from women involved in the study. The *Pfdhfr* triple mutation (codons 51, 59 and 108) was detected in over 90% of *P. falciparum* isolates. No *Pfdhfr* mutations were observed at codon 164. *Pfdhps* mutations were detected in *P. falciparum* isolates at codons 436, 437, 581 and 613. No *Pfdhps* K540E mutation was observed among study women. The prevalence of triple *Pfdhfr* mutations was almost 80%. There was also a high prevalence of quintuple and septuple *Pfdhfr-Pfdhps* mutations. There was no significant difference in the distribution of mutant alleles between both treatment groups.

## 5.2 LIMITATIONS OF THE RESULTS

Limitations of the study include the lack of a complete set of observations for all women. Most of these women contributed data to the birth weight analysis, but about 43% did not contribute data to third-trimester maternal anaemia which was the primary outcome. This loss of data is attributable to several national strikes embarked upon by different cadres of health workers that disrupted antenatal clinic attendance and adversely affected follow-up visits and the recording of related outcomes because the health facility was locked up over these periods. With the exception of high level of data loss for the primary outcome, the overall rate of loss to follow-up rate was 8.5% and did not differ between the control and intervention group. Additionally, due to logistic constraints, we were unable to collect placental samples from all study women who had facility deliveries. The West Africa Ebola Epidemic in 2014 also adversely affected blood sample collection for molecular quantification of gametocytaemia by QT-NASBA. As Nigeria experienced a small outbreak, it was agreed with the supervisors to suspend the collection of blood samples for QT-NASBA as the analyses could not be carried out in Munich under the newly necessary security measures.

Furthermore, the prevalence of malaria parasitaemia observed in this clinical trial was very low. This prevalence may have been underestimated due to the low

sensitivity of microscopy and RDTs. Perhaps, if a more sensitive method such as loop-mediated isothermal amplification (LAMP), with an estimated lower limit of detection 1-5 parasites per µl of blood (Oriero et al., 2015), was used, the prevalence might have been higher due to an identification of more sub microscopic parasitaemia.

### **5.3 OVERALL INTERPRETATION**

The overall incidence of malaria and severe anaemia in study women was observed to be much lower than expected and relative to previously reported estimates from Nigeria (Olubukola et al., 2011, Nwizu et al., 2011, Agan et al., 2010). All the malaria infections among study women were mild, and none required hospital admission. Apart from the fact that pregnant women often have low-density asymptomatic malaria infections this finding may be explained by continued decline in the incidence of malaria cases and the sustained scale-up in coverage of preventive interventions, most especially LLINs. All study women received a LLIN at enrolment and were encouraged to continue sleeping under them at scheduled follow-up visits. The Cross River State government has also provided free medical care to pregnant women and under-fives since 2009. These may have also contributed to the low prevalence of malaria observed in the trial.

The ISTp intervention was well received by most pregnant women and health providers in this study. This is similar to findings from a trial in West Africa (Smith et al., 2010). A few study women, however, withdrew consent from this study as a result of repeated blood draws and finger pricks. However, ISTp-AL is likely to be more expensive than IPTp-SP because of the cost of RDTs and doses of artemether-lumefantrine which are higher than the cost of a dose of SP. AL is also more complex to administer being a three-day treatment. Thus, adherence monitoring is more difficult and would mostly rely on self-report by pregnant women or home visits by health workers. From a programmatic perspective, to be able to deliver ISTp through antenatal clinics, health managers, and health policymakers will need to take into consideration the workload and changes in health provider responsibilities and issues around the sustainability of funding.

The transmission of parasites from humans to mosquitoes requires the presence of infectious gametocytes in the human peripheral blood. Consequently, pregnant women with asymptomatic parasitaemia could constitute a reservoir of parasites for the inoculation of mosquitoes (Kern et al., 2011). It is important to note that women in the IPTp-SP treatment group had a higher risk of parasitaemia compared to women in the ISTp-AL treatment group. AL has been shown to be more effective at reducing gametocyte carriage and malaria transmission to mosquitoes compared to DHA-P (Sawa et al., 2013). ISTp with AL or other ACT regimens could reduce the incidence of malaria and anaemia which could bring immense health and socioeconomic benefits. These benefits could be considered to outweigh the lower cost-effectiveness of the approach in the long term.

