COMPARATIVE EFFICACY AND SAFETY OF PYRONARIDINE-ARTESUNATE VERSUS ARTEMETHER-LUMEFANTRINE IN THE TREATMENT OF ACUTE UNCOMPLICATED MALARIA AMONG CHILDREN IN SOUTH-WEST NIGERIA.

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Date last updated: 25 March 2019

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SUMMARY

Malaria is a common protozoan infection that poses a serious health problem in the world today especially in tropical Africa where morbidity and mortality is high among pregnant women and children under five years of age. In Nigeria, malaria is the commonest reason for outpatient clinic attendance in childhood and is responsible for about 20 % of childhood deaths. The emergence of strains of *P. falciparum* resistant to chloroquine and sulfadoxine-pyrimethamine led to severe worsening of the morbidity and mortality from malaria. As a result of resistance to previously used monotherapy, the World Health Organization (WHO) in 2001, recommended that malaria endemic countries experiencing drug resistant malaria infection adopt combination therapy. Artemisinin-based combination therapy (ACT) is preferred to non-ACT combination

This study is a randomized open label clinical trial that will compare the safety and efficacy of pyronaridine-artesunate and artemether-lumefantrine in the treatment of malaria among children aged 3 to 144 months who have microscopically confirmed symptomatic *Plasmodium falciparum* malaria. The study will be carried out at the Oni Memorial Children's Hospital, Ring Road Ibadan. One hundred and sixty-two children between 3 and 144 months who meet the inclusion criteria will be enrolled after obtaining written or witnessed signed informed consent from the parents or guardian. Details obtained from the enrolee will be entered into a case record form specifically designed for the purpose of accurate documentation. A detailed history and physical examination will be carried out on each enrolee. Finger prick blood samples will be taken from each enrolee for thick blood smear for malaria parasite, haematocrit and blood spots on filter paper. Five millilitres of venous blood will be taken from an arm vein for baseline liver function tests, creatinine and random blood glucose on days 0, 3, 7 and 28.

Enrolees will be randomized into one of two groups. Group one will receive pyronaridine-artesunate while group two will receive artemether-lumefantrine at standard doses. Enrolees will be seen daily from days 0-3, and on days 7, 14, 21 and 28. Study drugs will be administered supervised at standard dosage on days 0, 1, and 2. History taking, physical examination and blood smears will be done at each contact time. Special attention will be paid to adverse effects.

Parasite clearance time, fever clearance time and cure rates will be compared between the two groups. The data collected from the participants will be entered into Epi info version 6 databases and then transferred to SPSS Statistics Software version 20 for statistical analysis.

INTRODUCTION

Background of the study

Malaria is an important protozoan infection which poses a major health problem in the world today.[1] The infection is endemic in most parts of the tropics, including Nigeria with a greater percentage of malaria incidence and deaths occurring amongst pregnant women and children under five years of age.[2] In Nigeria, malaria is the commonest reason for outpatient clinic attendance in childhood and is responsible for about 20 % of childhood deaths.[3] Malaria is the only vector borne disease placed on the World Health Organization's disability adjusted life years (DALYS) list.[4] Of the approximately 3.4 billion people worldwide who are exposed to malaria yearly, the World Health Organization (WHO) states that there were about 219 million cases of symptomatic malaria in 2016.[5]

Although, mortality rates from malaria seem to have fallen globally and in the Africa region due to effective control measures, the disease continues to be a major threat to childhood health and development. In 2016 there were 445000 deaths with 80% of these occurring in children under 5years and pregnant women.[5] Chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) which were once very effective against the malaria parasite, have been replaced in many malaria-endemic countries by artemisinin-based combination therapies as first line drugs due to the emergence of resistance to these monotherapies in the malaria parasite following WHO recommendations[6]. Increasing parasite resistance to these old drugs and failure of malaria treatment with these single agents in many endemic countries of Africa led to a widespread promotion of artemisinin-based combination therapy (ACT) as a strategy for effective management of *Plasmodium falciparum* malaria. The World Health Organization (WHO) in 2001 recommended that malaria endemic countries change their treatment policies and adopt combination therapy, and in particular artemisinin-based combination therapy as the first-line antimalarial treatment.[1]

The artemisinins act by inhibiting *Plasmodium falciparum* encoded sarcoplasmic endoplasmic reticulum calcium ATPase. They inhibit development of gametocytes thus reducing transmission.[7] Gametocytes, the sexual stages of the malaria parasite, do not cause clinical disease but are vital to the maintenance of malaria transmission cycle. These gametocytes are present in symptomatic and asymptomatic parasitemic individuals.[7] The WHO defines artemisinin-based combination therapy for malaria as the simultaneous use of at least two efficacious schizonticidal drugs one of which is an artemisinin derivative. The two or more drugs must have different mechanisms of action and thus different biochemical targets in the parasite.[8] It is important that the two or more constituent drugs are efficacious and are used at individual full dosages. Artemisinin-based combination therapies (ACTs) are now the first-line treatment for acute uncomplicated malaria globally. They are more effective than the non-artemisinin–based combination or monotherapies, as they lead to rapid parasite clearance, hasten clinical recovery and reduce the chances of development of drug resistance in the parasite.[1]

Artemether-lumefantrine (AL) is the first fixed - dose ACT to be pre-qualified by the WHO and the most commonly used ACT as first-line drug for the treatment of uncomplicated falciparum malaria in sub-Sahara Africa.[6, 8]. It has also been extensively studied in Nigeria.[9-11] Artesunate-amodiaquine (ASAQ) the second most widely used ACT globally after artemether-lumefantrine is also currently used as a first line treatment in some countries in sub-Saharan Africa.[12-14] However, ASAQ is disadvantaged by the variability in dosing regimens between the different formulations of ASAQ.[15, 16] Dihydroartemisinin-piperaquine (DP) is another ACT that has been recommended by the WHO for use in the treatment of acute uncomplicated malaria.[17] However, DP is not widely used in sub-Saharan Africa compared to AL as its recommendation by the WHO is more recent.[1] Dihydroartemisinin-piperaquine has the advantage of daily dosing and does not require taking it with meal. The long half-life of piperaquine confers on DP a longer post treatment chemoprophylaxis when compared with AL.[18]

