A clinical phase III comparative, randomised, clinical trial to assess the safety and efficacy of a fixed dose of oral pyronaridine-artesunate granule formulation (60:20) (paediatric Pyramax®) versus artemether-lumefantrine (Coartem®) crushed tablets in infants and children with acute uncomplicated *Plasmodium falciparum* malaria in Lambaréné, Gabon

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2 Introduction

Worldwide, 3.3 billion people are at risk for malaria in 97 countries and territories and more than one third of those are at high risk of infection (>1 in 100 chance to get malaria in a year) [1]. According to the latest estimates,198 million cases of malaria occurred globally in 2013 (uncertainty range 124–283 millions), and the infection led to 584 000 deaths (uncertainty range 367 000-755 000) [1]. Malaria is due to human infection of Plasmodium sp. protozoan; nowadays species infective to humans consist in *P. falciparum*, *P vivax*, *P.malariae*, *P. knowlesii* and the newly proposed species of *P. ovale curtisi* (former classic type) and *P. ovale wallikeri* (former variant type) [2-4]. Most cases of malaria occur in Africa (80%) where the majority of infections are caused by *P. falciparum*, the most virulent of all the human malaria parasites. Moreover, of the global total of malaria deaths in 2013, 90% were estimated to occur in the Sub-Saharan Africa and 78% of the total deaths occurred in children under 5 years of age in the region [1]. Thereby it is not surprising that malaria nowadays is considered as the fifth global greatest cause of death in under 5 years old [5, 6].

Whilst malaria incidence and mortality has decreased in the recent years due to multidisciplinary and globally coordinated interventions [1], the spread of drug-resistant parasites is still one of the most important points at issue in malaria control strategies [7]. Former commonly used antimalarials, such as most aminoquinolines, have become increasingly ineffective, leading to an urgent need for new treatment options [8]. Children and pregnant women are inherently vulnerable to malaria infection and the need of treatment is of high importance in the fight against the disease in this populations. Presently, artemisinin-based combination therapy (ACT) is recommended by the WHO Global Malaria Program as the first-line treatment for malaria [8, 9], and there is a need for new regimens that are affordable, convenient, effective and safe. Yet appropriate drug formulation is an essential factor for the success of the strategy of artemisinin-based combination therapy in children and oral paediatric formulations are urgently needed.

2.1 Antimalarial Combination Therapy

Antimalarial combination therapy is defined as “the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and thus unrelated biochemical targets in the parasite” [8]. The principal assumption of combination therapy is that “the probability of resistance developing simultaneously to two chemotherapeutic agents with independent mechanisms of action is extremely low”, of the order of once in 10^{12} treatments [10]. This frequency is the product of the probabilities of the independent acquisition of a resistant mutation to each drug multiplied by the number of parasites in a typical infection, therefore reducing -at least in theory- the likelihood of resistant parasites being selected and transmitted.

Antimalarial combination therapies can be distinguished in two main groups: the artemisinin combination therapies (ACT) and the non-artemisinin based combinations (non-ACT). Currently available non-artemisinin based combination regimens include sulfadoxine-pyrimethamine, chloroquine plus sulfadoxine-pyrimethamine, quinine plus sulfadoxine-pyrimethamine, amodiaquine plus sulfadoxine-pyrimethamine, mefloquine plus sulfadoxine-pyrimethamine, quinine plus tetracycline-doxycicline, quinine plus clindamycin and atovaquone-proguanil. Chlorproguanil-dapsone has been withdrawn following increased evidence of haematological toxicity [11]. Quinine-sulfadoxine-pyrimethamine, amodiaquine-sulfadoxine-pyrimethamine and mefloquine plus sulfadoxine-pyrimethamine are regarded to have acceptable levels of efficacy while desirable efficacy has been shown for quinine-tetracycline, quinine-clindamycin, and atovaquone-proguanil [12]. However, effectiveness is sub-optimal for all treatments necessitating repeated doses over more than 3 days. The prevailing high levels of resistance to chloroquine and amodiaquine have compromised the efficacy of these two drugs even in combinations. There is no evidence that the combination of chloroquine plus sulfadoxine-pyrimethamine gives any additional gain over sulfadoxine-pyrimethamine alone, thus the combination is not recommended anymore; sulfadoxine-pyrimethamine plus amodiaquine has been observed to be more effective than either drug alone though the efficacy of this combination

is actually inferior to ACT so it is not recommended anymore for the treatment of *P. falciparum* malaria [13].

The effect of combination therapy is thus improved by including an artemisinin derivative. Parasite density is diminished more rapidly by artemisinin-based antimalarials than by any other antimalarial drug [14]. The period of parasite exposure to sub-therapeutic blood levels is minimized when artemisinin derivatives are used alone due to their short half-life. When combined with drugs with longer half-life, the rapid parasite clearance time of artemisinin derivatives and their short half-life imply that exposure to the partner drug after the artemisinin component is eliminated attains many fewer parasites. Moreover, parasite exposure to the companion drug occurs when blood levels are close to the maximum [10]. An additional benefit for treated patients with artemisinin combinations is the 90% reduction of gametocyte density [15]. Taken together, all these characteristics minimize the probability of a resistant mutant surviving antimalarial therapy and could thus reduce overall malaria transmission rates.

There are two additional reasons for the concept of combining antimalarials: to increase efficacy and to shorten duration of treatment. The enhancement of therapeutic efficacy has been a major issue since drug resistant strains of *P. falciparum* have rendered many former antimalarial monotherapeutic regimens ineffective. Simultaneous administration of two independent drugs has proven to overcome this decreased efficacy of monotherapy by acting on different biochemical targets. Finally, the potential of drug combinations to shorten duration of treatment has been shown in a number of clinical trials. A further advantage thereby is that patients' compliance is improved with shorter treatment courses, which is again related to effectiveness and development of drug resistance.

Artemisinins should not be used as a single agent, to prevent emergence of drug resistance and to avoid the need for prolonged therapy [9]. Artemisinin-based combination therapy combines the highly effective short-acting artemisinins with a longer-acting partner to protect against artemisinin resistance and to facilitate dosing convenience. ACTs are typically administered

for three days and have been recently developed mainly in fixed-dose tablets. Besides pyronaridine-artesunate, five ACTs have been recommended by the WHO for the treatment of uncomplicated malaria: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulfadoxine-pyrimethamine, and dihydroartemisinin-piperaquine [13]. Artemether-lumefantrine appears to be the most widely adopted ACT in Africa, followed by artesunate-amodiaquine [16]. Though the different artemisinin derivatives show some minor differences regarding oral absorption and bioavailability, these differences are not clinically significant within currently available formulations. The efficacy of the ACT is therefore mostly determined by the proprieties of the partner medicine and these proprieties are considered for the choice of combination. As resistance to the artemisinins' partner medicines may compromises the efficacy of the ACT [8], the proprieties of the partner drug of the artemisinin derivative in ACTs such as resistance and tolerability may affect choice. Artesunate- mefloquine, artemether-lumefantrine or dihydroartemisinin-piperaquine have the highest cure rates in many countries [17-21]. The use of artesunate-mefloquine in African children was subsequently restricted due to excessive vomiting associated with the recommended dose (25 mg/kg) of the partner drug. However, further evaluation showed that the tolerability to artesunate-mefloquine in children weighing between 10-20 kg was as good as with artemether-lumefantrine [18]. In some African regions, artesunate-amodiaquine or sulfadoxine-pyrimethamine are still considered effective options due to the low levels of resistance to amodiaquine and sulfadoxine-pyrimethamine. However, these two drugs remain widely available as monotherapy and despite the deployment of corresponding combination with artemisinin derivatives, resistance to amodiaquine and sulfadoxine-pyrimethamine will probably continue to worsen due to continue selection pressure.

A key to many limitations associated with artemisinin-based combination therapy seems to be the pharmacokinetic mismatch of the partner drugs [22]. A pharmacokinetic mismatch can also be a major factor contributing to resistance of the long-acting partner drug, which in the later stages of its presence in the blood stream is not protected by the short-acting artemisinins. This is not a

problem as long as both drugs are fully efficacious on their own and as long as the drug levels of both drugs remain above the minimum inhibitory concentrations until all asexual parasites have been cleared. However, with a reasonable duration of drug administration, short half-life drugs will not be able to cover the minimum duration of drug exposure. At the same time, long half-life drugs will inevitably result in drug levels of the partner drugs below the minimum inhibitory concentrations and without protection from the artemisinin compound. This particularly applies to the use of artemisinin-based combination therapy in high transmission areas [23], as widespread use of such combinations would result in exposure of parasites to low doses of those drugs in case of re-infection after treatment.

2.2 Drug Formulation of Combination Treatment Regimen

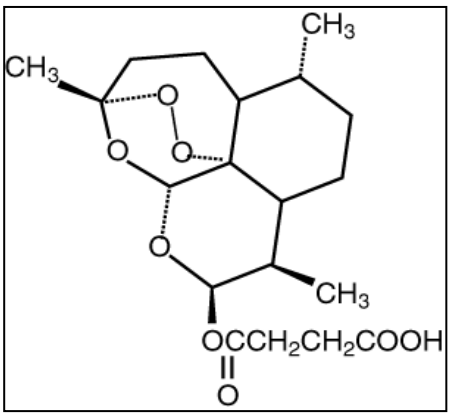
Besides effectiveness, safety, tolerability and accessibility of antimalarials, adherence to treatment has been characterized as a major determinant for successful treatment of malaria, especially when dealing with rural populations in developing countries [13]. In general, compliance with sequential combination regimen of antimalarial drugs tends to be problematic with patients unwilling to take antimalarials after clinical improvement. This is a particular danger for artemisinin-containing combinations, as they resolve clinical symptoms such as fever quicker than all other antimalarials. This relief of symptoms might easily be interpreted as cure, making patients reluctant to continue treatments as prescribed, which may quickly lead to recrudescent parasitaemia. Therefore, a potential problem of treating patients with more than one antimalarial drug is the fact that many currently available combination therapies are not co-formulated, greatly increasing complexity of treatment and the chances of misuse. In order to reduce the potential selective pressure of drugs used as monotherapy and to improve adherence to treatment, fixed-dose combination formulations are strongly advised and preferable over blistered loose tablets or even co-packaged combinations [9, 13]. Fixed-dose combinations are now available for the following recommended artemisinin-based combinations: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and dihydroartemisinin–piperaquine. However, the widely used co-formulation artemether–lumefantrine remains a relatively complex regimen (with a children dose of two tablets twice daily for 3 days) and compliance, and therefore programmatic effectiveness, is not optimal [24]. Yet artemether-lumefantrine dispersible tablets, artesunate-amodiaquine water-soluble tablets and dihydroartemisinin-piperaquine liquid formulation are the only drugs available for oral liquid administration for children included on the list of prequalified medicinal products of the WHO [25]. Fixed-dose combination formulations of artemisinin-based combinations are technically difficult, and therefore it is essential that any new combination is shown to have adequate ingredient compatibility, optimal stability, and similar oral absorption rate and bioavailability

to the loose or co-blistered tablets. Certainly, there is a need of new ACT formulations specifically developed for use in pediatric populations.

2.3 The Combination of Artesunate and Pyronaridine

2.3.1 Artesunate

The artemisinins are derived from the leaves of the Chinese sweet wormwood plant, *Artemisia annua*. They have been used in China by herbal medicine practitioners for many centuries as a treatment of fever and malaria [26] and came to attention outside this country in the 1970s and 1980s [27].



Artesunate [Public domain]
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Artemisinin containing endoperoxide, originally known as Qinghaosu (青蒿素) was firstly isolated in 1972 by Chinese scientists [28]. Since then and due to the emergence of *P. falciparum* resistance to commonly used antimalarials, artemisinin derivatives have become widely used. Nowadays they are the cornerstone of nearly all new combination therapies. Even in multidrug resistant *P. falciparum* malaria, artemisinin derivatives show remarkably high activity such as rapid resolution of fever and parasitaemia [29, 30] .

The mechanism of action of artemisinins seems to involve iron-binding and breaking down peroxide bridges leading to the generation of free radicals that damage parasite protein, being the SERCA-type PfATPase6, a sarco-/endoplasmatic reticulum Ca^{++}-ATPase the proposed specific target of the compound [31]. They act rapidly, killing blood stages of all Plasmodium species and reducing the parasite biomass [14]. Artemisinins have the fastest parasite clearance times of any antimalarial [32] and are most effective against late ring to early trophozoite stages. Artemisinins are active against gametocytes and their use has been associated with reduced malaria transmission [33]. Currently, artemisinin derivatives used in clinical practice include artemether, dihydroartemisinin and artesunate [13].

Artesunate is a water-soluble hemisuccinate ester of the original sesquiterpene lactone artemisinin. It can be administered orally, rectal, intravenously or

intramuscularly as artesunic acid. As for all artemisinin derivatives, dihydroartemisinin (DHA) appears to be the principal active metabolite of artesunate *in vivo* [34]. Biotransformation of artesunate to dihydroartemisinin is very rapid, yet the degree at which artesunate is transformed varies considerably [35, 36]. Hydroxylation of artesunate to dihydroartemisinin is pH dependent and seems to occur already in the gastrointestinal tract [37] , which means that the drug is partly absorbed in form of its metabolite. In the blood, hydrolysis of artesunate is catalysed by esterases [38]. Dihydroartemisinin binds moderately to human plasma proteins, predominantly to albumin [39]. It is quickly eliminated from plasma with an elimination half-life time of approximately one hour. Dihydroartemisinin undergoes glucuronidation in liver microsomes, and is mainly excreted via the urine as alpha-dihydroartemisinin-ß-glucuronide [34] .

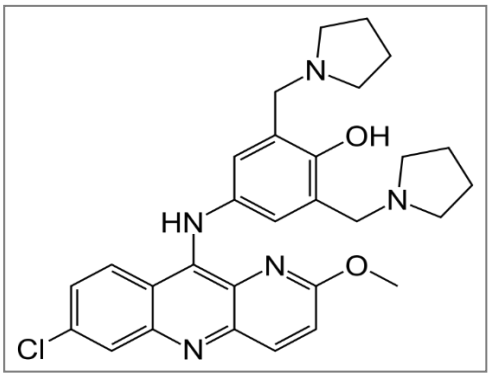
Intravenous artesunate alone is used as the initial treatment of severe malaria. It is superior to quinine for treatment of severe malaria with respect to clearing parasitaemia and reducing mortality [40]. Given the short half-life of artemisinins, intravenous therapy must be followed by a longer acting agent once the patient is able to tolerate oral medication. Treatment for less than five days of artesunate alone by any route (parenteral, rectal or oral) results in recurrent parasitaemia several weeks after therapy due to the very short duration of action, rather than to artemisinin resistance [41].

Artemisinins are generally well tolerated [42, 43]. Type 1 hypersensitivity to the artemisinin compounds has been reported (incidence 1:3000) [44]. A large-scale study on the adverse effects of orally-administered artemisinins demonstrated transient neurological abnormalities, including nystagmus and disturbances in balance; these effects resolved without lasting sequelae [43]. Transient neutropenia has been observed rarely in individuals receiving oral artesunate at doses higher than that typically prescribed [45]. Furthermore, attention has been recently drawn to the possible delayed hemolysis after artesunate in severe malaria [46-49]. While the pathophysiology of this phenomenon has not yet been fully elucidated, the necessity of standardized follow-up after treating with artesunate those patients suffering of severe

malaria has been therefore underlined [50]. Regarding resistance to artemisinins derivatives, reduced susceptibility as evidenced by delayed parasite clearance time, has been demonstrated in western Cambodia [51] and the bordering regions with Thailand [52]. The underlying mechanism and its long term clinical implications are not known.

2.3.1 Pyronaridine

Pyronaridine, 2-methoxy-7-chloro-10 [3,5-bis-(pyrrolidinyl-1-methyl4-hydroxyphenyl] aminobenzyl-(b)-1,5-naphthyridine, is a highly active blood schizonticidal Mannich-base antimalarial drug. It was firstly developed in China in 1970 [53].