Previous trials (Tagbor et al., 2015, Tagbor et al., 2010, Gonzalez et al., 2014, Clerk et al., 2008, Kakuru et al., 2016) have investigated alternative drug regimens and strategies to replace SP for intermittent preventive treatment of malaria in pregnancy. The most promising results are from Western Kenya (Desai et al., 2016). They found that IPTp with DHA-P was more effective than ISTp with DHA-P or IPTp with SP at preventing peripheral or placental malaria at delivery. This result suggests that ISTp as a strategy may not be suitable for settings with high transmission. A recently published trial (PREGACT Study Group, 2016) that evaluated four ACT combinations for the treatment of African pregnant women (second and third trimester) with malaria and found DHA-P to have the best efficacy and an acceptable safety profile. However, AL was associated with the fewest adverse effects and had an acceptable cure rate.

Moreover, the sensitivity of RDTs currently in use suggest that available malaria RDTs may be inadequate for the screening of asymptomatic women who typically have low-density infection which is often missed by RDTs (Desai et al., 2016, Ahmed et al., 2015). There is also a concern about the absence of a prophylactic effect in women who test negative. This allows low-density infections to persist and increases the chance of new infections occurring between scheduled antenatal visits.

More recently, there has been growing concern about reports of histidine-rich protein 2/3 (*pfhrp2*/*pfhrp3*) gene deletions. Most of the currently available RDT kits work by detecting histidine-rich protein II which is expressed only by *P. falciparum*. This preference was due to research findings that they are more sensitive and heat-stable

than RDTs that detect other malaria antigens, such as plasmodium lactate dehydrogenase (pLDH) or aldolase (World Health Organization, 2016a).

Since 2010, when Gamboa and colleagues (Gamboa et al., 2010) reported the first confirmed identification of *P. falciparum* parasites with *pfhrp2/pfhrp3* gene deletions there have been successive reports from Eritrea, Ghana, India, Mali and Senegal. This poses a serious public health challenge for parasitological confirmation of malaria as parasites with *hrp2*, or *hrp3* gene deletions cannot be detected by HRP2-based rapid diagnostic tests (RDTs) resulting in false-negative results.

There are other reasons for false-negative RDT results which still need to be taken into consideration such as poor transport and storage conditions of RDTs, sustained exposure to high temperature as well as host-parasite density. There is also the chance of human error in performing or interpreting the test result. Thick or thin films may also be incorrectly interpreted as positive by microscopy. Continued human capacity building for microscopist is required to ensure results from microscopy represent a reliable gold standard against which RDT results can be compared. NMCPs may also procure alternative RDTs such as the SD Bioline Malaria Ag P.f (HRP2/pLDH) test kits used in this trial, which do not rely exclusively on HRP2 for *P. falciparum* detection.

WHO recently recommended the continued use of IPTp-SP for the prevention of MiP as there is not sufficient evidence to suggest that it be replaced by ISTp or any other drug regimen replacing SP for use as IPTp. The WHO also recommends that National Malaria Control Programmes (NMCP) consider discontinuing IPTp-SP when the population prevalence of *P. falciparum dhps* mutation K540E is greater than 95%, and the prevalence of mutation A581G exceeds 10 %, as it is likely to be ineffective (World Health Organization 2016b).

In this study, among pregnant women, before the commencement of IPTp-SP, the *Pfdhfr* mutations 51I, 59R and 108N were almost fixed with all three mutations being present in more than 70% of the isolates. There was no I164L mutation in the study samples a finding similar to a previous study in Nigeria (Happi et al., 2005). However, the frequency of the triple *Pfdhfr* mutation was also high (70%). The *Pfdhfr* triple mutation is known to confer intense pyrimethamine resistance *in vitro* (Gregson

and Plowe, 2005). The *Pfdhfr* mutations are known to have emerged about a decade or two before the *Pfdhps* double mutant genotype in Africa (Talisuna et al., 2004) and are now well established across sub-Saharan Africa. The high level of triple *Pfdhfr* mutations found in this study could be explained in part by the fact that pyrimethamine was previously used as weekly chemoprophylaxis to prevent malaria in pregnancy (Fawole and Onyeaso, 2008, Yusuf et al., 2008). Also, cotrimoxazole use has been associated with the emergence, spread and intensification of the A437**G** and K540**E** mutations in the *Pfdhps* gene (Gesase et al., 2009). In Nigeria, there is a high burden of pneumonia and cotrimoxazole is commonly used as prophylaxis or treatment among HIV patients and children with pneumonia (Onyedum and Chukwuka, 2011).