There are limitations to the use of AL which is the commonly used ACT in Nigeria. These include high number of dosing (six) which is not convenient and the need to give the drug with fatty food for optimal absorption of lumefantrine because of its high lipophilic property.[19] Pyronaridine, a Mannich base 1-aza-acridine structurally related to mepacrine and amopyroquine, one of the earliest synthetic antimalarial drugs, was first developed in China over 30 years ago. It has been used as monotherapy in parts of Asia and China.[20] Pyronaridine, is a benzonaphthyridine derivative that is active against *Plasmodium falciparum* and *Plasmodium vivax.[21, 22]* [14]

Pyronaridine-Artesunate (PA) is a novel fixed dose ACT drug that received a Positive Opinion through Article 58 from the **European Medicine Agency[1] (EMA).** This Positive Opinion is a stringent regulatory review and approval granted for a specific group of drugs that will not be marketed in Europe. This rigorous review process is performed in conjunction with the WHO. At the time of the Positive Opinion (registration) PA also obtained WHO prequalification status. Pyramax received approval with EMA for the treatment of acute uncomplicated *P. falciparum* and *P. vivax* malaria. Subsequent EMA approvals have been granted for repeated dosing and for the paediatric formulation of granules in sachets. pyronaridine-artesunate has been found to have good efficacy and tolerability and is cost effective.[23-26] Like DP, pyronaridine-artesunate has a daily dosing regimen and can be taken with or without meals.[23] The studies conducted so far on the efficacy and tolerability of PA showed that other ACTs were not superior to it and there were strong recommendations that PA should be included as a standard antimalarial treatment.[27] The most commonly reported adverse effects were headache, eosinophilia, neutropenia, anaemia, increased platelet count, vomiting, abdominal pain, bradycardia, short lasting transaminase increases and hypoglycaemia.[16] Other studies reported vomiting which may be due to it bitter taste, nausea, loss of appetite and cough.[27] More recent studies provide increasing evidence on the safety of Pyramax.[28, 29]

1 RATIONALE FOR THE STUDY

The World Health Organization currently recommends the use of one of five different artemisinin-based combination therapies (ACTs) for the treatment of acute uncomplicated *falciparum* malaria [1]. Studies conducted on pyronaridine-artesunate (Pyramax™ Shin Poong) showed that it compared well in terms of efficacy and safety with other ACTs. EMA granted positive opinion through Article 58 to Pyramax tablet in 2013 and to Pyramax granules in 2015, this stringent regulatory authority recognition confirms that Pyramax can be used in malaria patients and, in particular, in African children. However, additional studies are needed to confirm, in parallel to the launch of Pyramax, the good safety and efficacy observed in the Phase III program and in the peri-approval (WANECAM) study. The WHO is however yet to make a statement on the unconditional inclusion of pyronaridine-artesunate in the treatment guidelines, which may not be unconnected with limited amount of data available for a new product. Hence the more the observations recorded across different malaria endemic countries and in real life scenarios the greater the degree of confidence on the safety of the drug. This will enhance the possibility of having a greater role in the management of uncomplicated malaria.

The need for a cost effective and user friendly antimalarial with good efficacy and tolerability is highly desirable to expand the number of ACTs in Nigeria and the need to continue efforts to optimize efficacy, tolerability, costs, and treatment regimens has led to this clinical trial. Whereas the therapeutic and efficacy studies are often used to select ACTs for programmatic deployment, the National Malaria Elimination Programme (NMEP) in Nigeria has restructured and streamlined the conduct of **Therapeutic Efficacy Studies (TES)** restricting each cycle to just about 3-4 sentinel cites per year. Under the current arrangement, Pyronaridine-artesunate will not be included in the country's 2018 TES. A TES efficacy study conducted that is well aligned with the national TES protocol will provide important opportunity for expanding the use of pyronaridine-artesunate in Nigeria.

It is also important to continue to assess the efficacy of AL, the ACT of choice in Nigeria in order to detect early loss of efficacy as the country is an endemic region for malaria. In the results of the WANECAM study, the efficacy PCR corrected (recrudescence of the initial infection) is the same between Pyramax and AL, while PCR uncorrected (new infection) is different and Pyramax is better than AL. In addition, the safety and efficacy of pyronaridine-artesunate in children less than 20kg shall be studied, especially in children under 1year of age which this study intends to cover.

2 OBJECTIVES

The aim of the study is to compare the safety and efficacy of Pyronaridine-Artesunate (PA) and Artemether-Lumefantrine (AL) in children aged between 3 months and 12 years with acute uncomplicated malaria in south-west Nigeria.

2.1 SPECIFIC OBJECTIVES

1. Primary:

To determine the comparative efficacy of pyronaridine-artesunate and artemether-lumefantrine in the treatment of acute uncomplicated malaria in children in south-west Nigeria.

