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# Efficacy, tolerability and safety of fosmidomycin and piperaquine as a non-artemisinin combination therapy in children with uncomplicated malaria

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# Abbreviations

| | | |
|---|---|---|
| 4ABC | - | *Four Artemisinin-Based Combinations* |
| ACPR | - | *adequate clinical and parasitological response* |
| ACT | - | *artemisinin combination therapies* |
| AE | - | *adverse events* |
| AESI | - | *adverse events of special interest* |
| A-L | - | *artemether-lumefantrine* |
| ALAT | - | *alanine aminotransferase* |
| AS-AQ | - | *artesunate-amodiaquine* |
| ASAT | - | *aspartate aminotransferase* |
| AS-MQ | - | *artesunate-mefloquine* |
| AS-SP | - | *artesunate-sulfadoxine-pyrimethamine* |
| CERMEL | - | *Centre de Recherches Médicales de Lambarené* |
| CRF | - | *case report form* |
| CRMN | - | *Centre de Recherches Médicales de la Ngounie* |
| CTCAE | - | *The Common Terminology Criteria for Adverse Events* |
| D14 | - | *day 14* |
| *D21* | - | *day 21* |
| *D28* | - | *day 28* |
| *D35* | - | *day 35* |
| *D42* | - | *day 42* |
| *D63* | - | *day 63* |
| *D7* | - | *day 7* |
| *DHA-PQ* | - | *dihydroartemisinin-piperaquine* |
| *DNA* | - | *deoxyribonucleic acid* |
| *DOXP* | - | *1-deoxy*-D-*xylulose 5-phosphate* |
| *FCT* | - | *fever clearance time* |

| | | |
|---|---|---|
| *FOS-AS* | - | *fosmidomycin-artesunate* |
| *FOS-CM* | - | *fosmidomycin-clindamycin* |
| *FOS-PQ* | - | *fosmidomycin-piperaquine* |
| *GCLP* | - | *good clinical laboratory practice* |
| *GCP* | - | *good clinical practice* |
| *Hb* | - | *hemoglobin* |
| *HIV* | - | *human immunodeficiency virus* |
| *ITT* | - | *intention to treat population* |
| *MedDRA* | - | *Medical Dictionary for Regulatory Activities* |
| *MMV* | - | *Medicines for Malaria Venture* |
| *MSP1* | - | *merozoite surface protein 1* |
| *MSP2* | - | *merozoite surface protein 2* |
| *nPCR* | - | *nested PCR* |
| *P. falciparum* | - | *Plasmodium falciparum* |
| *P. knowlesi* | - | *Plasmodium knowlesi* |
| *P. malariae* | - | *Plasmodium malariae* |
| *P. ovale* | - | *Plasmodium ovale* |
| *P. vivax* | - | *Plasmodium vivax* |
| *PCR* | - | *polymerase chain reaction* |
| *PCT* | - | *parasite clearance time* |
| *PfEMP1* | - | *erythrocyte membrane protein 1* |
| *PfHRP2* | - | *Histidine rich protein 2* |
| *PfRh5* | - | *recticulocyte binding protein homologue 5* |
| *PP* | - | *per protocol population* |
| *QTc* | - | *QT interval corrected for heart rate* |
| *QTcB* | - | *QT interval corrected for heart rate by the Bazett formula* |
| *QTcF* | - | *QT interval corrected for heart rate by the Frederica formula* |
| *$R^2$* | - | *coefficient of determination* |
| *RDT* | - | *rapid diagnostic test* |
| *SP* | - | *sulfadoxine-pyrimethamine* |
| *sum FIC* | - | *sum of fractional inhibitory concentrations* |

# 1 Introduction

## 1.1 Malaria

Malaria is one of the most challenging infectious diseases known to mankind. The most recent World Malaria Report by the World Health Organization (WHO) estimates that 228 million malaria cases and 405,000 malaria deaths occurred worldwide in 2018 [1]. Most deaths occur in young (<5 years) Sub-Saharan African children [1]. Malaria has been successfully eradicated from the USA, Canada, Europe and Russia but transmission is still ongoing in 95 mostly tropical or sub-tropical countries inhabited by roughly 3 billion people [2–4]. After a resurgence of prevalence from 1970 to 2000 many financial and political efforts led by the Millennium Development Goals from 2000 onwards have been made to fight malaria [4].