Pyronaridine [Public domain]
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Pyronaridine was one of the first synthetically developed antimalarial drugs after chloroquine [54]. Structurally, pyronaridine is related to the aminoacridine drug quinacrine. The drug is highly effective against erythrocytic stages of *P. falciparum* and *P. vivax* [55, 56] and had been used against these Plasmodium species for over thirty years as a monotherapy in China [57].

Early studies showed that pyronaridine seems to affect the food vacuole of the parasite [58, 59], followed by the fast formation of an structure of multilamellar whorls in the trophozoites' pellicular complexes [59]. In infected primates a single membrane surrounding undigested endocytic vesicles of parasites exposed to pyronaridine was observed. The most specific and earliest effect of therapy was shown to interfere with the parasite food vacuole of schizonts and late trophozoites [60]. The anti-plasmodial activity of the compound seems to involve interference with the glutathione-dependent detoxification of haem [61, 62] and targeting of β-haematin formation [63]. Pyronaridine has been shown to inhibit β-haematin production with a 50%-inhibitory concentration similar to that of chloroquine. Moreover, forming complexes with β-haematin enhance haematin-induced human blood cell lysis by approximately 1/100 of the

concentration needed with chloroquine [61]. Furthermore, *P. falciparum* DNA topoisomerase II seemed to be inhibited by pyronaridine and anilinoacridine analogs [63]. However, it appeared that the compound does not elicit formation of a protein-DNA complex *in situ* but the formation of a drug-haematin complex [61] .

Advantages of pyronaridine include its structural differences to other antimalarial drugs, long shelf-life and once-daily dosing [57]. In animal models of malaria infection, pyronaridine showed synergistic activity with artesunate against strains resistant to either component [64]. Furthermore, it is efficacious in combatting chloroquine-resistant strains of falciparum malaria both *in vitro* and *in vivo* in human patients [65, 66]. However, there have been concerns about the emergence of resistance if used as monotherapy [64, 67].

Pyronaridine monotherapy seems to have no relevant effect on gametocyte carriage of *P. falciparum* in children and adults. However, in patients who had chloroquine-resistant infection, chloroquine has been associated with a higher relative risk for gametocytaemia after treatment compared to pyronaridine [68]. It has been therefore suggested a relative small benefit of pyronaridine for gametocyte carriage compared to chloroquine in areas where chloroquine-resistant *P. falciparum* is present. Pyronaridine has been also investigated in *P. ovale spp* (n = 8) and *P. malariae* infection (n =14) [69]. Mean parasite and fever clearance times were respectively 50 h and 33 h, while no patient had fever after Day 4. All patients were considered clinically and parasitological cured at Day 14.

The tolerability and safety profile of pyronaridine as monotherapy has shown the drug to be generally well tolerated. The common adverse events described after oral pyronaridine therapy have been usually similar to the symptoms of malaria, i.e. dizziness, nausea, vomiting and abdominal discomfort [57] Transient ECG changes as well as palpitations and allergic skin reaction have also been noted at higher doses of pyronaridine [53]. Pruritus, an adverse reaction seen related to many antimalarials in patients of African origin, has been described in a cohort in Cameroon patients treated with pyronaridine, though 2.5 times lower than in chloroquine-treated patients [70]. In this study,

seven patients treated with pyronaridine showed increased serum transaminases transiently after treatment as well as four patients treated with chloroquine. Moreover, in five patients receiving pyronaridine total bilirubin was slightly elevated. In Thailand, seven patients receiving 1,200 mg-1,800 mg pyronaridine had increased transaminases at Day 7; all cases resolved within five weeks after treatment start [71]. Finally, there is some evidence of successfully cured malaria in pregnancy, treated with pyronaridine at their mid- and late-trimester with no known adverse effects [72].

2.3.2 Combination Therapy in Malaria

Pyronaridine-artesunate (3:1 ratio, fixed dose once daily for three days) has been developed as an artemisinin-based combination for the treatment of uncomplicated *P. vivax* and *P. falciparum* malaria [73]. In a Phase II open-label study in 60 children with falciparum malaria in Gabon, pyronaridine-artesunate (tablets or granules) given in ratios of 6:2 mg/kg, 9:3 mg/kg, or 12:4 mg/kg once daily for 3 days showed good tolerability and safety profile, with a PCR-corrected cure rate of 100% on Day 28 [73]. Furthermore, rates of adequate clinical and parasitological response (ACPR) on Day 28 for pyronaridine-artesunate tablet formulation therapy (corrected for re-infection with polymerase chain reaction genotyping) were respectively 99.5% (780/784) and 99.2% (743/749) in two clinical trials, both conducted in adults and children with *P. falciparum* malaria in Africa and Asia, [74, 75]. Antimalarial therapy with pyronaridine-artesunate was non-inferior to that with artemether-lumefantrine and artesunate plus mefloquine, respectively. Moreover, the profile of adverse events of pyronaridine-artesunate in these clinical trials was considered to be adequate, though some patients had transient increased liver transaminases.

2.4 Study Objectives

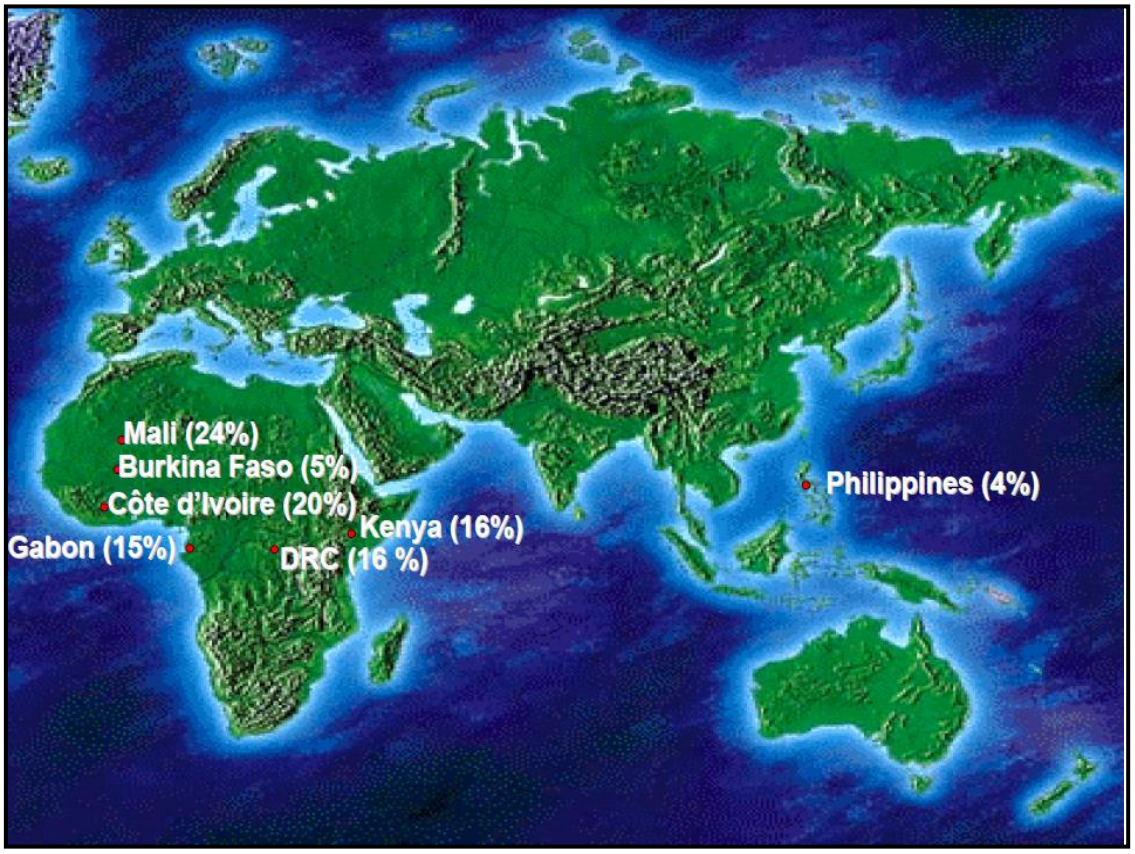
Artemisinin-based combination therapy is now the recommended treatment for uncomplicated *P. falciparum* malaria. Appropriate drug formulation is an essential factor for the success of the strategy of artemisinin-based combination therapy and oral paediatric formulations are urgently needed.

The primary objective of this clinical study was to demonstrate the efficacy of a fixed combination of pyronaridine-artesunate granule formulation (60:20 mg) by showing a PCR-corrected adequate clinical and parasitological cure rate of more than 90% in African children of 5-20 kg body weight and 1-12 years old. The secondary objective of this study was to compare the efficacy (non-inferiority) and safety of pyronaridine-artesunate granule formulation compared to Coartem® crushed tablets in a pediatric population and to assess the safety of pyronaridine-artesunate granule formulation. Both treatments are a three days regimen course and are formulated specially for the use in pediatric patients. Pyronaridine-artesunate granule formulation additionally addressed the need for an ACT regimen for young children with difficulties to swallow tablets, facilitating compliance and thereby increasing effectiveness of treatment and reducing the risk of emergence of resistance.

3 Methods

The study was part of a multicenter, comparative, randomized, open-labeled, parallel group study on the efficacy, safety and pharmacokinetics of pyronaridine-artesunate conducted in a total of 534 male and female infants and children (between ≥ 5 kg and < 25 kg body weight) suffering from acute symptomatic uncomplicated *P. falciparum* malaria recruited from study sites located in Africa and Philippines [76]. Patients were randomized to either pyronaridine-artesunate granules (paediatric Pyramax®) or artemether-lumefantrine (Coartem®) crushed tablets in a 2:1 ratio. A total number of 80 children suffering from acute symptomatic *P. falciparum* malaria were included at the "Centre de Recherches Médicales de Lambaréné" (CERMEL) situated at the Albert Schweitzer Hospital in Lambaréné, Gabon.

Figure 1. Location of centres of recruitment in Africa and Philippines and percentage of total included patients recruited at each site



3.1 Study Site

The study was conducted at the Albert Schweitzer Hospital in Lambaréné, Gabon. Lambaréné is a town of about 20.000 inhabitants and is located in a region of dense rainforest. The predominant Plasmodium species in Gabon is *P. falciparum*, though infection by *P. ovale* and *P. malariae* can be found. *P. falciparum* mean parasite densities are typical for areas with stable perennial hyperendemic malaria [77-79]. The entomological inoculation rate is around 50 bites per person per year [79]. *In vitro* and *in vivo* high-resistance to chloroquine has been shown in clinical specimens in and around the area [80-82]. A decreasing sensitivity to antifolates has been likewise reported [83, 84]. Susceptibility to mefloquine [77] and quinine remains high despite broad administration for a long time [83-85]. Regarding the activity of artemisinins derivatives, early *in vitro* studies reported high susceptibility of *P.falciparum* isolates to artesunate [83]. Moreover, consistent 50% and 90% effective concentrations with dihydroartemisinin have been reported against fresh isolates [86]. In clinical trials, a 3-day course of artesunate at a dose of 4 mg/kg in children with *P. falciparum* malaria failed to achieve sufficiently high cure rates (92% by Day 14 and 72% by Day 28 [41]. Although resistance to artemisinin derivatives has been induced *in vitro*, treatment failure of artesunate seemed to be of limited clinical relevance in the region due to high recrudescence rates when too short course of artesunate is used in monotherapy as a 5-day course of oral artesunate yielded a cure rate of 90% on Day 28. [41, 87].

3.2 Trial Population

Infants and children with acute symptomatic uncomplicated *P. falciparum* malaria were recruited in the vicinity of Lambaréné. Patients were deemed eligible for the study if they met the following inclusion criteria:

- Male or female patients < 12 years of age.
- Body weight > 5 kg and < 25 kg with no clinical evidence of severe malnutrition (defined as a child whose weight-for-height is below 3 standard deviations or less than 70% of the median of the NCHS/WHO normalised reference values).
- Presence of acute uncomplicated *P. falciparum* monoinfection confirmed by:
    - Presence of *P. falciparum* with parasite density between 1,000 and 200,000 asexual parasites/µl of blood and
    - Fever, as defined by axillary temperature above 37.5°C or oral/tympanic/rectal temperature above 38°C, or documented history of fever in the previous 24 hours
- Written informed consent provided by parent/guardian. If the parent/guardian was unable to write, witnessed consent procedures were performed. Where possible, patient assent was sought.
- Ability to swallow pre-specified volume of liquid in which medication was suspended.
- Female patients of child-bearing potential were neither pregnant (as demonstrated by a negative pregnancy test) nor lactating, and willing to take measures to not become pregnant during the study period.
- Ability and willingness to participate based on information given to parent or guardian and access to health facility. The patient was to comply with all scheduled follow up visits until Day 42.

Patients who met the following exclusion criteria were not enrolled:

- Patients with signs and symptoms of severe/complicated malaria requiring parenteral treatment according to the World Health Organization Criteria 2000.
- Mixed Plasmodium infection.
- Severe vomiting, defined as more than three times in the 24 hours prior to inclusion in the study or inability to tolerate oral treatment, or severe diarrhoea defined as 3 or more watery stools per day.
- Known history or evidence of clinically significant disorders such as cardiovascular (including arrhythmia, QTc interval greater or equal to 450 milliseconds), respiratory (including active tuberculosis), history of jaundice, hepatic, renal, gastrointestinal, immunological (including active HIV-AIDS), neurological (including auditory), endocrine, infectious, malignancy, psychiatric, history of convulsions or other abnormality (including recent head trauma).
- Presence of significant anaemia, as defined by Hb < 8 g/dL.
- Presence of febrile conditions caused by diseases other than malaria.
- Known history of hypersensitivity, allergic or adverse reactions to pyronaridine, lumefantrine or artesunate or other artemisinins.
- Patients with known disturbances of electrolytes balance, e.g., hypokalaemia or hypomagnesaemia.
- Use of any other antimalarial agent within 2 weeks prior to start of the study as evidenced by reported patient history.
- Pregnant or breast feeding.
- Patients taking any drug which is metabolised by the cytochrome enzyme CYP2D6 (flecainide, metoprol, imipramine, amitriptyline, clomipramine).
- Received an investigational drug within the past 4 weeks.
- Known active Hepatitis A IgM (HAV-IgM), Hepatitis B surface antigen (HBsAg) or Hepatitis C antibody (HCV Ab).

- Known positive for HIV antibody.
- Liver function tests [ASAT/ALAT levels] more than 2.5 times upper limit of normal range.
- Known significant renal impairment as indicated by serum creatinine of more than 1.4 mg/dl.
- Previous participation in any clinical study with pyronaridine-artesunate.

3.3 Investigational Plan

The study was designed as a comparative, randomized, open-labeled, parallel group study. Patients were randomized to receive either oral pyronaridine-artesunate granule formulation (60:20 mg sachet) once a day for 3 consecutive days (Day 0, 1 and 2) or artemether-lumefantrine (20:120 mg crushed tablets) twice a day for 3 consecutive days (Day 0, 1 and 2). For pyronaridine-artesunate, the actual range covered by this regimen was 7.0:2.3 mg/kg to 13.3:4.4 mg/kg. The dose range had been shown to be well tolerated from phase I studies conducted in healthy volunteers and effective and well tolerated in phase II studies in malaria patients, including children.

Posology was based on body weight ranges for both pyronaridine-artesunate combination and the comparator regimen.

The efficacy endpoints and schedule of assessments selected followed the WHO guidelines for monitoring anti-malarial drug efficacy [88]. Patients were followed for 42 days, with the primary efficacy endpoint occurring at 28 days after initiation of study drug administration (Day 28). Patients were confined to the study facility for at least 4 days (Study Day 0, 1, 2 and 3) and remained in the vicinity (to rapidly address any safety issue) of the study site for a further 3 days or when fever and parasite had been cleared for at least 24 hours (see definitions, page 71), whichever was the earlier. The patient returned to the study site for scheduled follow up visits until completion of the study on Day 42. In the case of adverse events were reported and unresolved at visit Day 42, patients were followed up for up to a further 30 days, or until resolution of the event whichever was the earlier. Severe adverse events were followed up to resolution.