2. Secondary:

i. To compare the efficacy and safety (crude – ACPR) of pyronaridine-artesunate and artemether-lumefantrine in the treatment of acute uncomplicated malaria in children in south-west Nigeria
ii. To determine and compare the parasite clearance time and the fever clearance time of pyronaridine-artesunate and artemether-lumefantrine in the treatment of acute uncomplicated malaria in children in south-west Nigeria
iii. To determine and compare gametocyte carriage between pyronaridine-artesunate and artemether-lumefantrine during treatment of acute uncomplicated malaria in children in south-west Nigeria
iv. To compare the PCR Corrected cure rates of pyronaridine-artesunate and artemether-lumefantrine in the treatment of acute uncomplicated malaria in children in south-west Nigeria

3 INVESTIGATION PLAN

3.1 Study Design

3.2 This is a randomized open label trial that will compare the safety and efficacy of PA and AL in patients of both sexes between the ages of 3 months and 10 years who have symptomatic microscopically confirmed acute uncomplicated *Plasmodium falciparum* malaria. Patients will be randomized following a pre-generated randomization code. On the basis of the results of these assessments, the patients will be classified as having therapeutic failure (early or late) or adequate clinical and parasitological response (ACPR). The proportion of patients experiencing therapeutic failure during the follow-up period will be used to estimate the efficacy of the study drug(s). The primary outcome will be PCR-adjusted adequate clinical and parasitological response (ACPR) i.e., absence of patent parasitaemia, regardless of axillary temperature and without evidence of previous treatment failure up to day 28. Secondary outcomes will be adequate clinical and parasitological response without correction for reinfection, parasite clearance time, fever clearance time and the proportions of patients who are aparasitaemic or apyretic on days1, 2, and 3.

3.3 Study Site

The study will be conducted in Oni Memorial Children's Hospital, Ring Road, Ibadan. Ibadan, the capital of Oyo state is a highly populated city located in the rain forest belt of south-western Nigeria. Malaria transmission is intense and occurs all year round in the study area. The total population of Oyo state is estimated to be about 5.5 million with about 3.8 million people in

Ibadan alone. Ibadan has a land mass of 28,245.26 km. Oni Memorial Hospital serves a mix of urban and rural populations as rural patients are referred to it, though more of its patients are from the urban populations.

3.4 Timing and Duration of Study

The study will be conducted during the high malaria transmission season, from April 2019 to September 2019.

3.5 Study Population

The study population will consist of patients with uncomplicated *P. falciparum* malaria attending the study hospital who are aged between 3 and 144 months of age.

3.6 Sample Size Calculation

$$n = \frac{\left(Z_{1-\alpha} + Z_{1-\beta}\right)^2 \left[P_1(1-P_1) + P_2(1-P_2)\right]}{\left[\varepsilon - (P_1 - P_2)\right]^2}$$

For Ref – Chow et al, 2008 (see attachment)

Where

$Z_{1-\alpha}$= standard normal deviate for level of significance; at α=0.05, it is 1.96

$Z_{1-\beta}$= standard normal deviate for power (1-β); at β=0.2 (80% power), it is 0.84 but at β=0.1 (90%), it is 1.28

P_1: 28-day cure rate in AL = 96.4%

P_2: 28-day cure rate in PA = 98.9%

ε: non-inferiority margin= 5%

Applying the formula and parameters above, sample size for each group

90% power: 70 per group [total 140]

80% power: 51 per group [total 102]

Assuming a difference of 2.5% in cure rate between PA and AL (98.9% vs 96.4%) [REF- Roth et al, 2018], 140 patients are required to be 90% sure that the upper limit of a one-sided 95% confidence interval will exclude a difference in favor of the standard group of more than 5%

Total N = 140. Allowing for 20% loss to follow up, the total number of patients required would be 168 to give 84 patients per arm. (See sample size calculation attachment)

3.6.1 Inclusion criteria

- Individuals of either gender between the ages of 3months (but weight ≥5 kg) and 12 years who present with symptoms compatible with acute uncomplicated malaria
- Minimum asexual parasite density of 1000/µl. This will be done at enrolment for all study participants.
- Fever with an axillary temperature≥ 37.5°C or history of fever within 24hours of presentation
- Residence within 15 kilometers to study site
- Ability to take drugs orally
- Absence of history of ACT intake in the two weeks prior to enrolment
- A signed informed consent from parents or guardian of prospective enrollee to participate in the study

3.6.2 Exclusion criteria

- History of allergy to study drugs i.e. artemisinins, lumefantrine and pyronaridine
- Any concurrent illness that could hamper evaluation of response e.g. bacterial infections, viral infections, severe gastrointestinal disease, malnutrition (weight for height <70%)
- Presence of clinical evidence of severe malaria such as prostration, inability to drink or breast feed, persistent vomiting, convulsion, severe anemia hemoglobin <5 g/dl), unarousable coma
- Patients with known chronic diseases like chronic kidney disease, chronic liver disease, malnutrition, cardiac failure, Sickle Cell haemoglobin (HbSS) etc.
- mixed or mono-infection with another Plasmodium species detected by microscopy;
- presence of severe malnutrition defined as a child aged between 6-60 months whose weight-for-high is below –3 z-score, or has symmetrical edema involving at least the feet or has a mid-upper arm circumference < 115 mm)
- Parent or guardian who in the judgment of the investigator will not comply with protocol in the opinion of the investigator

3.7 Sample Collection Procedure

Enrollees who have fulfilled the inclusion criteria would have the details of the study explained thoroughly to them. The parent or guardian will be required to sign an informed consent form once they agree to participate in the study. All enrollees will have a case record form (CRF). The CRF will be used to collect the following details.

1. Socio-demographic, clinical and laboratory details of all enrollees in the study. The CRF will also have the contact information of each enrollee as well as the past medical history.

2. Record details of each visit: These will include clinical details that would be assessed at each clinic visits, including physical examinations, blood film for malaria parasite, and hematocrit values for each enrollee. Any change in contact details or living situation would be noted.

For the enrollees, a medical history will be obtained from parent/guardian including presenting symptoms and current medications. Physical examination will be performed; weight and axillary temperature recorded. A finger prick blood sample will be obtained for thick blood smears and blood spots on filter paper for parasite genotyping. Five milliliters of venous blood sample will also be obtained for assessment of hematological and biochemical parameters.