Malaria is caused by a group of sporozoan parasites called plasmodia that are transmitted to humans via the female Anopheles mosquito [5]. Plasmodia reproduce in the red blood cells of its host and cause fever as well as potential multiple organ failure in the afflicted [3]. There are 5 different species of the genus plasmodia parasites causing malaria infection in humans: Plasmodium falciparum (P. falciparum), Plasmodium vivax (P. vivax), Plasmodium ovale (P. ovale), Plasmodium malariae (P. malariae) and the simian Plasmodium knowlesi (P. knowlesi) [5]. Most human infections are caused by P. falciparum and P. vivax [1]. The disease caused by P. falciparum is called malaria tropica and accounts for the great majority of malaria deaths [5]. The following dissertation will only deal with P. falciparum infection.

### 1.1.1 Epidemiology

The main determinants of the transmission rate of malaria are related to the mosquito vector. The malaria-transmitting Anopheles species mostly thrive in tropical and sub-tropical regions, especially Sub-Saharan Africa. In countries with stable transmission (much of Sub-Saharan Africa) P. falciparum predominates and it is young children that are most affected [3].

#### 1.1.1.1 Global

The epidemiologic data presented in this section refers to the World Malaria Report 2019 published by the World Health Organization (WHO) [1] if not otherwise noted.

Regarding global prevalence and mortality malaria is the most dangerous human parasitic infection. Most of the cases fall on the African region* (93%), followed by the South East Asian region (3%) and the Eastern Mediterranean region (2%). The great majority of cases is caused by P. falciparum infections, especially in the African region (99.7%). Globally about 3% of cases are caused by P. vivax infection. Notably, in regions other than the African P. vivax accounted for an estimated 36% of malaria cases [6]. In the African region the incidence rate was highest with 229 cases per 1000 population in 2018. Regarding mortality an estimated 405,000 deaths were caused by malaria worldwide of which approximately 380,000 deaths fell on the African region (94%) in 2018. 272,000 deaths (67%) were estimated to be in children below 5 years.

In the Millennium Development Goals established by the Millennium Summit of the United Nations in 2000 one of the goals were to halt and begin to reverse the incidence of malaria by 2015. This goal has been achieved following the Millennium Development Goals Report 2015 [7]: between 2000 and 2015 the global incidence and mortality rate of malaria have decreased by 37% and 58% respectively. Unfortunately, this decline in global malaria incidence has stalled since 2014 and the decline of global mortality has slowed down significantly in the period between 2016 to 2018. In the light of current trends the 2020 milestones defined in the WHO Global Technical Strategy for malaria (GTS) [8] of a 40% reduction in global incidence and mortality when compared to 2015 will not be met. The latest developments therefore highlight the importance to intensify the efforts in the fight against malaria.

#### 1.1.1.2 Gabon and the area around the study site in Lambaréné

Gabon is a country with stable and high transmission throughout the year due to its tropical climate. The often poor housing and health standards when compared to global standards are another factor why malaria aside other infectious diseases like tuberculosis and HIV accounts for much of the high burden of disease in Gabon [9]. According to the latest WHO country profile of Gabon [10] the malaria incidence (per 1000 population at risk in one year) was estimated to be 248 in 2018 which is slightly higher than the African

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*All the regions in this section refer to the regions defined by the WHO: http://www.who.int/about/regions/en/ [last accessed: August 22, 2018]

average of 229 [1]. Gabon had an estimated 342,000 malaria cases and 505 malaria deaths in 2017 [10]. A study conducted by Zoleko Manego et al. [11] in the area around Lamabaréné shows the epidemiological features of malaria representative for the study site. In this study the overall malaria infection prevalence was 37% and the infection rate in 2-10-year-old asymptomatic children was 46%. The predominant species causing malaria infection was P. falciparum with 79%.

## 1.1.2 Biology

The following sections regarding the biology of malaria refer to Manson's Tropical Diseases (23rd edition) from 2014 [5] if not otherwise noted.

### 1.1.2.1 Plasmodia

Plasmodia are protozoan or more specifically sporozoan parasites of the phylum Apicomplexa that infest red blood cells of mammals, birds and reptiles. All Plasmodium species are transmitted via mosquito bites. There are 4 Plasmodium species that preferentially befall human (Plasmodium (P.) falciparum, P. vivax, P. ovale and P. malariae) and one simian species that can also befall human (P. knowlesi). Since the study was only aimed at curing uncomplicated malaria tropica caused by P. falciparum this introduction will furthermore only deal with the characteristics of P. falciparum.