3.4 Investigational Drugs

Patients who met all entry criteria and presented no exclusion criteria were included in the study and randomized to receive either the study drug or the comparator drug.

3.4.1 Drug Administration

Study drug and comparator drugs were administered to patients by a qualified member of the study site and who was designated by the Principal Investigator. The medication was taken under the supervision of an investigator who documented drug administration time. The investigator who dispensed and administered the study medication did not participate in any assessment of the patients. The remainder of the site staff was blinded to treatment and was not informed of treatment allocation unless the blind needed to be broken for a medical emergency. Patient weight recorded during the physical examination at screening was used to calculate the number of tablets to be administered.Study drug and comparator drug were given to each patient, with up to 150 ml (full glass) of not carbonated liquid. All patients took the medication in an upright position (seated or standing) and immediately after preparation.

If a patient vomited first dose of treatment within 30 minutes after administration, the full dose was replaced. The dose was not replaced if vomiting occurred more than 30 minutes after intake. If a patient vomited twice the first dose or any of the subsequent doses within 30 minutes, the study medication was discontinued and a rescue treatment with a standardized alternative antimalarial was initiated. Any new additional medication taken during the 42-day study period was documented in the case report form. Medication with antimalarial activity was not used within the 14 days period prior to enrolment or during the entire study period.

3.4.2 Study Drug: Pyronaridine-Artesunate Granules

Pyronaridine artesunate was supplied by Shin Poong Pharm Co in aluminium sachets. The active ingredients of the investigational product were pyronaridine tetraphosphate and artesunate. Each sachet contained 60:20 mg pyronaridine artesunate. Depending on their body weight, patients had received 1 sachet (5-9 kg) 2 sachets (9-17 kg) or 3 sachets (17-25 kg) per day. The dose-level range covered by this regimen was between 7.0:2.3 mg/kg to 13.3:4.4g/kg pyronaridine-artesunate, respectively.

3.4.3 Artemether-Lumefantrine Crushed Tablets

The comparator drug Coartem® was supplied by Novartis SA. The active ingredients of Coartem® are artemether 20 mg and lumefantrine 120 mg in a fixed dose combination.

The posology was 1 crushed tablet for patients weighting 5 to <15 kg, 2 crushed tablets for patients weighting 15 to <25 kg twice a day for three days, the second dose given 8 hours after the first one and the third dose 24 hours after the first. The following 3 doses were given every 12 hours.

Table 1 Dosing scheme

	Treatment Group 1 60:20 mg Pyronaridine Artesunate Sachet	Treatment Group 2 20:120 mg Artemether Lumefantrine Oral Fixed Tablet
Day 1	1 , 2 or 3 sachets depending on body weight	1-2 crushed tablet depending on body weight 1-2 crushed tablet 8 hours after first dose
Day 2	1 , 2 or 3 sachets depending on body weight	1-2 crushed tablets 24 hours after first dose 1-2 crushed tablets every 12h
Day 3	1 , 2 or 3 sachets depending on body weight	1-2 crushed tablets every 12h

3.5 Study Design

Study Endpoints

The primary efficacy endpoint of the study was pyronaridine-artesunate Day 28 PCR-corrected adequate clinical and parasitological response (ACPR) >90%.
The main secondary efficacy endpoint was non-inferiority of pyronaridine-artesunate to artemether-lumefantrine for Day 28 PCR-corrected ACPR [88].
Treatment failures were classified as early treatment failure, late clinical failure, and late parasitological failure according to WHO criteria [88].

Other secondary efficacy outcomes were:

- Day 28 crude (non-PCR corrected) ACPR
- Day 42 PCR-corrected and crude ACPR
- parasite clearance time (time from first dose until clearance of parasitaemia, i.e. two consecutive negative readings taken between 7 and 25 h apart)
- fever clearance time (time from first dose to apyrexia, i.e. two consecutive normal readings taken between 7 and 25 h apart)
- proportion of patients with parasite clearance or fever clearance on Day 1, 2, and 3

Exploratory efficacy outcomes were:

- gametocyte density and proportion of patients with gametocytes
- gametocyte clearance time (defined as for parasite clearance time)

Safety outcomes were:

- adverse events, categorized using MedDRA (version, 10.1)
- laboratory abnormalities graded using the Division of Microbiology and Infectious Diseases Toxicity Scale (February, 2003)
- ECG abnormalities.

Visit Schedule

The study was organized in a baseline assessment, a treatment period and a follow-up period (Table 2).

Baseline assessment (Day 0). Baseline examination was done within 12 hours before the first dose of study medication. The informed consent was obtained prior to any study related activity or evaluation. After having performed a blood smear for two thick blood smears and two thin blood smears, blood spot on filter paper for polymerase chain reaction analysis (PCR), physical examination, vital signs, laboratory tests, and electrocardiography (ECG) the patient could be included according to the inclusion and exclusion criteria.

Treatment period (Day 0 – Day 3). The treatment period started with the first administration of the study medication, defined as “Day 0 hour 0”. Starting from hour 0 on, every 8 hours two thick blood smears were done and temperature was measured. Vital signs clinical signs and symptoms of malaria assessment and physical examination were done every 24 hours (Days 1, 2 and 3). ECG was done within 2-4 hours after third dose of pyronaridine or fifth of Coartem® (day 2). At day 3 were also performed laboratory tests.

Follow up period (Day 4 – Day 42). Follow-up visits at the were done on Day 7 (+/-1), 14(+/-1), 21(+/-1), 28(+2), 35(+/-2) and 42(+/-2) On all follow up visits two thick blood smears, one thin smear and blood sample on filter paper for PCR analysis were performed and vital signs and temperature taken. Follow up visits on Day 7 included in addition a physical examination and laboratory tests. Day 28 visits required an additional physical examination as well as a haematologic assessment.

	Screen	Inclusion	Treatment Period								
Schedule	D0	D0	D1	D2	D3	D7	D14	D21	D28 or day of failure	D35	D42
Informed Consent	●										
Study Drug Administration Once or Twice Daily (Reference time point)="1d 0h")		●	●	●							
Inclusion/Exclusion Criteria, Medical History/Contact details, Demography	●										
Weight and Height	●										
Urine hCG Pregnancy Test [a]	●								●		●
Physical Exam	●		●		●				●		
Urinary Test for antimalarial drug / patient's history	●										
Vital Signs	●		●	●	●	●	●	●	●	●	●
Temperature [b]	●	●	●	●	●	●	●	●	●	●	●
Clinical Signs and Symptoms of malaria	●		●	●	●	●[d]	●[d]	●[d]	●[d]	●[d]	●[d]
Clinical Biochemistry Tests/urinalysis	●				●	●			●[c]		●[c]
Haematology	●				●	●			●		●[c]
12 lead ECG	●			●		●[d]	●[d]		●[d]		
Thin blood films [i]	●	●				●	●	●	●	●	●
Thick blood films [e] (x 2)	●	●	●	●	●	●	●	●	●	●	●
PCR		●				●[f]	●[f]	●[f]	●[f]	●[f]	●[f]
Adverse Event		●	●	●	●	●	●	●	●	●	●
Concomitant Medication	●	●	●	●	●	●	●	●	●	●	●
Pyronaridine and artesunate concentration		●[g]	●[g]	●[g]	●[g]	●[g]	●[g]	●[g]	●[g, h]	●[g]	●[g]

Table 2 Visit schedule for baseline, treatment and follow-up period

a) In females of childbearing potential
b) Every 8 hours over at least 72 hours following first study drug administration or temperature normalization for at least two readings between 8 and 24 hours apart, (when dosing and assessments coincided, assessments was done before dosing), then at each visit and as clinically indicated
c) Haematology mandatory on Day 28
d) If clinical abnormalities were observed
e) Thick blood films were examined every 8 hours until at least 72 hours or until 2 negative smears had been recorded for at least between 8 hours and 24 hours apart (when dosing and assessments coincided, assessments were done before dosing); then at Day 3, 7, 14, 21, 28, 35 and 42 or any other day if the patient returned.
f) As a reserve sample for PCR to assess status of recrudescence or re-infection in case of re-appearing parasitaemia
g) Samples for pyronaridine and artesunate levels were taken for population PK at selected sites and selected time points (two time points per patient).
h) A sample of blood for pyronaridine levels was taken in the event of treatment failure
i) Thin blood films taken from Day 7 whenever parasitological blood samples were taken, and reserved in the event of recrudescence, to confirm Plasmodium species

All patients and respective guardians were asked to present at the study center in case of any new or worsening disease or injury occurring in-between the scheduled visits.

The following events were considered withdrawal criteria and sufficient reason to discontinue the study treatment:

1. Adverse event(s) of such a nature or intensity which withdrawal was advisable (including abnormal laboratory findings)
2. Vomiting as described in study drug administration
3. Protocol violation
4. Patient withdrawal of consent
5. Lost to follow up
6. Death

If the patient discontinued study treatment before Day 3 and/or observation before Day 28, every effort was made to get follow up information on the status of the patient. The only events considered as sufficient reasons for a patient to discontinue observation in the clinical study were loss to follow up, death, and withdrawal of consent.

3.6 Efficacy Assessments

3.6.1 Parasitologic Assessments

Microscopy

Parasite density, expressed as the number of parasites per microliter (µl) of blood, was measured serially to determine parasite clearance time (PCT).

Asexual parasite and gametocyte counts were recorded separately throughout the study by means of finger prick.

Blood smears preparation, staining, examination and interpretation were in accordance with the WHO guidelines [88]. Briefly, thick blood smear for initial screening was examined by counting the asexual parasites and the white blood cells in a limited number of microscopic fields. Parasite density was then calculated by counting the number of asexual parasites against a set number of white blood cells (WBCs) — typically 200 or 300 — in the thick blood film, using a hand tally counter. Once a field was started, it was counted to completion. If more than 500 parasites were counted before 200 WBCs were reached, the count was stopped after the reading of the last field was completed. Parasite density, expressed as the number of asexual parasites per microliter (µl), was calculated by dividing the number of asexual parasites by the number of WBCs counted and then multiplying by an assumed WBC density (typically 6000–8000 WBCs/µl). The same technique was employed for establishing parasite counts on each of the subsequent blood film examinations. When the number of asexual parasites dropped below 10 per 200 WBCs, counting was done against at least 500 WBCs (i.e. to the completion of the field in which the 500th WBC were counted). A blood slide was considered negative when the examination of 100 thick-film fields did not show the presence of asexual parasites. The presence of gametocytes on any enrolment or follow-up slide should was noted. In addition, 100 fields of the second thick film were examined to exclude mixed infections; in case of any doubt, the thin film was examined for confirmation. If examination of the thin film was not conclusive, the patient was excluded from the study after complete treatment.

Thick and thin blood films for parasite count was obtained and examined at screening on Day 0 to confirm inclusion/exclusion criteria. Thick blood films were examined every 8 hours (+/- 1 hour) following first dose administration and for at least 72 hours or until the parasites had cleared as evidenced by two consecutive negative readings taken 8 to 24 hours apart. Microscopy was conducted before any dose of clinical trial medication on each of the first three days, or until parasites had been cleared as defined above. Thick blood films were also examined on Days 3, 7, 14, 21, 28, 35 and 42 or on any other day if the patient spontaneously returned. Additionally, blood films were obtained whenever parasitological reassessment was required. A thin blood smear was also taken from Day 7 whenever parasitological blood samples were taken and kept in the event of recrudescence, to confirm Plasmodium species.

Local quality control of slides was assured by reading of slides by 2 different qualified microscopists, reporting independently, with the arithmetic mean of the 2 counts. In the case of discrepancy, a third microscopist reviewed. An external quality control of slide reading was established by an independent laboratory at Swiss Tropical Institute (STI), Basel, Switzerland. The external quality control was blinded to treatment assignment. STI examined all slides in the case of suspected recrudescence plus a proportion of slides from each study site.

3.6.2 Genotyping Studies by PCR

Genotyping studies by PCR were used to differentiate new infection from recrudescence. Samples were taken for PCR assessment prior to initial dosing on Day 0. Blood samples were added directly from finger pricks to FTA Cards.

PCR samples were also taken from Day 7 at each study visit and whenever parasitological blood samples were taken, and preserved. The PCR analysis was performed in the case of reappearance of parasites as judged by a positive microscopy slide. PCR samples were shipped by courier for centralized assessments at the Swiss Tropical and Public Health Institute (Basel, Switzerland) and PCR genotyping using *P. falciparum* glurp, msp1 and msp2 genes was then performed at the central laboratory in Basel [89]. Briefly, a small FTA-card disk was punched out of the dried blood spot of the FTA card

and washed extensively with DNA purification solution. After washing with ethanol the disks were dried. A nested PCR was conducted for the msp2 locus using primary primers (S2: 5'- GAA GGT AAT TAA AAC ATT GTC -3'; S3: 5'- GAG GGA TGT TGC TGC TCC ACA G -3') with the following PCR conditions: 30 cycles of (94ºC - 5 min 94ºC - 30 sec 42ºC - 1 min, 65ºC - 2 min). Nested PCR was conducted with nested primers S1: 5'- GAG TAT AAG GAG AAG TAT G -3'; S4: 5'- CTA GAA CCA TGC ATA TGT CC -3'. PCR working conditions are 30 Cycles of (94ºC - 5 min 94ºC - 30 sec 50ºC - 1min 70ºC - 2 min). After PCR the amplified DNA was digested with the restriction endonuclease HinfI and subsequently run on a 1.5% agarose gel. The gel was electronically documented using an Omnilab Gel Documentation system.

Samples showing a banding pattern different from the banding pattern of Day 0/Day 1 were considered as new infections and no further analysis was required. Samples which show a banding pattern identical to the banding pattern on Day 0/Day 1 or show a banding pattern which contains identical alleles observed at Day 0/Day 1 were considered as potential recrudescence and were processed further. Identical alleles were further discriminated by size and by allelic family using the following PCR conditions: Primers for primary PCR: M1-OF and M1-OR M1-OF: 5'-CTA GAA GCT TTA GAA GAT GCA GTA TTG-3'; M1-OR: 5'-CTT AAA TAG TAT TCT AAT TCA AGT GGA TCA-3'; with the following PCR conditions: 25 Cycles of (94ºC - 5 min 94ºC - 30 sec 55ºC - 1 min 70ºC - 2 min). Family specific nested PCRs will be set up with the following primers: K1 allelic family: M1-KF and M1-KR: M1-KF: 5'-AAA TGA AGA AGA AAT TAC TAC AAA AGG TGC-3'; M1-KR: 5'-GCT TGC ATC AGC TGG AGG GCT TGC ACC AGA -3'; MAD20 allelic family: M1-MF and M1-MR M1-MF: 5'-AAA TGA AGG AAC AAG TGG AAC AGC TGT TAC-3'; M1-MR: 5'-ATC TGA AGG ATT TGT ACG TCT TGA ATT ACC -3'; RO33 allelic family: M1-RF and M1-RR M1-RF: 5'-TAA AGG ATG GAG CAA ATA CTC AAG TTG TTG-3'; M1-RR: 5'-CAT CTG AAG GAT TTG CAG CAC CTG GAG ATC -3' with the following PCR conditions 30 Cycles of (94ºC - 5 min 94ºC - 30 sec 58ºC - 1 min 70ºC - 2 min).

After PCR the samples were run on a 1.5% agarose gel and documented as above. Recurrent samples with identical banding patterns as on Day 0/Day 1 or with banding patterns contained in samples from Day 0/Day 1 were considered as true recrudescence and were documented as treatment failures. Recrudescence was then defined as one or more superposable allelic bands in all three markers between samples from Day 0/Day 1 and samples obtained after Day 7. Completely different patterns were classified as new infections.