Blood film for malaria parasite: Thick blood film will be prepared from finger prick sample of each enrollee at each contact time, stained with fresh 10% Giemsa stain at pH 7.2 and read under a light microscope at X1000 magnification. Gametocyte carriage will be specifically looked out for in the thick film on all contact days. Blood spots on filter paper will be obtained at enrollment and at follow up. This will be used for DNA analysis by polymerase chain reaction (PCR) to differentiate re-infection from recrudescence

Blood chemistry and hematology: 5mls of venous blood samples would be taken for clinical chemistry (electrolytes, urea and creatinine as well as a liver function test) evaluation at day 0 and on the follow up days.

Hematocrit measurement: This will be done by the micro-hematocrit method and the result recorded in percentage. The hematocrit will be done at enrolment and at each clinic visit.

3.7.1 Drug administration

Study participants will then be assigned into one of the two treatment groups according to a pre-generated randomization code. Drug treatment will be given in the clinic supervised.

Pyronaridine-artesunate:
Children randomized to group 1 will receive three doses of pyronaridine-artesunate granules or tablets (Pyramax®) manufactured by Shin Poong Pharmaceuticals depending on their body weights. Pyramax® granules comes in sachets with each containing 60mg of pyronaridine/20mg of artesunate while Pyramax tablets contain180mg pyronaridine/60mg artesunate. Pyramax dosing is once daily for three days. Dosing will be according to the chart below:

Body weight	No of sachets or Tablets	Regimen
5 - < 8kg	1 Sachet	Daily for 3 days
8 - < 15Kg	2 sachets	Daily for 3 days
15 - < 20 Kg	3 Sachets	Daily for 3 days
20 - < 24 Kg	1 Tablet	Daily for 3 days
24 - <45 Kg	2 Tablets	Daily for 3 days

Artemether-lumefantrine:

The study participants randomized to group 2 will receive the standard six-dose regimen of artemether-lumefantrine dispersible tablets (Coartem® Novartis pharma) twice daily according to body weights. Each dispersible tablet of Coartem® contains 20mg of artemether/120mg of lumefantrine) and patient will be dosed as follows

Body weight	**No of dispersible Tablets**
5 -<15 Kg	1 tablet
15 - < 25 Kg	2 tablets
25 - <35 Kg	3 Tablets
≥35 Kg	4 Tablets

5 -<15Kg one tablet, 15 - <25 Kg two tablets, 25 - <35 Kg three tablets, and ≥35 Kg four tablets at the following dosing intervals

- 0 hour - 1st dose;
- 8 hours – 2nd dose;
- 24 hours – 3rd dose;
- 36 hours – 4th dose;
- 48 hours – 5th dose
- 60 hours – 6th dose.

The first, third and fifth doses will be administered supervised in the clinic with water (very young children or a glass of milk while the second, fourth and sixth doses will be given by the care-giver at home 8 hours and 12 hours respectively after the first and subsequent morning doses. Mothers will be encouraged to breastfeed very young babies still on the breast soon after drug administration. Parent/guardian will be provided a sachet of milk after consultation on days 0, 1 and 2 for the 2nd, 4th and 6th doses of AL. Parents and care-givers will be also advised to give Coartem™ with a fatty meal e.g. fried plantain or bean porridge (local fatty meals) for the older children especially those who cannot tolerate milk.

Each enrollee will then be observed in the clinic for 1 hour after drug administration for vomiting. If vomiting occurs within 30 minutes of drug administration, the full treatment dose (PA or AL) will be re-administered. If vomiting occurred between 30-60 minutes of drug administration, half the treatment dose will be re-administered. Any enrollee who vomits the repeat dose will be withdrawn from the study and treated with another ACT.

No other antimalarial therapy will be allowed/given throughout the study period (28 days) except there is parasite recurrence. Paracetamol – an antipyretic drug will be administered to study participants with axillary temperatures ≥38.5°C at standard dosage.

3.7.2 Follow-up

Clinical and parasitological evaluation will be done on each day of follow-up or on any other day if the patient feels unwell. Patients will be reminded by phone calls 30minutes before the next dose of AL and on the day before the follow up visit (all enrollees i.e. PA and AL arms) and those who fail to attend follow up will be visited at home. Transportation fare will be given as incentives to the parents or care givers to encourage attendance for follow up. During each visit, a brief clinical history will be obtained to assess new complaints and possible side effects of medications and a physical examination will also be performed. Hematocrit and chemical analysis (bilirubin, blood glucose, creatinine and liver enzymes- AST, ALT & Alkaline Phosphatase) will be done on at enrolment and follow up on days 3, 7 and 28 to monitor possible changes.

Filter paper blood samples for parasite genotyping will be obtained on day 0, 3, 7, 14, 21 and 28 or earlier if enrollee is symptomatic. A finger prick blood sample will be taken to prepare thick blood smears on days 1, 2, 3, 7, 14, 21, 28 and on any other (unscheduled) visit. The slides will be air-dried away from direct sunlight, stained with fresh 10% Giemsa at pH 7.2 for 15 to 20 minutes and read independently by two microscopists. Parasite density (the number of asexual parasites per µl) will be calculated by counting parasites against about 200 leukocytes and assuming a leukocyte count of 8,000/µl of blood [36]. A smear will be considered negative only after screening at least 200 high power fields. However, a slide will be read against 500 leukocytes before it is declared negative. All discordant smears and slides with a parasite density difference >10% will be read by an independent microscopist and the PI. For quality control 5% of randomly selected slides will be read by an independent microscopist not involved in the study.

Paired samples (D0 and Day of recurrence) of blood spots on filter paper collected from patients with parasite recurrence on or before D28 will be analyzed using PCR techniques for parasite DNA.

Enrollee will be withdrawn from the study if the parent/care giver withdraws consent, enrollee develop serious concomitant illness or report taking other unauthorized anti-malarial medication apart from the study drugs during the follow-up period. Those who withdraw consent will be revolved back to the hospital care while those who develop serious concomitant illness would have immediate medical care given to them and thereafter be referred for specialist care at Oni memorial children's hospital or University College Hospital, Ibadan at no cost to them. Enrollees who fail treatment with either of the two ACTs being evaluated will receive the alternative ACT under supervision.