In the *Pfdhps* gene, the frequency of the core mutation, A437**G**, was over 90%. Although the K540**E** mutation is very frequently found in association with the A437**G** mutation (Pearce et al., 2009, Kublin et al., 2002), the K540**E** mutation was absent from all the *P. falciparum* isolates in this study. Thus, there was no *Pfdhps* double mutation, at codon 437 and 540 which is a predictor of post-treatment SP resistance (Kublin et al., 2002, Plowe et al., 2004). Although *Pfdhfr/dhps* quintuple mutants are rare in West Africa, recent studies have reported the emergence of the K540**E** mutation. This mutation is known to be a reliable marker for parasites carrying the quintuple mutants. Studies from the western part of Nigeria have found an emergence of mutant *P. falciparum* isolates carrying sulfadoxine resistance associated A437**G** and K540**E** mutations in the *Pfdhps* gene (Iwalokun et al., 2015, Olasehinde et al., 2014, Happi et al., 2005).

However, the occurrence of A437**G** combined with A581**G** mutation confers higher levels of SP resistance (Pearce et al., 2009), and this combination of A581**G**/A437**G** mutations was present in twenty of twenty-eight isolates. The prevalence of the S436**A** mutation, which is an additional mutation that follows the emergence of A437**G** mutation was over 80%. This additional mutation corresponds to an increase in the degree of resistance to SP.

The combination of A437**G** mutation with the *Pfdhfr* triple mutation (51**I**/59**R**/108**N**) is considered to be associated with SP treatment failure (Mockenhaupt et al., 2005) and was detected in over 70% of the isolates.

The prevalence of the triple *Pfdhfr* and A437**G**/A581**G** *Pfdhps* mutations were very high; suggesting that the efficacy of SP as IPTp in Southeast Nigeria may be severely threatened. However, the K540**E** mutation was absent suggesting that SP may still be efficacious when used as IPTp.

ISTp remains a valuable approach to the control of MiP that needs to be further explored. IST may have other malaria control applications in endemic countries as they approach pre-elimination phase of malaria control, characterised by low-density infections in asymptomatic carriers. The intermittent screening may serve as a malaria surveillance tool for monitoring malaria levels in the population. Active detection of malaria infection has been demonstrated to have an impact on miscarriage and preterm delivery (Bardaji et al., 2011). ISTp may also be recommended in the first trimester of pregnancy when IPTp-SP is not recommended or restricted to the first ANC visit. Screening at initial ANC visit has been shown to detect up to 50% of all the malaria infections diagnosed throughout the course of pregnancy (Williams et al., 2016).

Furthermore, combining IPTp-SP and ISTp may result in lower fitness of SP-resistant parasites as there may be a fitness cost of the *Pfdhfr* and *Pfdhps* mutant haplotypes in the absence of a strong SP pressure (Zhou et al., 2008, Bacon et al., 2009).

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## 6 CONCLUSION

Malaria is a major parasitic disease in sub-Saharan Africa and remains a major public health issue. Despite reported global decline in clinical cases of malaria and malaria deaths including in Africa pregnant women and children aged less than five years are remain particularly vulnerable to clinical disease when the get infected.

Intermittent preventive treatment (IPTp) with SP along with use of ITNs for vector control and prompt access to diagnosis and treatment of clinical malaria with ACTs are the key interventions currently recommended by WHO for the control of malaria in pregnancy.

However, there is widespread and established parasite resistance to SP in the Southern and Eastern parts of Africa. Reports indicate the emergence of SP resistance in West African countries too. Despite widespread parasite resistance to SP in Southern and Eastern Africa, SP remains the drug of choice for intermittent preventive treatment of malaria in pregnancy in West Africa. However, there have been increasing reports of emerging SP resistance even in West Africa. This emerging SP resistance calls to question the effectiveness of SP as a preventive intervention for malaria in pregnancy.

It has been argued that the continued use of SP for intermittent preventive treatment in pregnant women, may further increase the prevalence of SP-resistant parasites and lead to the selection of new mutations. It may be better to administer full malaria treatment doses to pregnant women only when they have parasitaemia as a mechanism for reducing drug pressure and resistance.