3.6.3 Clinical Efficacy Assessments

Body Temperature

Temperature was measured at screening on Day 0 (Pre-Dose) and every 8 hours over at least 72 hours following first study drug administration or when the temperature had normalized (less than 38.0°C oral/tympanic/rectal at readings taken between 8 and 24 hours apart). Temperature was measured at every visit thereafter and as clinically indicated. When the time of study drug administration and clinical assessments coincided on Day 2 and Day 3, the clinical assessment was performed before the study drug administration. Temperature methodology was according to local practice. Within an individual patient the same means of temperature measurement (oral, tympanic or rectal) were used throughout the entire study period. Patients who have had a documented reported history of fever (more than 38.0°C oral/tympanic/rectal) prior to screening were monitored closely for fever peaks.

Patients entered in the study on the basis of history of fever and who did not subsequently have at least one body temperature measurement indicating presence of fever were not included in the analysis of fever clearance time. Quality of temperature-taking technique and thermometers were checked and calibrated prior to study commencement and at regular intervals during study.

Clinical Signs and Symptoms

Clinical signs and symptoms of malaria were assessed during the treatment period, plus on any other occasion where the patient presented to the site, when clinically indicated. The following signs and symptoms were specifically

assessed: rigors/chills, sweating, headache, nausea, vomiting, cough, loss of appetite/anorexia, fatigue (asthenia/lethargy/malaise), myalgia (back and limbs), jaundice (hyperbilirubinaemia), hepatomegaly and splenomegaly. If the signs or symptoms of acute malaria represented a clinically significant worsening compared to the patient's baseline condition, and were considered to be more severe than normal daily fluctuation of the disease process, then this was recorded as an adverse event.

3.7 Safety Assessments

Safety was assessed using the following criteria:

- Nature, incidence, relationship and severity of Adverse Events (AEs) and Serious Adverse Events (SAEs). Adverse event reporting was assessed through indirect questioning.
- Clinically significant change from baseline clinical laboratory parameters.
- Incidence of and reasons for withdrawals.
- Physical examinations and vital signs.
- 12-Lead ECG assessments.

3.7.1 Clinical Safety Assessments

Clinical evaluations were undertaken in all patients using the following parameters:

Physical Examination

A standard physical examination was performed at baseline (Day 0 pre-dose), as well as on Day 1, 3 and 28, and in case of early termination. At a minimum the following body systems were examined: general appearance; head and eyes; ears, nose and throat; chest and lungs; cardiovascular; abdomen; neurological; lymphatic and musculoskeletal. A complete medical history, demography, and contact address and details were also taken at baseline.

Body Weight and Height

Body weight and height were recorded at screening on Day 0 (pre-dose). The screening weight was used to satisfy the inclusion/exclusion criteria for minimum weight as well as to calculate the dose to be administered. The reliability of weighing scales was verified prior to commencement of study and was checked and documented at regular intervals during the study.

Vital Signs

Vital signs (supine blood pressure and heart rate) were taken after the patient had been supine for 5 minutes. Vital signs were measured at baseline (Day 0 pre-dose) and on Day 1, 2, 3, 7, 14, 21, 28, 35 and 42 and in the case of early termination. In addition, vital signs could be measured during the study period, as clinically indicated.

3.7.2 Laboratory Assessments

Clinical laboratory tests were performed as indicated in Table 3. Haematology parameters were measured on Day 0, Day 3, Day 7 and Day 28, as well as Day 42 if clinically indicated (e.g., presence of anemia at Day 28). Biochemistry and urinalysis were measured on Day 0, Day 3, and Day 7, as well as Day 28 and 42 if clinically indicated. Female patients of child-bearing potential had a urine hCG test for pregnancy prior to inclusion and on Day 28 and 42. Clinical laboratory tests required 2 ml for haematology and 2 ml for clinical chemistry, plus a urine sample for dipstick analysis.

Table 3. Clinical laboratory tests performed

Haematology	**Clinical chemistry**
Haematocrit	Total bilirubin
Haemoglobin	Albumin
Erythrocyte count (RBC)	Alanin aminotransferase
Platelet count	Aspartate aminotranferase
	Creatin kinase
Leukocytes (WBC) with differential count including eosinophils	Alkaline phosphatase
Reticulocytes	Urea
	Creatinine
Pregnancy test (urine hCG)*	Sodium
	Potassium
	Glucose
*Females with childbearing potential	

3.7.3 ECG

The incidence and nature of clinically significant ECG abnormalities were assessed. A 12-lead resting ECG was obtained at screening on Day 0 (Pre-Dose), and approximately 2 - 4 hours after study drug administration on Day 2. An ECG was performed also on Day 7, 14 or 28 if clinically significant abnormalities were observed. Further ECG measurements at other times could be taken if clinically indicated. Clinical indications for further ECG recordings could be the finding of obvious abnormalities of rhythm, and/or obvious changes from the previous ECG or if the patient experienced cardiac-type symptoms such as chest pain, palpitation or shortness of breath. Only one recording was required, however after the recording was made on each occasion the ECG was inspected to ensure that it was of sufficient quality for interpretation (i.e. all of the leads were in their correct place and there was minimum artifact). The ECG recording was read and interpreted by the Investigator. A proportion of ECGs were sent to a central blind reviewer (at Fulcrum Pharma) for review.

The ECG was reviewed for:

1. Waveforms P, QRS and T as well as the presence of U waves.
2. Rhythm (abnormal rhythms as well as tachycardia and bradycardia).
3. Evidence of abnormalities of PR, QT, and ST segments.
4. An assessment of overall “normality” of the ECG and where borderline or abnormal whether the abnormality is clinically significant.
5. Any change from baseline or from one ECG to the next.
6. Where an ECG was considered abnormal at baseline consideration as to whether an exclusion criterion has been violated needed to be considered.
7. Where an ECG exhibited a change this needed to be recorded and clinical significance assigned (Yes/No); if clinically significant then an AE was recorded if appropriate.

3.8 Adverse Events

3.8.1 Adverse Event Definition

The following definitions were employed for the reporting of adverse events:

A) *Adverse Event*

An adverse event was defined as any unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the period of observation in the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) which were considered by to be detrimental were recorded as adverse events whether or not they had a causal relationship with the study drug. Where the laboratory result was a sign of another clinical condition, the clinical condition itself was the reported adverse event. An event unequivocally caused by a significant deterioration of the underlying condition related to malaria was regarded as an adverse event. Recrudescence or new infection during the course of the study was not reported as adverse event unless the resulting malaria was of an unexpected severity.

B) *Unexpected Adverse Event*

Any adverse experience that had not been previously observed, (i.e. included in the protocol), whether or not the event was anticipated because of the pharmacologic properties of the study drug.

C) *Serious Adverse Event*

Any adverse experience occurring at any dose that resulted in any of the following outcomes:

- Death
- Life threatening defined as an experience that placed the patient at risk of death at the time of the event.
- Required inpatient hospitalization or prolongation of existing hospitalization.
- Resulted in a congenital anomaly or birth defect.
- Resulted in a persistent or significant disability or incapacity.

- Important medical events that did not result in death, were life-threatening or required hospitalization were considered as a serious adverse drug experience when, based upon appropriate medical judgment, they jeopardized the patient and required surgical or medical intervention to prevent any of the above-listed outcomes.

The following were not considered as serious adverse events: cases of hospitalization for elective surgery, need to observe high risk patients to prevent serious events, and visiting the emergency room due to suffering from adverse events but discharged after treatment without hospitalization.

D) Alert terms and other reasons for expedited reporting to Pharmacovigilance.
No specific events were subject to reporting as alert terms in this study.

Pregnancy did not constitute an adverse event. When occurred, the pregnant patient had to be followed until birth of the child. However, any adverse outcome to the mother, fetus or newborn had to be regarded as a serious adverse event. Worsening of the baseline conditions that were judged to be more significant than normal daily fluctuation of the disease symptoms were to be regarded as adverse events.

3.8.2 Assessment of Adverse Events

A) Severity
The severity/intensity of the adverse reactions and clinical laboratory changes were assessed using the “DMID Toxicity Grading Scale for Determining the Severity of Adverse Events” [90].
If an adverse event was not listed in the DMID table, the severity was assessed using the following guidelines:
1 = Mild: awareness of sign or symptom, but easily tolerated
2 = Moderate: enough discomfort to cause interference with usual activity
3 = Severe: incapacitating with inability to work or do usual activity
4 = Life-Threatening

B) Relationship or Association with the Use of Study Drug or Study Procedure

The relationship was between an event and the study drug was assessed using the following guidelines and terminology:

Definite: clear-cut temporal association, with a positive re-challenge test or laboratory confirmation.

Probable: clear-cut temporal association, with improvement upon drug withdrawal, and not reasonably explained by the patient's known clinical state.

Possible: less clear temporal association; other etiologies are possible. Other possible etiologies were recorded on the CRF.

None: no temporal association with the study drug; related to other etiologies such as concomitant medications or conditions, or subject's known clinical state.

C) Action Taken

- Study drug discontinued
- Patient withdrawn from study
- Concomitant Medication required
- Hospitalization required or prolonged (this was to be reported as a serious adverse event
- Other

D) Outcome

The patient was followed until AE resolution or until no further medically relevant information could be expected. AE outcome was classified as follows:

• Resolved
• Resolved with sequelae
• Continuing
• Death

3.9 Analytical and Statistical Plan

The Intention-To-Treat (ITT) population included all randomized subjects who received any study antimalarial medication. The Safety population was considered the same as the ITT-population. The Per-Protocol (PP) population was defined as all patients receiving a full course of study medication with recorded primary endpoint on Day 28 and had no protocol deviation/violation that could impair evaluation of Day 28 primary endpoint. The Efficacy Evaluable (EE) population included all patients receiving a full course of study medication and had a known 28 and/or 42 efficacy endpoint. Cure rates were also calculated for the ITT population employing an extreme case scenario. All patients who received any amount of study medication and who were not evaluable on Day 28 were considered as treatment failures.

The exact binomial test was used to evaluate the primary efficacy endpoint in the PP population (significance limit ≤ .025). The associated exact (Pearson–Clopper) two-sided 95% confidence interval (CI) was calculated (Graphpad Prism 6.0).

In order to evaluate the main secondary efficacy endpoint in the PP population, pyronaridine-artesunate was considered as non-inferior to artemether-lumefantrine when the lower limit of the two-sided 95% confidence interval for the difference between treatments was not lower than −10 % (Newcombe-Wilson score method without continuity correction [91, 92], Graphpad Prism 6.0). The non-inferiority margin of 10% was primarily specified for the total patient population of the study, as with 480 evaluable patients (randomized 2:1) the secondary endpoint of non-inferiority of pyronaridine-artesunate would be demonstrated with >99% power [76]. The analysis was repeated for the ITT population (extreme case scenario), Day 28 crude ACPR, Day 14 and Day 42 PCR corrected and crude ACPR. A post-hoc Kaplan-Meyer analysis of parasite clearance in the Efficacy Evaluable population was conducted. Treatments were compared by using the log-rank test. Kaplan–Meyer analysis and log-rank were also used to compare fever clearance between treatments (Graphpad Prism 6.0).

3.10 Patient Confidentiality

The CRF as well as all reports and communications relating to patients in the study identified each patient only by the patient's initials (first, middle, last) and by the patients' identification number. The investigator maintained a current confidential Patient Identification Code List of names of all patients allocated to patient identification numbers in this study. This list allowed the investigators to reveal the identity of the patients in the event that they needed to be contacted for safety reasons. This information was held in the strictest confidence and was used only for emergency purposes, if needed.

3.11 Ethics and Good Clinical Practice

The protocol and informed consent were reviewed and approved by the Ethics Committee of the International Foundation of the Albert Schweitzer Hospital before the study was initiated. The study was carried out in compliance to the protocol, in accordance with the Declaration of Helsinki and in adherence to Good Clinical Practice guidelines [93].

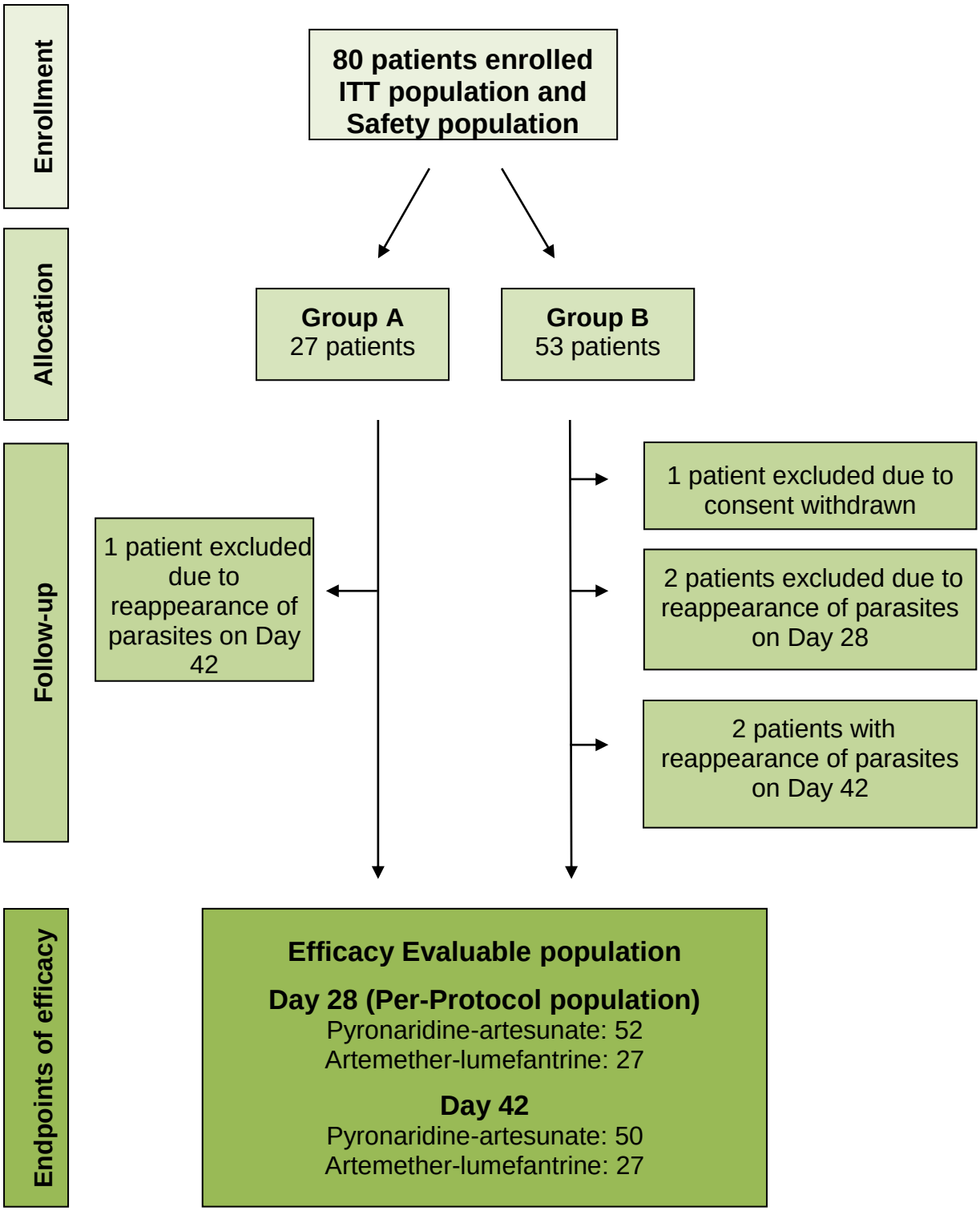
4 Results

4.1 Study Flow

The study took place from the 4th October 2007 to the 5th September 2008 comprising a total of eleven months. From 134 children and infants presenting at the study site with fever or/and history of fever and a positive thick smear for *P. falciparum*, 80 children were enrolled after screening procedures into the present study. The exclusions were mostly due to low haemoglobin (<8 g/dl), low parasitaemia (<1000 parasites/mL), high parasitaemia (>200,000 parasites/µl), prior antimalarial treatment, liver function over 2.5 times ULN range, severe vomiting and lack of parents' willingness to comply with all planned visits until Day 42. All patients not included in the study received medical assistance and treatment according to the clinical guidelines of the hospital.