Daily checks and corrections will be carried out on the CRF of enrollees seen to ensure correctness and accuracy. Blood samples collected from study participants will be transported to the respective laboratories in the University College Hospital Ibadan for analysis of blood chemistry and hematology parameters the same day they are collected. Efficacy parameters will be evaluated as follows:

- **Efficacy evaluation** will be based on WHO criteria of treatment outcome as follows; (1) Early treatment failure (ETF; danger signs or complicated malaria or failure to adequately respond to therapy on days 0–3); (2) Late clinical failure (LCF; danger signs or complicated malaria or fever and parasitemia on days 4–28 without previously meeting criteria for ETF or LPF); (3) Late parasitological failure (LPF; asymptomatic parasitemia on days 7–28 without previously meeting criteria for ETF or LCF); (4) Adequate clinical and parasitological response (ACPR; absence of parasitemia on day 28 without previously meeting criteria for ETF, LCF, or LPF).
- **Recrudescence** will be defined as the recurrence of asexual parasitemia within 28 days following antimalarial treatment comprising the same genotypes that caused the original illness. This is believed to result from incomplete clearance of asexual parasitemia because of inadequate or ineffective treatment.
- **Parasite clearance time**: time from first dose of ACT until first total and continued disappearance of asexual parasite forms.
- **Fever clearance time**: time from first dose until the first time the body temperature (for those with a raised temperature at enrolment) decrease to below 37.5^{o}C and remain so for at least 24 hours.
- **Gametocyte carriage**: proportions of patients with gametocyte at a given point in time using the Area Under the Curve Graph for PA will be compared with that of AL.

3.7.3 Safety parameters will be evaluated as follows:

- Safety will be analyzed in terms of adverse events and severe adverse events using clinical signs, symptoms and laboratory parameters.
- Liver enzymes (ALT/AST) >3 times upper limit of normal (ULN) plus peak total bilirubin >2 times ULN in the absence of a significant alkaline phosphatase increase (= Hy's law) will be deemed as serious adverse effects (SAE).
- ALT and AST >5 times ULN will be deemed an AESI. (see below)

Serious Adverse Events

An adverse event will be considered serious is it fulfils one of the following criteria:

- causes death or
- is life-threatening or necessitates hospitalization or prolongs hospitalization or results in persistent or significant disability/incapacity or is a medically important event constitutes a possible Hy’s Law

An example of an important medical event is one that while not-immediately life-threatening might become so if there had been no intervention

In case of SAEs, independent of causality, the healthcare personnel must immediately contact the PI / Investigator for validation of the seriousness and determination of the causality.

Subsequently, the procedure described below must be followed:

- SEND (within 24 hours and pdf scanned documents) the signed and dated copy of the "Adverse Event form" and the form "SAE complementary information" to Shin Poong Pharmacovigilance (PV) (Tel/Fax +44 203 291 3032 or email: safety@artemidapharma.com)
- Contact immediately (the same day) the medical monitor/Pyramax QPPV responsible for safety in case of death or life-threatening events.
- The follow-up of each fatal or life-threatening AE must be provided to the medical monitor and Shin Poong PV within the same timeline as the initial report (within 24 hours and preferably by email).
- ATTACH to the Case Record Form (CRF) the photocopy of all available results and examinations which were undertaken (and their date). Analysis results must be accompanied by the laboratory normal ranges. Special consideration shall be taken to ensure patient anonymity, and to the correct completion of the patient's study specific identifier in the copies of the source documents provided to the sponsor.

Causality

All adverse events must have causality assigned by the investigator. As a guide causality can be assigned as follows:

- Not related
    - The Investigator has determined that the adverse event is not related to the study medication
    - The current state of knowledge indicates that a relationship to the use of study medication is unlikely
- Related
    - The Investigator has determined that the event has a reasonable relationship to the use of the study medication
    - The Investigator has determined that the complication is more than likely related to the study medication

3.7.4 Adverse event of special interest

An adverse event of special interest (AESI) is an adverse event for which on-going monitoring is appropriate within the context of the study. These events necessitate complementary examinations in order to characterize and understand them.

AESIs in this study can be related to:

Hepatotoxicity
Presenting with fatigue, nausea, abdominal pain itching or signs of jaundice such as dark urine, putty or mastic colored stools, jaundice
And/or
ALT or AST >5 x **Upper Limit of Normal (ULN)** (Patients with normal baseline LFTS)
Or ALT or AST >3 x ULN and total bilirubin >2 x ULN (Potential Hy's Law)

Or ALT or AST >2 x the baseline value if >2 x ULN at baseline
If these occur then a hepatitis panel should be performed

- Hypersensitivity
    - Flushing, appearance of wheals/urticaria, breathlessness, Faintness and/or fall in blood pressure occurring soon after Pyramax administration

The study team, as well as the relevant referral facilities, should be trained to take particular notice of symptoms/signs suggestive of the AESIs in this study.

In case of an AESI confirmed by the study physician, Shin Poong PV shall be informed within 24 hours by fax or email using the contact details above, even if the event does not satisfy any condition of seriousness. Notification will occur through the use of an AESI form.

Note that AESIs should only be reported as a serious adverse event if they fulfill the criteria for seriousness.

3.7.5 Follow-up of Adverse Events

The healthcare personnel must take all appropriate measures to protect the safety of the patients. Personnel must ensure to document follow-up of the evolution of each adverse event (clinical, biological or other) until resolution or until the stabilization of the patient's status.

All new relevant information concerning the initial SAE shall be recorded on a form "SAE follow-up information form" by the nursing staff of the health centre, and shall be validated by the PI/co-PI who shall transfer the form to the local person/physician responsible for the pharmacovigilance of the project and Shin Poong PV.