#### 1.1.2.1.1 Life cycle

When a female anopheline mosquito blood feeds on a human it inoculates motile sporozoites into human vessel or tissue. The sporozoites are in the larger salivary ducts of the mosquito and approximately 8-15 sporozoites per blood feed are injected. The sporozoites are transported via lymph and blood circulation to the liver where they invade hepatocytes. Within the hepatocyte the sporozoites replicate asexually and become a hepatic schizont. One sporozoite can produce 10,000 to more than 30,000 merozoites within 5.5-8 days. The pre-erythrocytic or hepatic phase is asymptomatic for the human host.

When the hepatic schizont bursts all the merozoites are released into the bloodstream and start to invade erythrocytes. The merozoites resemble motile sporozoites and have specific erythrocyte binding surface proteins to attach to the erythrocyte. The intraerythrocytic merozoites are now called trophozoites. The trophozoites feed of the erythrocyte's contents, mostly hemoglobin and grow logarithmically in size. The parasite

disposes of the toxic by-product hem by transforming it into hemozoin. After 12-14 hours the parasite begins to exhibit the erythrocyte membrane protein 1 (PfEMP1) that facilitates cytoadherence to the endothelium of venules and capillaries [12]. By adhering to the endothelium maturing parasites vanish out of the free blood stream and thereby escape microscopic observation. This process is called sequestration. After approximately 36 hours the trophozoite starts to asexually reproduce by nuclear division and becomes a blood stage schizont. The erythrocyte ruptures and 6-36 new merozoites are being released into the blood, each of which can repeat another asexual cycle within an erythrocyte. One asexual life cycle takes approximately 48 hours and parasite expansion is logarithmic at approximately 10-fold per cycle.

After 7-10 days of asexual replication next to the asexual merozoites a subpopulation of parasites develops into sexual gametocytes [13]. Gametocytes can transmit the infection. When a mosquito blood feeds on the infected human, the male and female gametocytes enter the mosquito's gut and their respective haploid micro- and macrogametes form a zygote that becomes an oocyst in the mid-gut of the mosquito. When the oocyst ruptures millions of sporozoites are released into the mid-gut from where they migrate into the large salivary ducts. When the mosquito blood feeds again on a human the whole cycle begins anew.

#### 1.1.2.2 Host

In regions where malaria transmission is hyperendemic or holoendemic, as it is in most of tropical Africa, people are infected repeatedly throughout their lives from early infancy onwards with up to 3 infectious bites per day [5]. Babies often are protected from severe malaria in the first 6 months after birth by maternal immunity and a higher proportion of fetal hemoglobin (HbF) [14, 15]. After this protected period young children <5 years in highly endemic regions are at highest risk for developing severe malaria symptoms as they have not acquired a sufficient level of immunity yet [16]. Repeated and constant transmission lead to partial immunity and even premunition (asymptomatic infection) in older children and adults [17, 18].

### 1.1.3 Pathophysiology

One of the main reasons underlying the clinical features of malaria is the excessive cytokine release triggered by parasitic and erythrocyte material freely circulating in the

blood stream after erythrocyte rupture [19]. Aside other factors cytokine release accounts for the development of fever, hypersplenism and suppression of erythropoiesis [20–24]. Hypersplenism and suppression of erythropoiesis present important etiologic factors for the development of anemia [20, 25]. The etiology of anemia in patients with malaria is complex. One very important factor seems to be that aside infected many uninfected erythrocytes are being cleared by the reticuloendothelial system [26, 27]. Uninfected erythrocytes show a loss of deformability [5]. This seems to be due to increased oxidation of membrane components and hematin-membrane interaction due to parasitic hemozoin production (section 1.1.2.1.1) [28–30]. Also, uninfected erythrocytes often seem to be marked by the immune system for phagocytosis due to reasons that are not fully understood yet [31, 32].

Sequestration, meaning the process of parasitized erythrocytes binding to the endothelium of capillaries and venules, plays a key role in the development of severe malaria symptoms. The sequestered erythrocytes tend to bind other uninfected erythrocytes via a process called rosetting [33]. These erythrocytes clump together with adjacent platelets which leads to partial blood flow obstruction, endothelial barrier disorders and inflammation [34]. The organs most affected are the brain and the kidney leading to cerebral malaria and acute renal failure [35, 36].