After randomization procedures, 53 recruited subjects were enrolled in the pyronaridine-artesunate treatment group and 27 patients in the artemether-lumefantrine treatment group. All of the participants finished the 3 days treatment regimen and were included in the ITT population and in the Safety population. Three subjects in the pyronaridine-artesunate group did not finish the 42-days follow-up period. The early withdrawal of the participants in the pyronaridine-artesunate group was due to reappearance of parasites on Day 28 (two cases) and consent withdrawn on Day 14 (one case). Two participants in the pyronaridine-artesunate group and one in the artemether-lumefantrine group had reappearance of parasites on Day 42. All patients with reappearance of parasites received rescue medication with ACT according to the clinical guidelines (artemether–lumefantrine or artesunate-amodiaquine in the case of prior artemether-lumefantrine). Therefore, 79 (52 and 27, respectively) participants comprised the PP population on Day 28 and 77 patients were included in the EE population (50 and 27 participants, respectively) on Day 42.

Figure 1. Study flow



4.2 Demographic and Baseline Characteristics

Demographic and clinical characteristics at baseline of the ITT-population (all randomized subjects who received any study antimalarial medication) are shown in Table 4. Baseline clinical and demographic characteristics were comparable within the two treatment groups, as no significant differences were found. It is important to notice that no patient under 1 year was included in any group. Regarding laboratory findings, no differences were found with respect to baseline parasitaemia (asexual forms) as well as percentage of patients presenting gametocytes at baseline.

Haematology and biochemistry values were representative for children with acute uncomplicated *P. falciparum* malaria (Tables 14 and 15). Mean haemoglobin values were below normal range in both treatment groups, 10 g/dl in the pyronaridine-artesunate group and 10.2g/dl in the artemether-lumefantrine group. Platelets were also below normal range at baseline 179000/µl and 201000/µl respectively. Baseline mean values of albumin, total bilirubin, ALT, urea, creatinine, glucose, sodium and potassium were within normal ranges. Mean values of AST were slightly over normal range at baseline. None of the patients had any clinically significant ECG finding at baseline.

Table 4. Baseline characteristics

	Pyronaridine-artesunate n=53	**Artemether-lumefantrine** n=27
Gender M/F %	53/47	46/54
Age, months Mean (SD)	56 (26)	55(29)
Age category n (%)		
<1 year	0 (0)	0 (0)
1-5 years	34 (64%)	17 (63%)
5-12 years	19 (36%)	10 (37%)
Height, cm Mean (SD)	101 (15)	105 (18)
Weight, kg Mean (SD)	15.7 (3.9)	16.7 (4.5)
Asexual forms/µl Median (range)	25408 (1343 -170313)	23601 (1248 – 133839)
Patients with gametocytes n (%)	4 (8%)	3 (11%)
Fever at baseline n (%)	30 (57%)	15 (56%)
Temperature Mean (SD)	37.8 (0.9)	37.8 (1.1)
Malaria in last 12 months		
None	25 (47%)	12 (45%)
1	18 (34%)	10 (37%)
2	7 (13%)	2 (11%)
>2	3 (6%)	2 (7%)

Showing main clinical, demographic and parasitological baseline characteristics in the ITT population (gender, age, weight, height, parasitaemia, fever, mean temperature and previous malaria episodes <12 months). All findings were comparable between both treatment groups.

4.3 Efficacy

Efficacy measures were evaluated for the ITT population and the EE population.

4.3.1 Primary Efficacy Endpoint

PCR-corrected ACPR >90% at Day 28

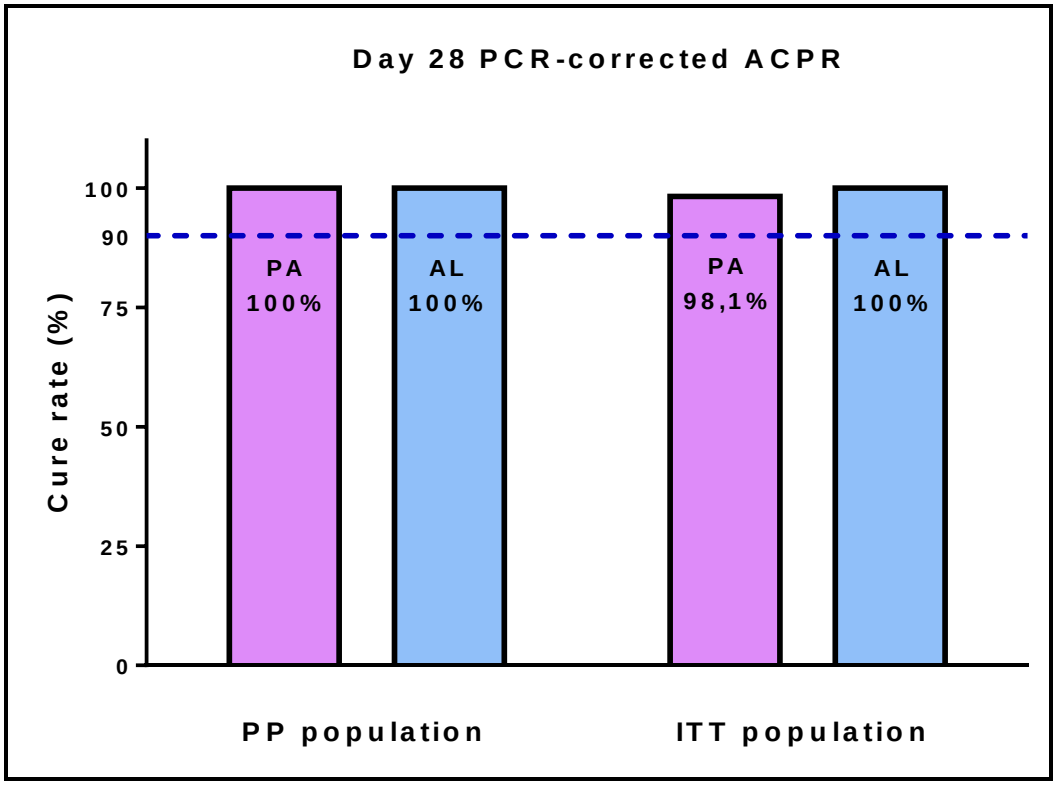
The primary outcome of this study was achieved. The cure rate at Day 28 (PCR-corrected ACPR >90%) served as primary outcome measure of efficacy in this clinical trial. In the EE population all patients were clinically and parasitologically cured on Day 28 (clearance of asexual parasitaemia within 72 hours and without subsequent PCR-corrected recrudescence within 28 days). PCR-corrected ACPR in the EE population was therefore 100% (52/52) with a 95% CI of 93 to 100%, and statistically significantly above 90% (p-value= 0.009; Table 5, Figure 2). Cure rates were also calculated for the ITT population employing an extreme case scenario (all patients who received any amount of study medication and who were not evaluable on Day 28 were considered as treatment failures). The intention-to-treat analysis was therefore supportive of the primary analysis, with a PCR-corrected ACPR of 98.1% (95% CI 90-99.9), p-value = 0.026).

Table 5. Primary endpoint

Day 28	**PP population**	**ITT population**
Day 28 PCR-corrected APCR (n/N)	52/52	52/53
percentage	**100%**	**98.1%**
95% CI	93-100%	90-99.9%
p-value (binomial exact test)	0.009	0.026

Table 5. Cure rate (adequate clinical and parasitological response) at Day 28 PCR-corrected in the PP and ITT population.

Figure 2. PCR-corrected ACPR on Day 28



Comparison of PCR corrected-ACPR on Day 28 between pyronaridine-artesunate (PA) and artemether-lumefantrine (AL) treatment groups in both PP and ITT populations. Both treatments showed >90% of cure rate.

4.3.2 Secondary Efficacy Endpoints

Non-inferiority of Day 28 PCR-corrected ACPR

The Day 28 PCR-corrected ACPR rate of the pyronaridine-artesunate group was comparable to that of the artemether-lumefantrine group, in both PP (100% versus 100%; treatment difference 0, 95% CI: -6.9 to 12.5) and ITT population (98.1% versus 100%; treatment difference -1.9, 95%CI: -9.9 to 10.7). The main secondary endpoint, non-inferiority of pyronaridine-artesunate compared to artemether-lumefantrine, was thus concluded for Day 28 PCR-corrected ACPR. However, as the lower limit of the 95% CI between difference was <10% in both PP and ITT populations (-6.9 and 12.5 respectively), non-inferiority was not demonstrated for Day 28 crude ACPR (PP population: difference -3.9, 95% CI: -12.9 to 8.9; ITT population difference -5.7, 95% CI: -15.3 to 7.4). It is important to note that the non-inferiority margin was primarily specified for the total patient population of the multicentre study (n=480).

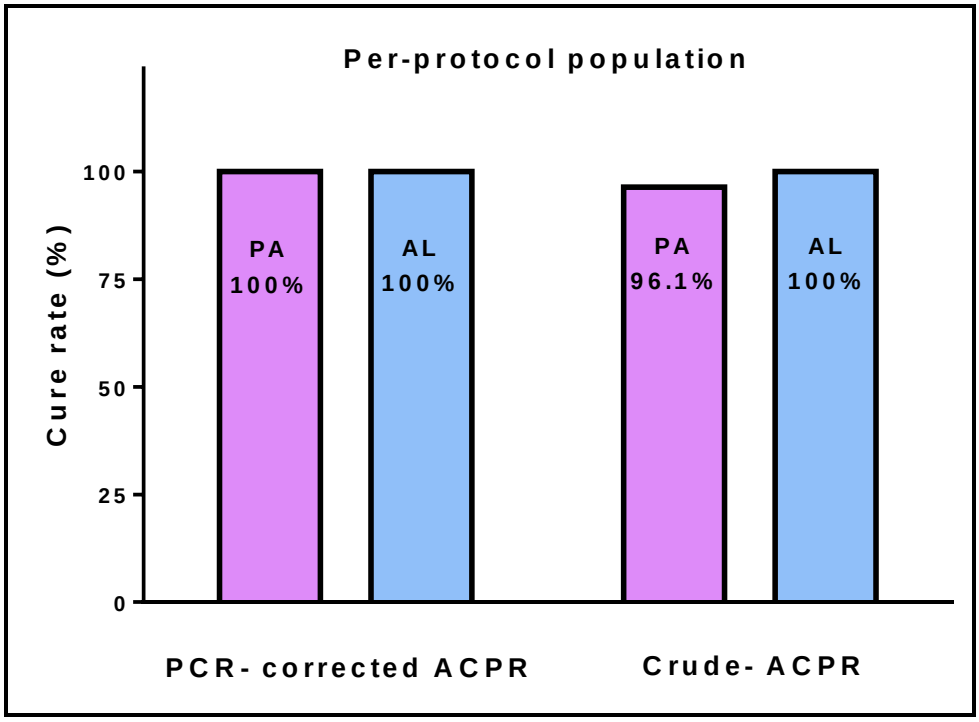
Table 6 Comparison of ACPR rates between treatment groups on Day 28

Day 28	**Pyronaridine - artesunate**	**Artemether - lumefantrine**	**Difference** (95% CI)
PCR- corrected ACPR (PP)	52/52	27/27	
	100%	100%	**0 (-6.9 to 12.5)**
PCR- corrected ACPR (ITT)	52/53	27/27	
	98.1	100%	**-1.9 (-9.9 to 10.7)**
crude ACPR (PP)	50/52	27/27	
	96.1%	100%	-3.8 (-12.9 to 8.9)
crude ACPR (ITT)	50/53	27/27	
	94.3	100%	-5.7 (-15.4 to 7.4)

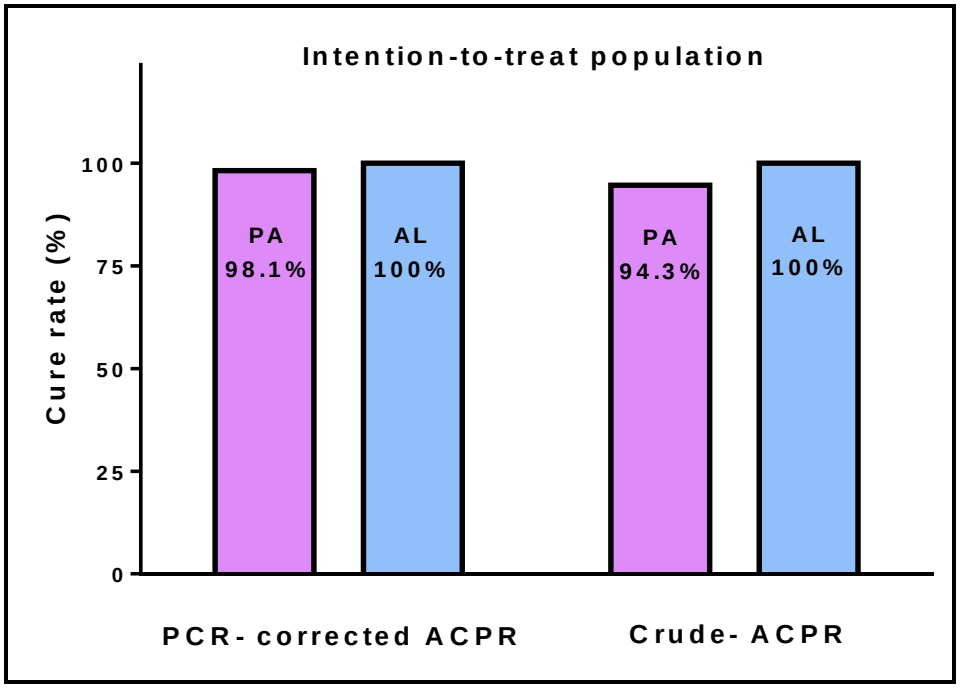
Non-inferiority analysis of pyronaridine-artesunate versus artemether-lumefantrine, concluded on Day 28 for PCR-corrected ACPR for both PP and ITT populations. Considered when the lower limit of the difference between cure rates >-10%.

Figure 3. Crude and PCR-corrected ACPR comparison between treatment groups in A) PP population and B) ITT population

A



B



Non-inferiority analysis of pyronaridine- artesunate against artemether-lumefantrine. Concluded for PCR-corrected ACPR in both PP (3A) and ITT (3B) populations.

Day 14 and Day 42 crude and PCR-corrected ACPR

Day 14 crude and PCR-corrected ACPR rates were 100% for the EE and ITT population in both treatment groups. The lower limit of 95% CI of the difference was >10% in both analysis and no significant differences between treatments were found (lower limit of 95% CI of the difference: 6.8). Day 42 PCR-corrected ACPR rates were 100 % in pyronaridine-artesunate and 100% in artemether-lumefantrine in the EE population while in the ITT population (extreme case scenario) the ACPR rates were 94.3% and 100% respectively. The crude ACPR cure rates were 96% and 96.3% in the EE population and 90.6% and 96.2% in the ITT population. Non inferiority was demonstrated for Day 42 PCR–corrected ACPR in the pyronaridine-artesunate group with a lower 95% CI of the difference against artemether-lumefantrine of -7.1.