In case of a serious adverse event the patient must be followed until complete resolution and normalization of all analysis results, or until chronicity of the patient's status. This can imply that the follow-up of the patient must continue beyond the period of follow-up per protocol, and that additional investigations could be requested.

DATA ANALYSIS.

Data will be entered into the computer using the Epi-data software. This subsequently would be cleaned and transferred to SPSS Statistic Software version 20 for analysis. The outcome of interest (dependent variable) presented as a categorical variable with, presence of malarial parasitemia, and the level of efficacy reported. The independent or explanatory variable of interest in this study would be use of chemotherapy. All independent variable would be reported as categorical. Proportions of treatment outcome will be compared by using chi square. Normally distributed continuous data will be compared using unpaired student t-test. Mean values will be given as mean ± SD and values of $P < 0.05$ will be considered statistically significant.

ETHICAL CONSIDERATIONS

This study, will be a prospective randomized open label clinical trial comparing the safety and efficacy of Pyronaridine-artesunate and Artemether-lumefantrine in the treatment of symptomatic acute uncomplicated malaria in Nigerian children aged 3 to 144 months who seek treatment in the study hospital. The studies will be conducted according to Good Clinical Practice and the Declaration of Helsinki.

Confidentiality of data: Personal details of enrollees in the study will be treated with utmost confidentiality, as it is against the principles of good clinical practice to make such information public. Also, codes will be assigned to all participants.

Translation of the informed consent to the local language: The informed consent has been translated to Yoruba (the local language) and is attached. Translation will has been verified for consistency and accuracy by an independent translator. The consent form will include the purpose of the study, risks and benefits to participants, confidentiality and voluntariness.

Beneficence to Enrollees: Enrollees in this study will have the advantage of been treated for malaria and any related febrile illnesses with no cost. Also, findings in the study will be useful in monitoring artemisinin resistance in Nigeria.

Non-maleficence to Enrollees: There is little or no risk involved in this study. Blood samples that will be collected from the prospective arm of the study will be via venipuncture technique which will be done by a qualified medical officer. The participant will only feel a mild pain and there may be a bruise which will go away in a few days.

Voluntariness: Enrollees in the study are absolutely free to decline or withdraw from the study without jeopardy and without loss of benefits to which they are entitled.

Summary of Activities

ACTIVITY	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Extra Visit	Extra Visit
Demographic data and clinical assessment	X	X	X	X	X	X	X	X	X	X
Blood smear – (Thick & Thin)	X	X	X	X	X	X	X	X	X	X
Blood spot on filter paper (for PCR)	X	X	X	X	X	X	X	X	X	X
Haematocrit	X	X	X	X	X	X	X	X	X	X
Full blood count	X	~~X~~	~~X~~	X	X			X		
Blood Chemistry – Creatinine & Random Blood glucose	X			X	X			X		
Liver Function test- AST, ALT, Bilirubin & Alkaline Phosphate	X			X	X			X		
Adverse events recording	X	X	X	X	X	X	X	X		
Concomitant treatments recording	X	X	X	X	X	X	X	X	X	X
Study medications (AL or PA)	X	X	X							

INFORMED CONSENT FORM

IRB Research approval number ----------

This approval will lapse on ----------

Title: **COMPARATIVE SAFETY AND EFFICACY OF PYRONARIDINE-ARTESUNATE VERSUS ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN SOUTH-WEST NIGERIA.**

***NAME AND AFFILIATION OF RESEARCHER*:**

This study is being conducted by Professor Catherine Falade of the Institute for Advanced Medical Research & Training, College of Medicine, University of Ibadan.

PURPOSE OF RESEARCH: The purpose of the research is to determine the safety and how well two important drugs named Pyronaridine-artesunate (PA) and artemether-lumefantrine (AL) work when they are given to children suffering from simple malaria.

***PROCEDURE OF THE RESEARCH*:** Malaria is a parasitic infection and an important cause of ill health in the world especially in tropical regions such as Nigeria. Children are particularly affected by malaria which can very easily become serious sometimes leading to hospitalization and even death. Illness and death from malaria has reduced in the last ten years because of the use of very effective antimalarial drugs called artemisinin-based combination therapy (ACT) and the use of protective measures such as insecticide treated nets (ITN).

AL is the first ACT approved by the World Health Organization and also the drug recommended in the Nigerian National Malaria Treatment Guidelines while PA is a newer ACT recently approved for the treatment of malaria by the WHO. During this study we will treat children aged between 3 and 144 months who have confirmed malaria with AL or PA. This is to allow us to determine the safety and efficacy of PA compared with AL. The results will also provide an evidence for use of another ACT for treatment of malaria among Nigerian children.

I am asking for your permission to allow your child to take part in this study because your child has malaria. Your child will be examined thoroughly by a doctor. A few drops of blood will be obtained by finger prick. This will be used to prepare blood smear on glass slides for malaria parasite, blood spots on filter paper for advanced biology studies and into a thin glass tubes to measure how much blood the child has. About a teaspoon of blood will be taken from an arm vein to assess the state of the liver and kidneys on the day of enrolment, on Days 3, 7 and 28 days after starting treatment to be sure the drug has no effects on the kidneys and liver of your child.

Your children will be treated with PA or AL given at the clinic daily for 3 days. The treatment of choice will be administered supervised. He/she will be will be expected to come to the clinic for follow up visit daily on days 7,14, 21 and 28. At every visit, you will be asked to tell us about the progress of your child's health and any unusual symptoms you have noticed. Your child will also be examined properly and a finger prick for blood tests as previously done. This is to find out how rapidly the parasites are clearing from the blood and also to check other parameters about your child.

EXPECTED DURATION OF RESEARCH: The study will run for 28 days for each child and you are expected to bring your child all through this period. It is important that they are brought to the clinic on these scheduled days and if you are not able to return on these days please let us know. The regular follow up visit will allow us to monitor the progress of your child and also to treat them if they are unwell at any time during the study. Do not hesitate to bring your child back to the clinic at any time if you have concerns.