## 1.1.4 Clinical features

### 1.1.4.1 Clinical course

The incubation time of a P. falciparum infection is about 2 weeks [5]. The clinical course of malaria can be categorized into uncomplicated and complicated or severe malaria.

#### 1.1.4.1.1 Uncomplicated malaria

Uncomplicated malaria is any symptomatic malaria infection that has been confirmed by an appropriate parasitological test and does not present signs of severe malaria [5]. The initial symptoms of uncomplicated malaria are non-specific. Patients can present with headache, malaise, abdominal discomfort and myalgias up to 2 days before fever sets in [5]. Then the fever comes with quickly and highly rising temperatures, in children and non-immune individuals often above 40°C [37]. These febrile paroxysms can be accompanied by chills with shivering, worsening headache, fatigue, arthralgias, dry cough, tachycardia, anorexia, diarrhea or constipation and vomiting [37, 38]. In P. falciparum malaria no distinct time rhythm of fever paroxysms can be observed. This is

because the life cycles are not synchronized as in the other malaria species causing human infection [5].

In the physical examination the patient can present signs of anemia, tachycardia, tachypnea, low blood pressure, spleno- and/or hepatomegaly [37].

In the laboratory assessment the patient may demonstrate normochromic normocytic anemia, thrombocytopenia, slight monocytosis and lymphopenia [37]. Inflammatory markers like C-reactive protein, procalcitonin, fibrinogen and immunoglobulin levels are often raised [5]. Liver as well as kidney functioning parameters such as bilirubin, transaminases, the hepatic alkaline phosphatase, serum creatinine and blood urea nitrogen can also be elevated [5].

##### 1.1.4.1.2 Severe malaria

In highly endemic areas especially young children <5 years, pregnant women, immune suppressed patients (e.g. AIDS patients) and outsiders without partial immunity are endangered to develop severe malaria [5]. The clinical course of malaria is judged severe if one or more vital organs are affected in a potentially life-threatening way in the presence of P. falciparum parasites or if the amount of infected erythrocytes is >10% [39]. Referring to the WHO research definition from 2014 [40] the following conditions define severe malaria: altered consciousness with or without seizures (cerebral malaria), severe anemia, acute kidney injury, pulmonary edema, metabolic acidosis and hypoglycemia among others. The mortality of severe malaria is approximately 10% in children and 15% in adults [5]. 1-3% of adults and 10-23% of children suffer from persistent neurological deficits such as language deficits, increased risk of epilepsy or even hemiparesis after surviving cerebral malaria [5]. Many of the substantial deficits recover rapidly but subtle impairment is estimated to persist in many cases [41].

##### 1.1.4.1.3 Malaria in children

As mentioned in section 1.1.2.2 young children have not acquired sufficient immunity yet and are therefore a lot more frequently affected by severe malaria than older children and adults in highly endemic regions [5, 16]. Common clinical features of severe malaria in children are convulsions, coma, hypoglycemia, metabolic acidosis, severe anemia, and defects in cognition and other neurodevelopmental capacities [5, 37].

#### 1.1.4.2 Diagnostic

Malaria infection is a parasitological diagnose. That means clinical malaria symptoms

must be confirmed by parasitological assessment. This can either be performed by light microscopy, rapid diagnostic test (RDT) or molecular tests. The clinical gold standard is the microscopic analysis of a thick and thin blood smear that is stained with *Giemsa* stain [5]. It allows differentiation between different parasite species and quantification of parasitemia. The detection threshold of light microscopy when performed by an experienced microscopist is around 4 to 20 parasites/µl [42].

Rapid diagnostic tests can detect parasite antigens by antibody binding. It is simple to perform and shows results within 15-30 minutes but quantification is not possible [43]. Most current tests for P. falciparum detect the Histidine-rich protein 2 (PfHRP2) which shows a sensitivity comparable to light microscopy [44, 45]. The specificity is generally also very good but because the PfHRP2 clearance from the blood is very slow these tests can remain positive for up to 1 month after acute infection [46]. So even though the infection has already been successfully treated and parasites have been eradicated the PfHRP2 rapid diagnostic test can show positive results for up to 1 month.

The gold standard to detect malaria parasites in a research setting is the molecular analysis, e.g. nested PCR [47]. Molecular methods are more reliable in quantification and differentiation of species than light microscopy, especially in very low parasite densities [48, 49].