As part of my PhD program, we undertook to investigate a different approach to preventing malaria in pregnancy. We conducted a clinical trial from October 2013 to November 2014. We designed a non-inferiority clinical trial described in chapter 3 to determine whether providing LLIN plus RDT screening and treatment (ISTp) is non-inferior to LLIN plus IPTp-SP in reducing the burden of malaria during pregnancy. The primary outcome of the trial was the prevalence of severe maternal anaemia (Hb<8g/dl) in the third trimester. We also determined the prevalence and density of gametocytaemia and parasitaemia at each visit. Other secondary endpoints included the prevalence of low birth weight, moderate anaemia (Hb < 11g/dl), placental

malaria, and the incidence of spontaneous abortions, intrauterine deaths/stillbirths, neonatal and maternal mortality, and developmental delays. A sub-study of the thesis was the determination of the prevalence of molecular markers of parasite resistance to SP among the pregnant women.

A total of 459 pregnant women were randomly assigned to receive either ISTp-AL or IPTp-SP. The study took place in the ANC clinic of the General Hospital in Calabar, the capital city of Cross River State in South eastern Nigeria. The interventions were given during the second and third trimesters with primary outcome evaluation at 36-40 weeks gestation.

The main results were reported in Chapter 4. ISTp-AL was non-inferior to IPTp-SP in preventing third-trimester maternal anaemia (RD -1.75% [-4.16% to 0.66%]) according to the non-inferiority margin of 5% set *a priori*. However, the results obtained for third-trimester anaemia are likely not powered to exclude small clinically significant differences based on the sample size calculation, and non-inferiority margin set *a priori*. However, the According-to-protocol (ATP) and modified intention-to-treat (mITT) analyses yielded similar results and loss to follow-up was 8.5%, and there was no significant difference in numbers of women lost to follow-up between the treatment arms.

The prevalence of moderate anaemia was lower in the ISTp-AL group but not significantly different from the IPTp-SP group. The risk of LBW was significantly lower in the ISTp-AL group in an adjusted analysis. Similarly, the risk of maternal malaria parasitaemia in the third trimester was significantly lower in the ISTp-AL group. Placental malaria was rarely found among the few samples obtained from study women. Gametocytaemia was not different between both study groups although the trial was not powered to detect small difference in gametocytaemia. The incidence of abortions, perinatal deaths and pre-term deliveries were comparable in both study groups. Other relevant findings of this trial were the high prevalence of the *Pfdhfr* triple mutation (51I59R108N). In the *Pfdhps* gene, the prevalence of the 436A, 437G, 581G and 613S mutations was also high.

ISTp as an approach to control malaria in pregnancy may be more effective when highly sensitive RDTs become available and if treatment can be with a single dose regimen, preferably produced from non-artemisinin derivatives, but effective against gametocytes. This will ensure therapy can be directly observed at the antenatal

clinic. The use of a non-artemisinin-based drug would prevent the potential development and spread of artemisinin resistance in sub-Saharan Africa.

This study showed non-inferiority of ISTp-AL to IPTp-SP for preventing maternal anaemia in pregnant women in an area of high malaria transmission and moderate SP resistance. ISTp remains one of the potential alternatives to IPTp-SP, and its relevance will become more evident when the sensitivity of current generation of RDTs are improved beyond present levels. In the meantime, high priority must be given to identifying safe and cost-effective alternatives to SP for use in IPTp. There is also need to ensure access for pregnant women to long-lasting insecticide-treated nets and prompt diagnosis and effective treatment.

## **6.1 AREAS FOR FUTURE RESEARCH**

This thesis has explored an alternative strategy for preventing malaria in pregnancy. However, several research questions remain and call for further investigation. Currently available rapid diagnostic tests cannot detect either the low-level blood-stage infections of any malaria species or the dormant liver stages of *P. ovale* and *P. vivax*. A highly sensitive point-of-care field test is needed to detect low-density parasitaemia rapidly and identify all infected individuals, enabling immediate treatment (Hemingway et al., 2016). Research in this respect is required and will determine the level of success that can be achieved with reducing the infectious burden of malaria.

Future research is also required to confirm and map HRP2/HRP3-deleted parasites across sub-Saharan Africa. There is also need to identify ways of improving the performance of pLDH based tests as well as identify new target antigens for use in RDTs.