Table 7. Comparison of ACPR rates between treatment groups on Day 14 and Day 42

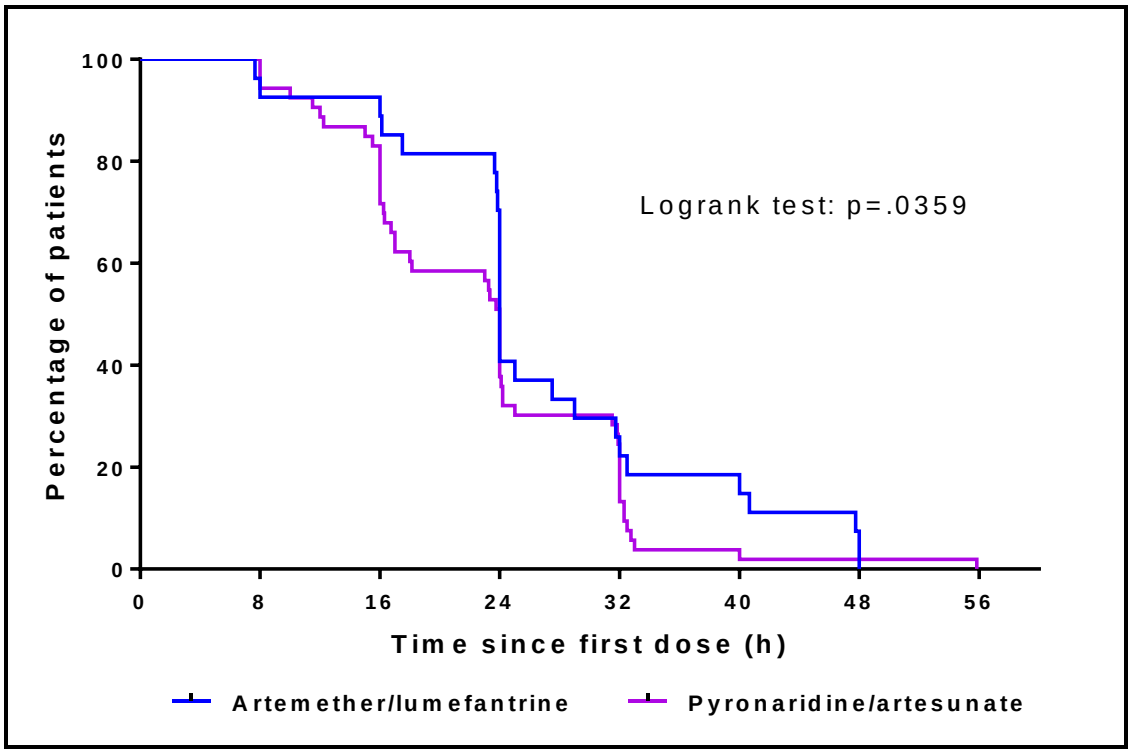
	Pyronaridine-artesunate	**Artemether-lumefantrine**	**Difference** (95% CI)
Day 14 crude ACPR			
EE	**53/53(100%)**	**27/27(100%)**	**0 (-6.8 to 12.5)**
ITT	53/53(100%)	27/27(100%)	0 (-6.8 to 12.5)
Day 14 PCR-corrected ACPR			
EE	**53/53(100%)**	**27/27(100%)**	**0 (-6.8 to 12.5)**
ITT	53/53(100%)	27/27(100%)	0 (-6.8 to 12.5)
Day 42 Crude ACPR			
EE	**48/50(96.0%)**	**26/27 (96.3%)**	**-0.3 (-10.2 to14.6)**
ITT	48/53(90.6%)	26/27 (96.2%)	-6.2 (-16.9 to 9.6)
Day 42 PCR-corrected ACPR			
EE	**50/50 (100%)**	**27/27 (100%)**	**0 (-7.1 to12.6)**
ITT	50/53 (94.3%)	27/27 (100%)	-5.7(-15.4 to 7.3)

Non-inferiority analysis, pyronaridine-artesunate versus artemether-lumefantrine on day 14 and 42 (crude and PCR-corrected ACPR, EE and ITT populations). Non inferiority was demonstrated on Day 14 for both crude and PCR-corrected ACPR and for PCR-corrected ACPR on Day 42 for EE population. Non-inferiority was defined as a lower limit of the difference between cure rates greater than -10%.

4.3.3 Parasite Clearance Time

Parasite clearance time was evaluated in the EE (PP) population. Median time to parasite clearance was significantly shorter in patients treated with pyronaridine-artesunate (23.9 h; 95% CI 17.0-24.1) compared to artemether-lumefantrine (24 h; 95% CI 23.9-31.8; p-value =0.036, log-rank test; Figure 3). Moreover, percentile-25 time to parasite clearance was 16h in the pyronaridine-artesunate group and 23h in the artemether-lumefantrine group, while percentile-75 times were 31.9h and 32h, respectively.

Figure 4. Parasite clearance time

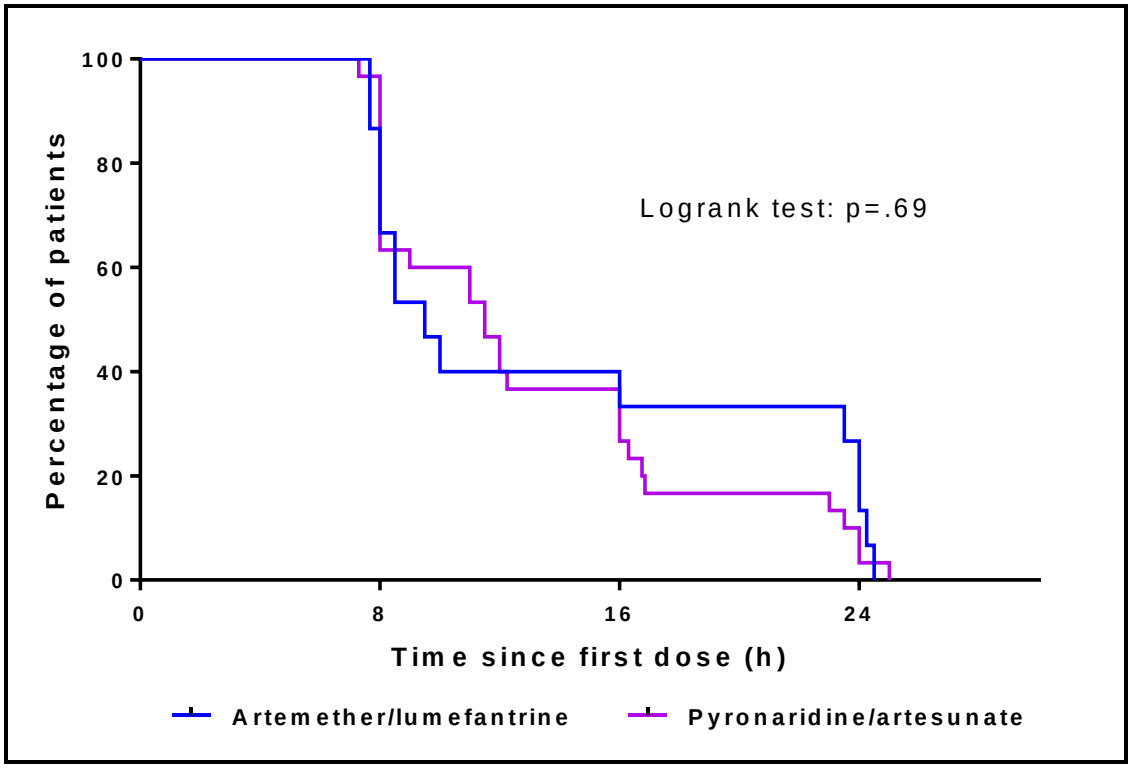


Parasite clearance time in EE population, showing significant shorter clearance in patients receiving pyronaridine-artesunate

4.3.4 Fever Clearance Time

Fever clearance time was evaluated in the PP population having fever at baseline. Fever was present in 55% (39/53) of the subjects receiving pyronaridine-artesunate while 56% (15/27) of the patients receiving artemether-lumefantrine had fever at baseline. Median fever clearance time was not significantly different between pyronaridine-artesunate (11.5 h; 95% CI: 8-16 h) versus artemether-lumefantrine (9.5 h; 95% CI: 8-24 h; p-value = 0.69, log rank test; Figure 5). Percentile-25 time to fever clearance was 8h for both pyronaridine-artesunate and artemether-lumefantrine, while the percentile-75 was 23.9 and 16.4, respectively.

Figure 5. Fever clearance time



Fever clearance time in EE population. No significant differences were found between treatment groups

4.3.5 Percentage of Patients with Gametocytaemia

Gametocytaemia at baseline as well as the percentage of patients cleared from gametocytes were evaluated on Day 3, 7, 14, 21, 28 and 42 (Table 8). At baseline, 9% (7/80) of the total included patients had gametocytes, 8% (4/53) in the pyronaridine-artesunate group and 11% (3/27) patients in the artemether-lumefantrine group. Patients with observed gametocytes at any point of the study were 17% (9/53) in the pyronaridine-artesunate and 22% (6/27) in the artemether-lumefantrine group. New appearance of gametocytaemia in patients that had none at baseline was found in 9.4% (5/53) of patients receiving pyronaridine-artesunate and in 11% (3/27) of those receiving artemether-lumefantrine. In the pyronaridine-artesunate group, complete gametocyte clearance was accomplished between Day 21 and Day 28 while patients in the artemether-lumefantrine group achieved clearance between Day 7 and 14.

Table 8. Patients with gametocytes over the course of the study

		Pyronaridine-artesunate	**Artemether-lumefantrine**
Patients with			
gametocytes at baseline	n (%)	4 (8%)	3(11%)
gametocytes at any time-point	n (%)	9 (17%)	6(22%)
gametocytes post-baseline	n (%)	5 (9%)	3(11%)
Gametocytaemia (sexual forms/µl)*			
Median (Min-Max)		76 (53 – 217)	95 (17 – 289)
Patients with gametocytes	n (%)		
Baseline		4 (8%)	3 (11%)
Day 0-3		9 (17%)	6 (22)
Day 7		6 (11%)	1 (4%)
Day 14		3 (6%)	0
Day 21		1 (2%)	0
Day 28		0	0
Day 35		0	0
Day 42		0	0

Number of patients with gametocytes at baseline, baseline gametocytaemia (defined as numbers of *P. falciparum* sexual forms per microliter of blood), and percentage of patients cleared from gametocytes.
*measured in patients showing gametocytes already at baseline

Table 9. Summary of the efficacy analysis

Outcome	Efficacy Evaluable population			Intention-To-Treat population		
	Pyronaridine-artesunate	**Artemether-lumefantrine**	**Difference (95% CI)**	**Pyronaridine-artesunate**	**Artemether-lumefantrine**	**Difference (95% CI)**
Day 14 PCR-corrected ACPR	53/53	27/27	0(-6.8 to 12.5)	53/53	27/27	0 (-6.8 to 12.5)
% (95% CI)	100 (93.3-100)	100 (87.2-100)		100 (93.3-100)	100 (87.2-100)	
Total failures	**0**	**0**		**0**	**0**	
Early treatment failure	0	0		0	0	
Late clinical failure	0	0		0	0	
Late parasitological failure	0	0		0	0	
Missing considered failure	-	-		0	0	
Day 14 crude ACPR	53/53	27/27	0(-6.8 to 12.5)	53/53	27/27	0 (-6.8 to 12.5)
% (95% CI)	100 (93.3-100)	100 (87.2-100)		100 (93.3-100)	100 (87.2-100)	
Total failures	**0**	**0**		**0**	**0**	
Early treatment failure	0	0		0	0	
Late clinical failure	0	0		0	0	
Late parasitological failure	0	0		0	0	
Missing considered failure	-	-		0	0	
Day 28 PCR-corrected ACPR	52/52	27/27	0(-6.9 to 12.5)	52/53	27/27	-1.9(-9.9 to 10.7)
% (95% CI)	100 (93.1-100)	100 (87.2-100)		98.1 (90.0-99.9)	100 (87.2-100)	
p- value binomial test	0.0086			0.0265		
Total failures	**0**	**0**		**1**	**0**	
Early treatment failure	0	0		0	0	
Late clinical failure	0	0		0	0	
Late parasitological failure	0	0		0	0	
Missing considered failure	-	-		1	0	
Re-appearance before Day 28	-	-		0	0	
Day 28 crude ACPR	50/52	27/27	-3.8(-12.9 to 8.9)	50/53	27/27	-5.7(-15.4 to 7.4)
% (95% CI)	96.1(86.8-99.5)	100 (87.2-100)		94.3 (84.3-98.8)	100 (87.2-100)	
Total failures	**2**	**0**		**3**	**0**	
Early treatment failure	0	0		0	0	
Late clinical failure	0	0		0	0	
Late parasitological failure	2	0		2	0	
Missing considered failure	-	-		1	0	
Day 42 PCR-corrected ACPR	50/50	27/27	0(-7.1 to12.6)	50/53	27/27	-5.7(-15.4 to 7.4)
% (95% CI)	100 (92.8-100)	100 (87.2-100)		94.3 (84.3-98.8)	100 (87.2-100)	
Total failures	**0**	**0**		**3**	**0**	
Early treatment failure	0	0		0	0	
Late clinical failure	0	0		0	0	
Late parasitological failure	0	0		0	0	
Missing considered failure	-	-		1	0	
Re-appearance before Day 42	-	-		2	0	
Day 42 crude ACPR	48/50	26/27	-0.3(-10.2 to14.6)	48/53	26/27	-6.3(-16.9 to 9.6)
% (95% CI)	96.0 (86.2-99.5)	96.3 (81-99.9)		90.6 (79.3-96.9)	96.3 (81-99.9)	
Total failures	**2**	**1**		**5**	**1**	
Early treatment failure	0	0		0	0	
Late clinical failure	0	0		0	0	
Late parasitological failure	2	1		4	1	
Missing considered failure	-	-		1	0	

4.4 Safety

All safety analysis was performed for the safety population, which comprised all randomized subjects who received any study antimalarial medication.

4.4.1 Incidence and Severity of any Adverse Event

A total of 135 adverse events were registered during the course of the study, 76 in the group receiving pyronaridine-artesunate and 40 in the group receiving artemether-lumefantrine. Experience of at least one adverse event of any cause occurred in 39/53 (74%) and in 21/27 (78%) patients receiving pyronaridine-artesunate and artemether-lumefantrine, respectively (Table 3). There were no deaths. Neither serious adverse events leading to study-drug discontinuation nor withdrawal occurred in the course of the study.

Table 11. Incidence of adverse events

	Pyronaridine-artesunate n=53		**Artemether-lumefantrine** n=27	
	n	**%**	**n**	**%**
Any AE	39	74%	21	78%
Any drug related AE	6	11%	6	22%
Any Serious AE	0	0%	0	0%
Any severe or life threatening AE	0	0%	0	0%
Any Death	0	0%	0	0%
Any AE leading to study drug discontinuation	0	0%	0	0%
Any AE leading to withdrawal of the study	0	0%	0	0%

Overall incidence and rates of adverse events in the Safety population

Regarding the incidence or nature of adverse events, there were no clinically important differences between the two study groups (Table 12). The majority of the adverse events were of mild intensity. However, 6% (3/76) of the total reported adverse events by patients receiving pyronaridine-artesunate and 8% (2/40) of those reported by patients receiving artemether- lumefantrine were

designated as of moderate intensity. The adverse events of moderate severity were: fever >40°C, anaemia and blood AST/ALT increased in the pyronaridine-artesunate group, and two cases of upper respiratory infection with fever >40°C in the artemether-lumefantrine group. None of the moderate adverse events were considered as possible related to the medication intake. All experienced adverse events are listed below in Table 12.