## 1.2 Antimalarial chemotherapy

The most essential pillar of the fight against malaria is still a highly effective and well-tolerated antimalarial chemotherapy. Observing current trends in malaria epidemiology and potential alternatives like vaccination antimalarial chemotherapy will most likely continue to be indispensable for many years to come. Therefore, clinical development of new antimalarial compounds must keep up with the surging drug resistance of P. falciparum parasites (section 1.2.2).

### 1.2.1 Pharmacological groups

There are 4 major groups of antimalarials: quinoline derivatives, artemisinin derivatives, antifolates and antimicrobials.

#### 1.2.1.1 Quinoline derivatives

Chloroquine, amodiaquine, quinine, quinidine, mefloquine, primaquine, lumefantrine, halofantrine and piperaquine belong to the group of Quinoline derivatives. The mode of action is not completely understood. According to the review by Kaur et al. [50] the

alkaline drugs accumulate in the acid food vacuole of the parasite and seem to selectively inhibit the hem degradation via different pathways. In the absence of hem detoxification free radicals develop and harm the parasite. Quinoline derivatives often have a long half-life and good efficacy so that they match well with the fast but short-acting artemisinin derivatives [5]. Therefore, many artemisinin combination therapies (ACT) have a quinoline-like partner drug such as artemether-lumefantrine (A-L), artesunate-amodiaquine (AS-AQ) and dihydroartemisinin-piperaquine (DHA-PQ).

#### 1.2.1.2 Artemisinin derivatives

Artemisinin derivatives are the most effective antimalarials discovered so far. Since 2006 ACTs are the first-line treatment for acute malaria by WHO recommendation and have been used excessively since then all over the world [39, 51].

The mechanism of action is still unclear. The generation of carbon-centered free radicals might play a role by alkylating critical proteins [52]. Parasite clearance is the fastest of all antimalarials and they show gametocytocidal activity [5, 53].

#### 1.2.1.3 Antifolates

Antifolates include pyrimethamine, sulfonamides and the antimalarial biguanides like proguanil. All of them inhibit folate synthesis and are only available in combination therapies such as atovaquone-proguanil or sulfadoxine-pyrimethamine (SP) [5].

#### 1.2.1.4 Antimicrobials

There are a few antibacterial agents that also have antimalarial activity. The most relevant pharmaceuticals in this group known so far are doxycycline and clindamycin which both inhibit prokaryotic protein synthesis [5]. Fosmidomycin also belongs to the antimicrobials but has a different mode of action (section 1.3.1).

### 1.2.2 Drug resistance

Since chloroquine resistance became evident in the end of the 1950s and resistance spread over the whole world in the following decades, antimalarial drug resistance has emerged as a serious threat to the fight against malaria [54]. The most serious threat to malaria control at the moment is artemisinin resistance. By now, complete or partial resistance against all groups of antimalarials can be found in P. falciparum [5]. In 2009 partial artemisinin resistance became evident on the Thai-Cambodian border and presented in markedly prolonged parasite clearance times and higher recrudescence rates [55]. Further studies have shown that partial resistance can be observed all over the WHO Greater

Mekong Subregion including Cambodia, Laos, Thailand, Vietnam, Myanmar, India and China [56]. Genotypical markers for molecular tracking of artemisinin resistance have been identified. All the validated markers are mutants of the Kelch13 gene [57]. Clinical artemisinin resistance has so far not been observed in Africa or South America [1, 58, 59]. Genetic markers of potential resistance (PfKelch13 mutations) on the other hand have already been detected at a significant prevalence (over 5%) in Guyana, Papua New Guinea and Rwanda [1]. In the WHO Greater Mekong Region P. falciparum strains can be found that show partial resistance against one (India, China, Myanmar) to up to 5 (Cambodia) ACTs [60]. Currently there are only 5 ACTs recommended by the WHO: A-L, AS-AQ, artesunate-mefloquine (AS-MQ), artesunate-sulfadoxine-pyrimethamine (AS-SP) and DHA-PQ [39]. DHA-PQ seems to be especially affected by drug resistance. In some regions like north-eastern Thailand treatment failure rates of up to 87% could be detected for DHA-PQ [61]. The highly drug-resistant parasites already pose a major threat to malaria control in these regions. If these multidrug resistant parasite strains should spread to Africa and South America, it might have disastrous consequences for global malaria control.