While screening for *Pf dhps* K540E, a predictor of the quintuple mutant should remain a priority in Nigeria and West Africa, research to evaluate the threshold of SP resistance above which IPTp-SP is no longer effective would also be useful. Also, the evaluation of alternative preventive treatment options for preventing malaria in pregnancy should be a priority. It may be worthwhile to evaluate the preference of pregnant women between a community-based and a facility-based approach to ISTp.

ISTp may be more appropriate for chemoprevention in HIV-infected women for whom SP use is contraindicated because of concomitant prophylaxis with cotrimoxazole. Clinical trials are required to evaluate the potential benefits and harms of ISTp in this peculiar group of pregnant women.

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## 7 REFERENCES

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## 8 ANNEX

### 9 CURRICULUM VITAE

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#### **Education**

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London School of Hygiene and Tropical Medicine, University of London	
<b>M.Sc. (Public Health in Developing Countries)</b>	<b>2008</b>
University of Ghana, Legon, Ghana	
<b>B.Sc. (Zoology)</b>	<b>2006</b>

#### **Work Experience**

##### **University of Calabar, Calabar, Nigeria**

- Lecturer II: 2013 - date
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##### **Cross River Health and Demographic Surveillance System, Calabar, Nigeria**

Field Epidemiologist/ Member, Scientific Board **2012 – date**

##### **Effective Health Care Research Consortium, Calabar, Nigeria**

- |                      |                    |
|----------------------|--------------------|
| ▪ Research Associate | <b>2010 - date</b> |
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##### **National Agency for the Control of AIDS, Abuja, Nigeria** **2008-2009**

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#### **Key Research Skills**

Malaria epidemiology; Evidence Synthesis & Meta-Analysis; Evidence-based Medicine;  
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## 10 LIST OF PUBLICATIONS

1. Meremikwu MM, Donegan S, Esu E. Chemoprophylaxis and intermittent treatment for preventing malaria in children. *Cochrane Database Syst Rev.* 2008 Apr 16;(2):CD003756. doi: 10.1002/14651858.CD003756.pub3.
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18. Oringanje C, Meremikwu MM, Eko H, **Esu E**, Meremikwu A, Ehiri JE. Interventions for preventing unintended pregnancies among adolescents. **Cochrane Database of Systematic Reviews** 2016, Issue 2. Art. No.: CD005215. DOI: 10.1002/14651858.CD005215.pub3.

## **11 STATEMENT ON PRE-RELEASE AND CONTRIBUTION**

I declare that parts of this study have been submitted for publication and is undergoing peer review.

I declare that this thesis is my own work, completed under the supervision of Professor Martin Meremikwu, Dr Nicole Berens-Riha, Dr Michael Pritsch and Professor Dr Thomas Loescher. I acknowledge the following assistance and collaboration: The laboratory analysis of molecular markers of SP resistance from asymptomatic pregnant women described in Chapter 4 was carried out by Costanza Tacoli and Prabhanjan Gai at the Institute of Tropical Medicine and International Health, Charité-Universitätsmedizin Berlin, Berlin.

Where results from the published or unpublished work of other people have been used in the thesis I have cited the appropriate references.

The trial in Nigeria was sponsored by University of Calabar, Calabar through a grant from the Tertiary Education Trust Fund.

I had primary responsibility for writing the protocol, preparing the Standard Operating Procedures and questionnaires, the ethics submissions to the Ethics board of the Medical Center of LMU, Munich, Germany and the Cross River Health Research Ethics Committee, registration of the trial, selecting the field sites, training the staff, organising the field work, supervising the enrolment and follow-up of study subjects, supervising data entry, writing the analysis plan and the statistical analysis, with guidance from Dr Nicole Berens-Riha and advice from Dr Michael Pritsch, Prof Dr Thomas Loescher and Prof Martin Meremikwu. I prepared the first draft of two manuscripts reporting the trial findings for submission for publication.

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Direct LMU – Supervisor: Dr Nicole Berens-Riha, Division of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich (LMU), Munich, Germany

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I hereby declare, that the submitted thesis entitled

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is the result of my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

The submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

I further declare that the electronic version of the submitted thesis is congruent with the printed version both in content and format.

Calabar, 30.09.2016

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