Table 12. List of all recorded adverse events

	Pyronaridine-artesunate n=53		**Artemether-lumefantrine** n=27	
Total AE	**76**		**40**	
	n	**%**	**n**	**%**
Upper Resp Tract Infection	35	46	22	55
Pyrexia	8	11	3	7
Vomiting	2	3	4	10
Abdominal pain	3	4	5	13
Diarrhea	1	1	1	2
Conjuntivitis	4	5	0	0
Skin infection	4	5	1	2
Skin eruption	2	3	1	2
Headache	0	0	2	5
Varicela	1	1	1	2
Tinea Capitis	2	3	0	0
Anorexia	2	3	0	0
Stomatits aphtosa	2	3	0	0
Splenomegaly	2	3	0	0
Anemia	2	3	0	0
Creatin-kinase increased	1	1	0	0
AST/ALT increased	1	1	0	0
Furuncle	1	1	0	0
WBC increased	1	1	0	0
Urinary tract infection	1	1	0	0
Hookworms	1	1	0	0

Number of cases and rate of all adverse events regardless of relationship to medication

4.4.2 Incidence of Drug-related Adverse Events

Adverse events designated by investigators as related to the study drugs occurred in 6/53 (11%) of the patients receiving pyronaridine-artesunate and 6/27 (22%) of those receiving artemether-lumefantrine. All of them were considered as “possibly” or “probable” related to the study drugs. The adverse events judged drug-related were gastrointestinal disorders (vomiting (3), abdominal pain (3) and diarrhoea (2)), anemia (1) headache (2), and blood creatin- phosphokinase increased (1). All adverse events were considered of mild or moderate intensity and resolved before Day 28 without sequelae. Neither serious nor AE leading to study drug discontinuation nor withdrawal were related to the study medications. The incidence of study drug related adverse events for each treatment group is listed below in Table 13.

Table 13. Drug-related adverse events

	Pyronaridine-artesunate n=53		**Artemether-lumefantrine** n=27	
	n	**%**	**n**	**%**
Any drug related AE	**6**	**11**	**6**	**22**
Vomiting	2	4	1	4
Abdominal pain	1	2	2	8
Diarrhea	1	2	1	4
Blood creatin-phosphokinase increased	1	2	0	0
Headache	0	0	2	8
Anemia	1	2	0	0

Incidence of adverse events considered drug-related by investigators.

4.4.3 Clinically Significant Abnormal Laboratory Parameter

Haematological and biochemical parameters were assessed for each patient in order to evaluate inclusion and exclusion criteria and to have reference values for evaluation of safety during the course of the study.

Haematology values at baseline were representative for children with acute uncomplicated *P. falciparum* malaria. Mean haemoglobin values were below normal range in both treatment groups, 10 g/dl in the pyronaridine-artesunate group and 10.2g/dl in the artemether-lumefantrine group. Furthermore, platelets were below normal range at baseline, 179000/µl and 201000/µl in the respective groups.

In both treatment groups, haematology results showed mean changes from Day 0 in consistence with effective anti-malarial therapy. In the pyronaridine-artesunate group, mean haemoglobin concentrations decreased on Day 3 by 0.5 g/dl and recovered by Day 28, while in the artemether-lumefantrine group haemoglobin decreased haemoglobin levels by -0.4 on Day 3 and recovered by Day 28. By Day 28, haemoglobin mean values reached significant higher levels than at baseline in both treatment groups (11.1 g/dl). Corresponding changes in RBC and haematocrit followed the same dynamics in both groups, with mean values under normal range at baseline (haematocrit: 30.2 and 30.8; RBC: 4.1 and 4.1, respectively) recovering by Day 28 and reaching higher values than in baseline (haematocrit: 33.7 and 33.7; RBC: 4.5 and 4.56, respectively).
Mean platelet values reached rapidly levels within normal ranges on Day 3 234000/µl in pyronaridine-artesunate group and 219000/µl artemether-lumefantrine group, respectively).

Mean values of white blood cell counts did not show major changes. However, mean values for percentage of eosinophils and lymphocytes rose from Day 3 until Day 28. Eosinophilia at Day 28 in both groups probably reflects endemic chronic helminthic infections of a large proportion of the patients.

Table 14. Haematology parameters

	Treatment Group							
	Pyronaridine-artesunate				Artemether-lumefantrine			
	D0	**D 3**	**D7**	**D28**	**D 0**	**D 3**	**D7**	**D28**
Haemoglobin (g/dl) *Mean(SD)*	10.0 *(1.0)*	9.7 *(1.1)*	9.65 *(1.1)*	11.1 *(1.0)*	10.2 *(1.1)*	9.8 *(1,3)*	10.3 *(1.28)*	11.1 *(1)*
Haematocrit (%) *Mean(SD)*	30.2 *(3.05)*	29.06 *(3.42)*	29.39 *(3.5)*	33.7 *(2.9)*	30.8 *(3.3)*	31.3 *(3.81)*	31.7 *(3.8)*	33.7 *(2.7)*
RBC (Mio/µl) *Mean(SD)*	4.1 *(0.5)*	3.95 *(0.55)*	4.0 *(0.6)*	4.5 *(0.5)*	4.1 *(0.53)*	4.15 *(3,81)*	4.28 *(0.61)*	4.56 *(0.49)*
Platelets (T/µl) *Mean(SD)*	179 *(95)*	234 *(96)*	360 *(145)*	277 *(90)*	201 *(73)*	213 *(98)*	361 *(121)*	292 *(81)*
WBC (T/µl) *Mean(SD)*	7.8 *(2.6)*	8.3 *(3.48)*	10.1 *(3.8)*	9.5 *(2.5)*	7.7 *(2.5)*	7.9 *(2.6)*	9.6 *(3.52)*	10.1 *(3)*
Neutrophils (%) *Mean(SD)*	39.1 *(14.4)*	27 *(9.57)*	36.6 *(37.9)*	27.7 *(10.5)*	37.8 *(15.7)*	24.65 *(8.5)*	27.3 *(7.9)*	26 *(8.8)*
Lymphocytes (%) *Mean(SD)*	39.3 *(12.6)*	48.3 *(9.59)*	45.0 *(10.6)*	47.2 *(10.1)*	37.9 *(13.5)*	50 *(10.1)*	47.9 *(9.3)*	48.9 *(9.7)*
Monocytes (%) *Mean(SD)*	9 *(2.3)*	12.4 *(4.65)*	12.5 *(3.5)*	11.9 *(9.9)*	13.1 *(4.3)*	11.4 *(3.6)*	10.9 *(4)*	9 *(2.3)*
Eosinophils (%) *Mean(SD)*	4.5 *(4.8)*	9.5 *(8.13)*	9.6 *(7.2)*	11.2 *(1.25)*	6 *(6.5)*	11.3 *(8.9)*	10.8 *(7.1)*	11.8 *(6)*
Basophils (%) *Mean(SD)*	1.27 *(0.83)*	1.3 *(0.7)*	1,4 *(0.7)*	1.25 *(0.7)*	1.12 *(0.76)*	1.25 *(0.6)*	1.14 *(0.35)*	1.11 *(0.32)*

Summary of haematology values showed by *mean* (*standard deviation*) at baseline, Day 3, Day 7 and Day 28 after treatment in the ITT population.

Clinical chemistry values were measured at baseline, Day 3, Day 7 and additionally Day 28 if needed. Clinical biochemistry results showed similar mean changes from baseline values in the two treatment groups. Baseline mean values of albumin, total bilirubin, ALT, urea, creatinine, sodium and potassium were as per-protocol criteria, within normal ranges. Moreover, creatinine-kinase, alkaline-phosphatase and glucose were also within normal ranges. Mean values of AST were slightly over normal range at baseline and normalized from Day 3 onwards. Laboratory values were classified with regards to their clinical

significance. One patient in the pyronaridine-artesunate group showed a peak alanine aminotransferase (ALT) and aspartate aminotransferase (AST) >3 times the upper limit of normal (136 IU/l and 126 IU/l respectively) on Day 3. However, total bilirubin was below 2 times the limit of normal (6.1 µmol/l) and values at baseline were already over normal range. From Day 7 and onwards values were within normal range. The episode was considered by investigators as clinically significant and classified as an Adverse Event of moderate intensity. However, the episode was considered as non-related to study medication. At the end of follow up no other abnormal laboratory value was classified as clinically significant.

Table 15. Biochemistry

	Treatment Group					
	Pyronaridine-artesunate			**Artemether-lumefantrine**		
	D0	**D 3**	**D7**	**D 0**	**D 3**	**D7**
Total Bilirubin µmol/L *Mean(SD)*	14.3 *(9.7)*	5.6 *(2.5)*	7.0 *(2.6)*	12.5 *(12.4)*	5.3 *(2.9)*	6.0 *(2.9)*
Albumin g/dl *Mean(SD)*	3.7 *(0.4)*	3.6 *(0.3)*	3.8 *(0.3)*	3.7 *(0.5)*	3.6 *(0.5)*	3.9 *(0.3)*
ALT IU/l *Mean(SD)*	19.2 *(13.7)*	21.1 *(25.5)*	17.4 *(9.6)*	19.8 *(14.3)*	16.9 *(8.6)*	16.4 *(6.3)*
AST IU /l *Mean(SD)*	38.2 *(26.6)*	32.5 *(17.8)*	30.0 *(10.7)*	37.2 *(18.8)*	33.3 *(12.6)*	30.4 *(6.5)*
Creatinine kinase IU/l *Mean(SD)*	85.9 *(31.3)*	72.0 *(69.7)*	98.3 *(81.4)*	77.0 *(39.9)*	55.8 *(29.9)*	73.0 *(39.7)*
Alk. Phosphatase IU/l *Mean(SD)*	193.4 *(57.8)*	172.6 *(59.9)*	171.5 *(55.3)*	204.9 *(56.6)*	189.0 *(53.1)*	190.3 *(49.8)*
Urea mmol/l *Mean(SD)*	7.8 *(8.9)*	7.6 *(7.6)*	7.6 *(9.2)*	7.2 *(7.4)*	7.4 *(7.5)*	7.6 *(8.0)*
Creatinine µmol/l *Mean(SD)*	34.2 *(12.3)*	34.5 *(10.9)*	35.6 *(18.3)*	36.3 *(8.4)*	35.9 *(8.4)*	35.2 *(9.9)*
Sodium mmol/l *Mean(SD)*	134.8 *(19.8)*	139.1 *(2.6)*	136.9 *(22.3)*	138.2 *(4.0)*	139.4 *(3.3)*	140.4 *(2.5)*
Potassium mmol/l *Mean(SD)*	3.9 *(0.4)*	4.0 *(0.5)*	5.1 *(0.5)*	3.9 *(0.4)*	4.1 *(0.5)*	4.4 *(0.5)*
Glucose mmol/l *Mean(SD)*	21.3 *(33.8)*	22.9 *(34.0)*	23.9 *(34.5)*	26.5 *(38.4)*	23.0 *(33.2)*	16.9 *(30.7)*

Summary of biochemistry values. *Mean* (*standard deviation*) at baseline, Day 3 and Day 7 after treatment in the safety population.

4.4.4 Vital signs, Physical Examination and ECG Abnormalities

Vital signs (blood pressure and pulse rate) were evaluated during the course of the study. Values were within the normal ranges for children and infants. As expected, mean pulse rate decreased in parallel to fever clearance from baseline in both treatment groups (117 bpm in the pyronaridine-artesunate group and 114 bpm in the artemether-lumefantrine group), reflecting the recovery from malaria episode due to effective anti-malarial therapy. None of the patients had a clinically significant ECG finding at baseline or during follow up. None of the patients had a significant medical history at inclusion.

Table 16. Vital signs

	Pyronaridine-artesunate n=53		**Artemether-lumefantrine** n=27	
	Mean systolic/diastolic blood pressure (mmHg)	Pulse rate (bpm)	Mean systolic/diastolic blood pressure (mmHg)	Pulse rate (bpm)
Baseline	94/57	117	92/54	114
Day 1	92/56	101	91/55	103
Day 2	89/54	100	92/57	101
Day 3	92/56	97	91/55	96
Day 7	89/54	95	89/53	98
Day 14	91/56	97	94/57	98
Day 21	92/58	95	92/57	103
Day 28	94/56	98	90/55	98
Day 35	92/57	95	90/53	97
Day42	89/54	95	91/53	95

Mean values of blood pressure and pulse rate in both treatment groups. Pulse rates decreased from baseline onwards probably due to fever clearance.

5 Discussion

During the past years, substantial progress has been made in the fight against malaria. In spite of this improvement, malaria remains an extraordinary cause of morbidity and mortality worldwide. In the present days, malaria is considered endemic in 97 countries and territories around the world. Estimates have shown that in 2013 malaria was the underlying cause of death for 584.000 individuals, yet children younger than 5 years in Africa accounted for 78% of all deaths [1]. Malaria is a curable disease provided prompt diagnosis and adequate treatment. It is well known that early diagnosis and rapid and effective treatment of malaria disease shortens its duration and prevents the development of complications and the vast majority of deaths from malaria. Great improvement has been made in the development of effective antimalarial medicines since the emergence of resistance to former drugs. At present, the World Health Organization recommends treatment of *P. falciparum* malaria with combination therapies, preferably those containing an artemisinin derivative in all countries experiencing resistance to monotherapies, such as chloroquine, sulfadoxine-pyrimethamine and amodiaquine. Artemisinin-based combination therapy has been demonstrated as a critical tool for the effective treatment and control of *P. falciparum* malaria. Development of efficacious ACT should take into account characteristics such as high schizontocidal and gametocyticidal activity, widespread accessibility and good tolerability. Moreover, short regimens, affordable price and simple administration are critical aspects for paediatric populations. However, there is a lack of adequate paediatric drug formulations for most current antimalarial treatment combinations. Evaluation of safety, tolerability, efficacy and effectiveness of artemisinin-based combination therapy in vulnerable populations living in high malaria transmission areas is one of the high priority issues for operational research. The relatively limited experience with implementation, registration, marketing, reliable supply and drug quality as well as costs and affordability of artemisinin based combination therapy result in a major challenge regarding the adequate treatment of malaria in African

regions. Moreover, the potential problems with adherence to co-administered non-fixed drug combinations need to be underlined [94].

This trial aimed to contribute to the knowledge on the use of pyronaridine-artesunate combination therapy in African children. The study comprised of 80 Gabonese children and infants <12 years old suffering from acute monoinfection of *P. falciparum* malaria. As young children have often a less favorable response to antimalarial drugs than older children and adults even in areas of low malaria transmission, the World Health Organization's recommendations are to emphasize treatment evaluations in young children [88]. Our trial population matched therefore with these recommendations targeting infants and children below 5 years of age.

Among all included patients, only one patient was lost to follow-up due to consent withdrawal. Follow-up rate was then 99 % over the whole study period in the clinical trial. Two patients had reappearance of parasitaemia at Day 28 and three at Day 42. The study could be therefore analyzed for efficacy and safety in 79/80 subjects. As expected in a randomized trial, the distribution of background characteristics was comparable between the two groups. Baseline clinical and laboratory characteristics as well as vital signs were found to be representative of children suffering from acute falciparum malaria in an endemic location (i.e. high frequency of heart rate, common hepato-splenomegaly anemia, and thrombocytopenia). No significant differences regarding clinical and laboratory findings were found between the two groups.

Patients in the pyronaridine-artesunate group received per dose 7.0: 2.3 mg/kg to 13.3: 4.4 mg/kg of pyronaridine tetraphosphate and artesunate sachet-contained and diluted in non-carbonated liquid up to 150 ml. Patients in the artemether-lumefantrine group received per dose 1.6: 9.6 mg/kg to 4.0: 24 mg/kg of artemether and lumefantrine respectively from crushed tablets and diluted in up to 150 mL of non-carbonated liquid. In the pyronaridine-artesunate group administration was once every 24 hours for three days.