## 1.3 Study drugs

### 1.3.1 Fosmidomycin

Fosmidomycin, a phosphonic acid derivative, was isolated for the first time in 1980 by the *Fujisawa Pharmaceutical Company* from the bacteria *Streptomyces lavendulae* [62]. It was originally designed as an antibacterial against uncomplicated urinary tract infections, but it proved to be ineffective against recurrent infections and was not further pursued. In 1999 then Jomaa et al. [63] discovered the antimalarial activity and specific mode of action of fosmidomycin against P. falciparum parasites described as follows: P. falciparum depends on a non-mevalonate pathway as the only pathway of isoprenoid synthesis. Isoprenoids act as essential precursors of steroids like cholesterol and other important lipid groups. Fosmidomycin competitively inhibits the second parasitic enzyme in the reaction cascade of the non-mevalonate pathway, the 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, and thereby isoprenoid synthesis. The non-mevalonate pathway is a specific drug target since human isoprenoids are synthesized via the mevalonate pathway.

Pharmacological studies conducted by Kuemmerle et al. [64, 65] showed that the

gastrointestinal absorption of fosmidomycin is about 30% and that fosmidomycin is not metabolized and nearly exclusively cleared by the kidneys. It has shown to be safe in all animal and clinical studies conducted so far and no genotoxic or teratogenic effect could be observed in animals [65; animal studies have not been published, data refers to the investigator's brochure].

Fosmidomycin was then tested in several Phase 2a clinical trials as monotherapeutic antimalarial and in combination with clindamycin as well as artesunate. In all studies it demonstrated rapid blood schizonticidal activity, but recrudescence rates in some studies were higher than commonly accepted [67–73]. The review by Fernandes et al. from 2015 [66] summarizes the clinical studies that assessed fosmidomycin. Fernandes et al. estimate that the overall cure rate on day 28 (D28) of all the pediatric studies was 85% (95%CI: 71-98%). However, one pediatric study assessing fosmidomycin-clindamycin (FOS-CM) combination therapy in Mozambican children <3 years with uncomplicated malaria stood out with unacceptably bad efficacy performance. It was the only study performed exclusively in children <3 years. The day (D) 28 polymerase chain reaction (PCR-) corrected cure rate, which represents the gold standard end point in antimalarial chemotherapy efficacy studies, was only 45.9% [74]. Most of the studies including the Mozambican study were performed in combination with clindamycin as partner drug because of promising pre-clinical synergistic activity and matching pharmacokinetic characteristics [75]. Clindamycin though is known to be a weak and slow antimalarial. The pharmaceutical company developing fosmidomycin (Jomaa Pharma GmbH) therefore decided to move forward with a more potent antimalarial partner compound, namely piperaquine. This dissertation deals with the second part (young pediatric cohort) of the first study assessing the efficacy and safety of a fosmidomycin-piperaquine (FOS-PQ) combination therapy in adults and children.

### 1.3.2 Piperaquine

Piperaquine chemically belongs to the Bis-4-aminoquinolone group, in short bisquinolines, and is a chemically modified 4-aminoquinoline [50]. As described in the review by Davis et al. [76] it was first synthesized in the 1960s by Chinese researchers. It was then used extensively in the following decades in China as an alternative to chloroquine. According to the review by Davis et al. [76] its use declined with upcoming piperaquine-resistant P. falciparum strains and the appearance of more effective

artemisinin derivatives. It was rediscovered by Chinese scientists in 1990 as a potent partner drug in ACTs and is now part of the ACT DHA-PQ recommended by the WHO [73]. Like the other 4-aminoquinolines, piperaquine targets the blood stages of the parasite's life cycle and probably acts by inhibiting the detoxification of hem (section 1.2.1.1) [50]. It has shown a strong antimalarial effect against P. plasmodium in various pre-clinical and clinical studies [78].

Piperaquine is slowly absorbed (around 9 hours) and absorption is slightly increased by fats. The mean elimination half-life is long, approximately 20 days. Clearance is faster and plasma levels are lower in children than in adults so that children require relatively higher doses [76]. Piperaquine is generally well tolerated but in combination with dihydroartemisinin it showed a QT duration prolonging effect comparable to chloroquine by blocking the repolarizing potassium channel hERG [78]. The prolongation can increase the proarrhythmic risk, i.e. leading to torsade de pointes. So far despite the worldwide application for many decades no arrhythmias or serious adverse events have ever been observed, neither with piperaquine nor with its predecessor chloroquine [79].