RISK: This study to the best of our knowledge will not expose your child unnecessarily to any danger. However, the child may experience slight pain and bruises from the needle prick and venepuncture. This pain will go away in a few days. There is also a possibility for the enzymes from the liver to rise temporarily. Usually this will correct in a few days.

COST TO PARTICIPANTS**:** All tests as well as treatment will not cost you any money. The cost of transportation to and from the hospital will also be refunded at each follow up visit.

BENEFIT: Your child will be evaluated, investigated and treated very closely. The data will be used in scientific publications and also for improving the healthcare of the children with malaria.

CONFIDENTIALITY: Data collected from this study will be treated with strict confidentiality. Your name will not be made public by the investigators and only authorized research staff will have access to your child's details.

VOLUNTARINESS: Taking part in this study is voluntary. You are free not to participate in this study.

ALTERNATIVES TO PARTICIPATION**:** If you choose not to allow your child participate in this study, the child will receive care from health care workers in the study center.

CONSEQUENCES OF WITHDRAWAL AND TERMINATION OF PARTICIPATION**:** You have the right to withdraw from the study at any time if you so wish without any repercussion. However, it may not be possible to remove all information and test results obtained before withdrawal. After withdrawal from the study, your child will continue to receive care from the study hospital (Oni memorial children's hospital).

If your child comes with or develop other serious illnesses or fails to respond to treatment given during this study, your child will be given an alternative drug. If your child develops a side effect or another illness, he/she will be given immediate medical care and referred to the appropriate specialist at Oni memorial hospital or UCH, Ibadan for further care.

IN CASE OF INJURY OR ADVERSE EFFECTS: All procedures in this study are not harmful and we will not involve your child in any harmful practices. In case of adverse events from the medications, your child will be treated at UCH at no cost to you.

AFTER THE RESEARCH, WHAT HAPPENS? After the study you may have access to information about your child's health status.

PYRONARIDINE-ARTESUNATE VERSUS ARTEMETHER-LUMEFANTRINE STUDY **Study ID- PAAL**: ………….

STATEMENT OF PERSON TAKING INFORMED CONSENT:

Do you have any questions?

I have fully explained this research to -- and have given sufficient information including the risks and benefits to make an informed consent.

STATEMENT OF PERSON GIVING INFORMED CONSENT:

I have read the above information or it has been read to me and I understand the contents. I have also asked questions about the study and my questions have been answered to my satisfaction.

I…………………………………………………of ----------------------…………………hereby consent

that my child/ward -------------------------------study ID----------- participate in this study.

I understand that I have the right to withdraw my child/ward from the study at any time without it affecting his/her medical care in any way.

-     -

Parents Name/signature, date   Investigators Name/signature, date

- -

Witness Name and signature & date

In case of emergency, please contact **Professor Catherine O. Falade Institute for Advance Medical Research and Training, College of Medicine, Ibadan**. Telephone 08033264593, E-mail – lillyfunke@yahoo.com

OR

Dr. Fiyinfoluwa Olusola, Institute for Advance Medical Research and Training, College of Medicine, UCH Ibadan. Telephone - 08099471018. Email – fiyinesan@gmail,com.

CASE RECORD FORM

COMPARATIVE EFFICACY AND SAFETY OF PYRONARIDINE-ARTESUNATE VERSUS ARTEMETHER-LUMEFANTRINE IN THE TREATMENT OF MALRIA AMONG CHILDREN IN SOUTH-WEST NIGERIA

PYRONARIDINE-ARTESUNATE VERSUS ARTEMETHER-LUMEFANTRINE STUDY

Study ID- PAAL: …….

Study ID: ……. Hosp.No: …… Date of enrollment: **……**

Sex**: ……..** Age (months): …….

Date of birth ……/……/……/ Weight (kg): …… Height (CM): ……

Temp (oC): …….. Phone no: …….....

Address (+ brief description):
……..……
……....……
……

Symptoms History

Record duration of symptoms in days (Today = 1) Fever: ( ) Vomiting: ( ) Chills & rigors ( )

Diarrhoea: ( ) Headache: ( ) Abdominal pains: ( ) Irritability: ( ) Loss of appetite ( )

Other complaint 1: ……. Other complaint 2: ……

Past medical history:

History of drug allergy: (Yes/No) If yes, name of drug: …….

Any other relevant history 1: ……

Any other relevant history 2: ……..

Treatment history: Has the child received any of the following drugs in the last 2 weeks (Yes/No)

CQ ( ) SP ( ) PCM ( ) ACT ( ) Specify.....

Antibiotic ( ) Specify..... Other: ……..

Clinical examination:

General: Palor (Yes/No) Icterus (Yes/No) Fever (Yes/No) Dehydration (Yes/No)

CVS: Pulse/min……..……. Apex Beat……..…….....Heart sounds…….....

Respiratory system: Resp. rate……Breath sound…….....

Abdomen: Liver size (Below costal margin)…… Spleen size…….. Kidneys……..

Muscle tone: (1) Normal (2) Hypotonia (3) hypertonia Eyes: conjuctivitis/Discharge (Yes/No)

Ears: Discharge (Yes/No)

MP result: …….. PCV.....

Study ID ----------

Study Drug Schedule

Study Day	**Date**	**Drug [PA or AL] Name of Drug …….**	**Dose**	**Time Drug Given**
Day 0			1st	
			2nd	
Day 1			1st	
			2nd	
Day 2			1st	
			2nd	

Comments:
Any vomiting? Yes/No
Time ……

Any Redosing?
Time ……..
Any vomiting? Yes/No

Any other comments

PYRONARIDINE-ARTESUNATE VERSUS ARTEMETHER-LUMEFANTRINE STUDY **Study ID- PAAL**: …….