The definition for the primary efficacy endpoint was Day 28 PCR-corrected ACPR higher than 90%. The Per-Protocol population analysis showed that

treatment with pyronaridine-artesunate granules of acute uncomplicated *P. falciparum* malaria in children had a Day 28 PCR corrected ACPR of 100% (95% CI 93.2 to 100). Introduction of a new antimalarial requires an efficacy of >95% [13]. This trial was statistically powered as part of the entire multicentre study (n=600) to demonstrate whether a cure rate higher than 90% could be achieved. This was based on the assumption that cure rates would achieve 95% of efficacy, based on previous studies [76] and because of practical reasons in setting a reasonable sample size. Results in our study site were highly reassuring by reaching the primary efficacy endpoint and when considering the characteristics of the site and trial population: age of the target population and high transmission area hyperendemic for *P. falciparum* malaria [79]. Moreover, these results are consistent with the high rates of efficacy reported in previous Phase III efficacy trials conducted in children and adults with uncomplicated acute *P. falciparum* malaria and treated with pyronaridine-artesunate [74, 75]. In 2012, Rueangweerayut et al showed that pyronaridine–artesunate was non-inferior to mefloquine plus artesunate against *P. falciparum* malaria for the primary outcome: Day 28 adequate clinical and parasitological response in the Per-Protocol population, PCR-corrected for reinfection. Efficacy in the group treated with pyronaridine– artesunate tablets was 99.2%. Moreover, Tschefu et al showed in 2010 that PCR-corrected ACPR cure rate at Day 28 of patients with uncomplicated *P falciparum* malaria was 99·5% (780 patients; 95% CI 98·7–99·9) in the pyronaridine-artesunate group , in a multicentre clinical trial in Africa comparing the safety and efficacy of pyronaridine-artesunate with that of artemether-lumefantrine.

The primary efficacy outcome in this clinical trial was not assessed in the youngest age group as no infants under 1 year old were included. Children aged 1–5 years who met the primary endpoint and outcomes were similar to the older children. Thus, further data to assess to efficacy and safety in very young children (<1 year old) are still needed. More extensive clinical use of pyronaridine-artesunate in older children and adults should be evaluated to demonstrate safety before further studies are conducted in very young children.

Regarding the main secondary objective, this study showed that the efficacy of pyronaridine-artesunate is non-inferior to that of artemether-lumefantrine for treating acute uncomplicated *P falciparum* malaria in children, measured by PCR-corrected ACPR rate at Day 28. This is consistent with previous studies [75]. Both treatments were highly efficacious with cure rates of more than 95% and no early clinical failures. The efficacy of artemether-lumefantrine was consistent with recent reports from other studies in the African and southeast Asian countries [95-98]. Additionally, the Day 14 PCR corrected ACPR, as a secondary efficacy objective, was equally high in the per protocol population of both treatment groups (100%).

Similarly to the results of the multi-centric study, PCR-corrected rates at Day 42 were non-significantly different in the pyronaridine-artesunate group than in the artemether-lumefantrine group [76]. This is in contrast with previous Phase III clinical trials with pyronaridine-artesunate (tablet formulation) where crude ACPR rates at Day 28 and PCR-corrected and crude ACPR rates at Day 42 were higher in the pyronaridine-artesunate group than in the artemether-lumefantrine group [75]. Moreover, shown in the previous Phase III study, the prophylactic effect at Day 42 of pyronaridine-artesunate tablet formulation was significantly greater when compared to artemether-lumefantrine (p-value=0.007). This could be attributed to differences in the transmission rates within both studies, or because in the previous study more than half of the patients (57%) were older than 12 years and no children under 5 years was included. The greater immunity in older children to *P. falciparum* infection may have led to a more prolonged prophylactic effect.

As expected, both treatments reduced parasitaemia rapidly. Parasite clearance was more rapid in the pyronaridine-artesunate group than in the artemether-lumefantrine group, with the greatest difference between groups seen before Day 2 (p-value=0.036), consistent with previous observations [74, 75]. The artemisinin component has been pointed out as mostly responsible for the rapid parasite clearance. This has been described in studies of other combinations of ACT containing dihydroartemisinin or artesunate when compared with artemether [97, 98]. Conversion of artesunate into the active form of

dihydroartemisinin is faster and more complete than that of artemether. There is evidence to suggest that the relative oral antimalarial bioavailability of artemether compared with artesunate is lower on the first day of treatment (58%, 95% CI 40–76) than on the second day (72%, 44–118; p=0.018) [99]. Food intake might also have an effect; artesunate is water soluble, whereas artemether is lipid soluble and its absorption is increased when taken with food. As the effect of fat for optimizing the absorption of artemether-lumefantrine is well-known, this study followed the local recommendations with regards to food or milk at the time of drug administration. Though the different local recommendations might potentially introduce some variability, results showed that the efficacy of artemether-lumefantrine was high across all sites included in the trial. Moreover, patients with malaria are often sick on the first day of treatment and might be unable to eat, potentially reducing absorption of artemether. However, even with the six-dose artemether-lumefantrine regimen, doses of artemether might not be completely effective in some patients. In this study, the mean artemether dose received was 1.7 mg/kg, ranging from 0.9 mg/kg to 2.4 mg/kg; artesunate doses ranged from 2.3 mg/kg to 4.7 mg/kg. Pyronaridine-artesunate can be given regardless of food intake, as there is no known significant food effect associated with the drug.

Fever clearance rate was clinically satisfactory in both treatment groups and did not differ between groups. Median fever clearance time was not significantly different with pyronaridine-artesunate (11.5 h; 95% CI 8-16h) versus artemether-lumefantrine (9.5 h; 95% CI 8-24; p-value=0.69). However, due to concomitant antipyretic treatment in more than half of all patients, no difference would be expected.

None of the patients showed parasitaemia on Day 2 higher than on Day 0, nor parasitaemia on Day 3 with temperature > 37.5°C, nor parasitaemia on Day 3 > 25% of count on Day 0. Thus, no patient met the World Health Organization criteria for early treatment failure with regards to parasite and fever clearance. Similarly, no World Health Organization criteria for late clinical failure (presence of parasitaemia on Day 3 with development of severe malaria or presence of parasitaemia an temperature > 37.5°C on any day from Day 4 to Day 14) or late

parasitological failure (presence of parasitaemia on Day 14 and temperature < 37.5°C) was met by any patient [8, 100].

All patients presenting gametocytaemia at baseline and patients presenting new occurrences after inclusion were cleared before Day 28. Complete gametocyte clearance was achieved on Day 28 in the pyronaridine-artesunate group on Day 14 in the artemether-lumefantrine group. The percentages of patients cleared from gametocytes are in line with the good gametocyticidal effect of artemisinins leading to reduced infectivity to mosquitoes by gametocytes in peripheral blood [101, 102]. The gametocyticidal effect of artemisinins is one of their proposed advantages compared to other drugs such as chloroquine or sulfadoxine-pyrimethamine known to induce gametocytogenesis [103-105]. However, transmission is only reduced but not prevented by artemisinin-based treatments [106] and some authors even claim that the gametocyticidal effect of antimalarials favor selection of resistant strains and therefore spread of resistance [107].

Both treatments were found to be well tolerated. Overall tolerability of pyronaridine-artesunate was consistent with that found for both components given in monotherapy [43, 67, 70, 71], as well as with that found in previous clinical trials with fixed oral tablet and granule formulations [74, 108, 109]. The most common drug-related complaints recorded in previous studies evaluating pyronaridine-artesunate were mild gastrointestinal disturbances such as mild abdominal pain, diarrhea, nausea and vomiting [110]. Other complaints usually included dizziness, headache, palpitation and pruritus. Regarding the comparator drug, artemether-lumefantrine has been widely and consistently studied and is registered in accordance with internationally recognized guidelines [111-114], The most commonly reported as possibly related adverse effects following artemether-lumefantrine therapy have involved the gastro-intestinal system (abdominal pain, anorexia, nausea, vomiting, diarrhoea) and central nervous systems (headache, dizziness). Pruritus and rash have been reported by < 2% of patients [115]. One case-control study found irreversible

hearing impairment associated with this treatment combination [116]. However, subsequent studies could not reproduce these findings [117].

The known adverse event profiles for both pyronaridine-artesunate and artemether-lumefantrine were similar to those found in the study. The drug-related adverse events were found to be similar between the two groups though the drug-related adverse events incidence was significantly lower in the pyronaridine-artesunate group (11% versus 22% respectively). What is more, no clinically relevant differences were found according to the age group analysis of adverse events. Nevertheless, when reviewing the safety data of the whole multicentre study, distribution and incidence of adverse events were found to be similar in both treatment groups [76]. One patient treated with pyronaridine-artesunate showed a peak of AST and ALT >3xULN, while bilirubin remained below 2 times the limit of normal. Values returned within normal ranges from Day 7 onwards. The episode was considered by investigators as clinically relevant though not study drug related. Regarding the outcome peak of ALT >3xULN plus peak of total bilirubin >2xULN, the incidence in the whole multicentre study was 0.3% (1/355) in the group receiving pyronaridine-artesunate and 0.6% (1/180) in the group receiving artemether-lumefantrine [76]. Increased transaminases have been observed with pyronaridine-artesunate tablets (as ALT elevations with increased bilirubin) consistently at a similar incidence in two out of four of the preceding Phase I studies and across all the previous Phase II/III pyronaridine-artesunate clinical trials. In all patients, rises in transaminases peaked around Day 7 and levels were decreasing or even had normalized by Day 28. At this time point, no instances of Grade 3 and/or 4 toxicity were observed. No clinical sequelae related to these changes in liver function were noted in any patient. Additionally, an Independent Data Monitoring Committee (IDMC) examined the safety liver function data from all of the studies. The Committee comprised six members and included three experts in hepatotoxicity. The IDMC concluded that pyronaridine-artesunate treatment is associated with transient elevated transaminases and that the early onset between Day 3 and Day 7 and its rapid resolution were consistent with a direct low-level toxicity. Furthermore, as pyronaridine-artesunate is dosed only for

three days, the risk of increasing liver injury is small [110]. Further studies should be conducted in patients as well as in healthy volunteers in order to evaluate the hepatic safety profile of pyronaridine-artesunate when administered more than once.

Abnormal haematology values at baseline were representative for children with acute uncomplicated *P. falciparum* malaria and consisted mostly of low haemoglobin, haematocrit and platelet counts rising after effective malarial therapy. Nowadays, anaemia in falciparum malaria is explained not just because of haemolysis of infected erythrocytes but also because of haemolysis of non-infected cells [118], splenic and reticulo-endothelial hyperactivity [119, 120], suppression of erythropoiesis along with dyserythropoiesis and erythrophagocytosis [121-123]. The increase of haemoglobin towards normal values is of high importance in malaria endemic areas as chronic anemia determines cognitive functioning and development of the children. Thrombocytopenia is one of the most common complications *P. falciparum* malaria [124-129]. Despite the dysfunction in the coagulation pathway and the prominent thrombocytopenia seen in severe malaria, haemorrhagic complications are relatively rare [130]. The speculated mechanisms leading to thrombocytopenia are: platelet destruction by antibody-mediation, bone marrow alterations, coagulation disturbances, splenomegaly, oxidative stress and the waste of platelets as co-factors in triggering severe malaria [131]. All patients from both treatment groups showed platelet counts within normal ranges from Day 3 onwards and therefor consistent with the recovery from malaria.

Electrocardiograph results showed no cardiac safety concerns with regarding to pyronaridine-artesunate therapy. Prolonged QT interval has been uncommonly observed with pyronaridine-artesunate (0.07%) [110]. Prolonged QT has been more frequently observed associated with the use of quinoline derivatives such as mefloquine (0.7%) and particularly chloroquine (2.7%) [132]. In 1.1% of patients receiving pyronaridine-artesunate and 0.8% with artemether-lumefantrine, bradycardia has been shown as an adverse event [110]. Even so, the finding of bradycardia in a cohort of children with acute uncomplicated febrile *P. falciparum* malaria after initiating effective treatment is probably

associated with the resolution of the fever and thus the associated tachycardia. To support this presumption, across all treatment groups decreases in mean heart rate were noted. This effect has been noticed in many other clinical trials of anti-malarial therapy while as patients become afebrile, heart rates return to the normal baseline values [99, 133-135].

The overall safety and tolerability profile of fixed oral pyronaridine-artesunate granulate formulation in acute uncomplicated *P. falciparum* malaria has been confirmed for children and infants less than 12 years old. This is in consistency with previous reports for both tablets [74, 75] and granulate formulation [73] of the study drug. In a meta-analysis by Kurth et al, it was shown that, rates of drug-related gastrointestinal adverse events including drug-induced vomiting were lower with ACT pediatric formulations compared to tablets [136]. In a previous trial, drug-related gastrointestinal adverse events in adults and children receiving pyronaridine-artesunate tablets were more common (vomiting 3.3%, other gastrointestinal adverse events 6.6%) compared to artemether-lumefantrine (1.9% and 5.2%, respectively) [75]. In this trial, the trend has been shown to reverse with pediatric pyronaridine-artesunate granule formulation (vomiting 2.0%, other gastrointestinal 2.0%) compared to artemether-lumefantrine crushed tablets (3.3% and 3.9% respectively).

Appropriate and registered pediatric drug formulation available for the treatment of young children is a major aim of drug-development programs against malaria. Young children and pregnant women are particularly vulnerable to malaria and clinical research needs to be encouraged in these target populations. Pyronaridine-artesunate pediatric granules were efficacious, safe and well tolerated in this study in Gabonese children under 12 years of age with uncomplicated *P. falciparum* malaria. Considering these data and in consistence with those of the other efficacy trials [74-76], pyronaridine-artesunate granule co-formulation strikes as being a useful ACT for treating uncomplicated *P. falciparum* malaria in children and infants.

6 Conclusion

The study assessed the efficacy and safety of a fixed dose of oral pyronaridine-artesunate granule formulation (60:20) (paediatric Pyramax®) compared to artesunate-lumefantrine (Coartem®) crushed tablets in infants and children with acute uncomplicated *P. falciparum* malaria. The study was part of a multicenter, comparative, randomized, open-labeled, parallel group study on the efficacy, safety and pharmacokinetics of the investigational drug conducted in a total of 534 male and female infants and children (between ≥ 5 kg and < 25 kg body weight) suffering from acute symptomatic uncomplicated *P. falciparum* malaria recruited from study sites located in Africa and the Philippines.

A total of 80 subjects with similar demographic and clinical characteristics at baseline were enrolled in the study. Fifty-three patients received pyronaridine-artesunate granule formulation (60:20 mg) daily for 3 days and 27 received artemether-lumefantrine crushed tablets as drug comparator. The primary outcome was achieved as the cure rate at Day 28 in the pyronaridine-artesunate group (PCR-corrected ACPR in the efficacy-evaluable population) was of 100%. Moreover, non-inferiority of the investigational drug compared to artemether-lumefantrine was demonstrated on Day 28 and on Day 42 in the efficacy-evaluable population. Furthermore, parasite clearance was significantly shorter with pyronaridine-artesunate. Overall adverse events incidence and severity were similar between groups. Neither serious nor adverse events leading to study neither drug discontinuation nor withdrawal occurred in the course of the study. Adverse events considered by investigators as drug related occurred in 11% of the patients receiving pyronaridine-artesunate and were mostly mild gastrointestinal disorders. Pyronaridine-artesunate pediatric granules were efficacious, safe and well tolerated in this study in children under 12 years of age with uncomplicated *P. falciparum* malaria. Pyronaridine-artesunate granule co-formulation appears to be a valuable ACT for use in children and infants with uncomplicated *P. falciparum*.

7 Definitions

Adequate Clinical and Parasitological Response (ACPR):

ACPR is defined as patients with clearance of asexual parasitaemia without recrudescence within 28 days of initiation of study treatment and not meeting other criteria of early treatment failure, late clinical failure and late parasitological failure.

Parasite clearance:

At least two consecutive negative smears for asexual parasites obtained within an interval of 8 to 24 hours post-dosing.

Fever clearance:

At least two consecutive normal body temperature measurements obtained within an interval of 8 to 24 hours post-dosing.

New Infection:

New infection is defined as the appearance of asexual parasites after clearance of initial infection with a genotype different from those parasites present at baseline. New infection must be confirmed by microscopy (positive blood smear) and PCR analyses. Confirmed new infection will not be regarded as treatment failure or recrudescence.

Recrudescence:

Recrudescence is defined as the appearance of asexual parasites after clearance of initial infection with a genotype identical to that of parasites present at baseline.

Recrudescence must be confirmed by microscopy (positive blood smear) and PCR analyses. Confirmed recrudescence is regarded as treatment failure.

Treatment failure:

Treatment failure is classified as: early treatment failure, late clinical failure and late parasitological failure and is based on WHO Guidelines 2005.

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