CLINICAL FOLLOW UP RECORD

	D0	**D1**	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**	**Day 35**	**Day 42**	**Other visit**
Date											
Temp (°C)											
Pallor											
Jaundice											
HR/Pulse Rate											
Resp Rate											
Lung Fields											
Liver (cm)											
Spleen (cm)											
Weight (kg)											
PCV (%)											
Parasite Density/µL											
Gametocyte Count/ µL											

TREATMENT EMERGENT SIGNS AND SYMPTOMS (TESS)

Features	**Day 0**		**Day 1**		**Day 2**		**Day 3**		**Day 7**		**Day 14**		**Day 21**		**Day 28**		**Day 35**		**Day 42**		**Extra Visit**		**Extra Visit**	
	P	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**
Date																								
Chills/Rigors																								
Fever																								
Anorexia																								
Nausea																								
Vomiting																								
Diarrhoea																								
Abd. Pains																								
Cough																								
Palpitation																								
Insomnia																								

Present (P): 0 = Not present, 1= Present, 2 = Not evaluable Severity (S): 1 = Mild 2 = Moderate 3 = Severe

TREATMENT EMERGENT SIGNS AND SYMPTOMS (TESS)

Features	Day 0 P	S	Day 1 P	S	Day 2 P	S	Day 3 P	S	Day 7 P	S	Day 14 P	S	Day 21 P	S	Day 28 P	S	EXTRA VISIT	
Date																		
Pallor																		
Yellow eyes																		
Headaches																		
Rash																		
Reflex																		
Diplopia																		
Fatigue																		
Other																		
Other																		

Present (P): 0 = Not present, 1= Present, 2 = Not evaluable Severity (S): 1 = Mild 2 = Moderate 3 = Severe

LABORATORY RESULTS: LFT & BLOOD CHEMISTRY

PARAMETER	DAY 0	DAY 3	DAY 7	DAY 27	DAY 35	DAY 42
Date						
Urea						
Creatinine						
Random Blood sugar						
Total bilirubin						
Conjugated bilirubin						
ALT						
AST						
ALP						
GGT						

<u>LABORATORY RESULTS: HAEMATOLOGY</u>

PARAMETERS	**D0**	**DAY 3**	**DAY7**	**D28**	**EXTRA VISIT**	**EXTRA VISIT**
White blood cell count						
Eosinophils						
Platelet count						
Neutrophils						
Basophils						
Lymphocytes						

ADVERSE EVENTS RECORD

ADVERSE EVENT	**DAY 0**	**DAY 3**	**DAY 7**	**DAY 28**	**EXTRA VISIT**	**EXTRA VISIT**

SUMMARY: EFFICACY

: D14 [ ], D21 [ ], D28 [ ]. {ETF = 1, LPF = 2, LCF = 3, ACPR = 4, Withdrawn = 5, Lost to follow up = 6}

Treatment outcome	**ETF**	**LPF**	**LCF**	**ACPR**	**WITHDRAWN**	**LOST TO FOLLOW UP**
Day 7						
Day 14						
Day 21						
Day 28						
Day 35						
Day 42						
Extra visit (Date----------)						
Extra visit (Date----------)						

Enrollee's Initials ……..

STUDY OUTCOME

1. **COMPLETED**
2. **LOST TO FOLLOW UP**
3. **WITHDRAL OF INFORMED CONSENT**
4. **PROTOCOL VIOLATION**

STUDY CONCLUSION

1. **ACPR**
2. **LPT**
3. **LCF**
4. **ETF**

Investigator - Name ……

Signature & Date ……

Principal investigator - Name ……

Signature & Date ……

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TABLE OF NORMAL VALUES – IBADAN, SOUTHWEST NIGERIA

HAEMATOLOGY

PARAMETR	NORMAL VALUE	UNIT OF MEASUREMENT
Red Blood Cell Counts		
Male	4.0 - 6.5	x 10^{12}/l
Female	3.5 - 5.5	x 10^{12}/l
Hemoglobin		
Male	130 – 180	g/l
Female	115 - 165	g/l
Packed Cell Volume		
Male	39 – 54	%
Female	36 - 46.	%
Mean Cell Volume	80 - 100	Fl
Mean Cell Hemoglobin (MCH)	27 -32	P/g
Mean Cell Hemoglobin Concentration (MCHC)	300 – 350	g/l
White blood cell count (WBC)	150 - 350	x 10^{9}/l
Platelet count	90 – 300	x 10^{9}/l

LIVER FUNCTION TEST

PARAMETER	NORMAL RANGE	UNIT OF MEASUREMENT
Total Bilirubin	0.2 -1.0	mg/dl
Direct Bilirubin	0 – 0.4	mg/dl
Alkaline phosphatase (ALP)	35 - 105	IU/L
Aspartate transaminase (AST)	0 - 37	IU/L
Alanine transaminase (ALT)	0 - 40	IU/L
Gamma glutamate transaminase (GGT)	7 - 50	IU/L
Total protein	3.5 -8.0	g/dl
Albumin	3.2 – 5.7	g/dl

PLASMA GLUCOSE

PARAMETER	NORMAL RANGE	UNIT OF MEASUREMENT
Fasting plasma glucose	65 - 110	mg/dl
2 Hours Post prandial plasma glucose	60 -140	mg/dl

FASTING LIPID PROFILE

PARAMETER	NORMAL RANGE	UNIT OF MEASUREMENT
Total cholesterol	<200	mg/dl
Triglyceride	0 - 200	mg/dl
HDL – Cholesterol	35 - 86	mg/dl
LDL - Cholesterol	80 - 120	mg/dl

ELECTROLYTE & UREA

PARAMETER	NORMAL RANGE	UNIT OF MEASUREMENT
Sodium	130 -145	mmol/L
Potassium	3.5 – 5.0	mmol/L
Chloride	95 – 110	mmol/L
Bicarbonate	20 - 30	mmol/L
Urea	15 - 45	mg/dl
Creatinine	0.5 – 1.5	mg/dl