## 1.4 Study rationale

In the light of surging artemisinin resistance in Southeast Asia effective antimalarial agents with new modes of action are needed. Fosmidomycin is one of the most advanced candidates. As described in the introduction very young children are the most endangered population in highly endemic malaria regions. Fosmidomycin has generally shown good efficacy but failed to convince in very young children when combined with clindamycin (section 1.3.1). Piperaquine is a strong antimalarial compound (section 1.3.2) and shows no relevant interaction nor toxic effect in combination with fosmidomycin in rats.

The FOS-PQ combination fulfills the non-artemisinin combination therapy (NACT) criteria by the WHO [80]: Both drugs have different modes of action and have not shown antagonistic antimalarial effect in vitro with the sum of fractional inhibitory concentrations (sum FIC) being 1.1-1.5 against chloroquine-sensitive and chloroquine-resistant strains of P. falciparum [unpublished data referring to the IB]. Fosmidomycin has rapid blood schizonticidal activity and piperaquine has a prolonged elimination half-life. Also, both have shown to be well-tolerated in other combination therapies. The study FOSPIP Part 2 and this thesis is therefore trying to assess the efficacy, tolerability and safety in the most vulnerable population, namely children aged ≤5 years. The hypothesis

was therefore defined as: The combination FOS-PQ is effective at ≥95% for the treatment of uncomplicated P. falciparum malaria in children aged ≤5 years.

# 2 Methods

## 2.1 Study design and objectives

The FOSPIP study (Evaluation of Fosmidomycin and Piperaquine in the Treatment of Acute Falciparum Malaria) was a Phase 2a open-label, uncontrolled proof of concept clinical trial (ClinicalTrials.gov Identifier: NCT02198807). Its overall objectives were to explore the efficacy, tolerability and safety of fosmidomycin sodium when administered with piperaquine tetraphosphate in a 3-day oral treatment to adults and children with acute uncomplicated Plasmodium falciparum malaria. The evaluated doses of fosmidomycin and piperaquine were administered orally in doses of 30mg/kg twice daily and 16mg/kg once daily for three days respectively.

The study was split into two parts. Each part consisted of 50 participants. 31 evaluable participants were required for a representative statistical efficacy analysis based on an anticipated cure rate of 95%, a significance level of 5% and 90% power with 95% confidence. A total of 50 patients was enrolled for additional safety data. In Part 1 only adults and children >5 years were enrolled. After analysis of the overall outcome of Part 1, Part 2 was launched. In Part 2 only children from 1 to 5 years were included. This dissertation will only describe the procedures used and assess the data obtained in Part 2 of the FOSPIP study. For safety reasons an age-step-down procedure was applied. The enrolment of the first 10 patients was initially restricted to subjects aged 3 to 5 years and with a minimum bodyweight of 10kg. The investigators, the clinical monitor and the nominated consultant were to conduct an interim review after acquiring the safety data and parasitological response at day 7 (D7) for the first 10 patients. With the agreement of all parties that it was safe, children below the age of three years with a minimum bodyweight of 5kg were also to be included.

The primary endpoint was defined as: per-protocol PCR-corrected adequate clinical and parasitological response (ACPR) at D28 (definition of ACPR see below).

Secondary endpoints were defined as:

- ACPR at D7 and day 63 (D63) in the per protocol population (PP) with and without PCR-correction

- ACPR at D7, D28 and D63 in the intention to treat population (ITT)
- Parasite clearance time
- 0-48 Log10 parasite reduction ratio
- Parasite elimination half-life
- Kaplan-Meier analyses for recurrent infections - new and recrudescent
- Kaplan-Meier analyses for subjects with:
    - gametocytes at baseline: to time of clearance of gametocytes
    - no gametocytes at baseline: to time of appearance of gametocytes
- number and severity of adverse events (AE)

Adverse events (AE) of special interest were defined as:

- Hemoglobin drop ≥ 2mg/dl
- QT interval prolongation >60ms compared to baseline or QT interval >500ms; all QT intervals were corrected for heart rate by the formulas of Frederica and Bazett (QTcF and QTcB respectively) (section 2.3.1.4)

PCR-corrected ACPR was defined as the absence of clinical or parasitological signs of malaria infection caused by the same genetic strain of parasites being present in the initial infection. The term PCR-corrected refers to the genotypical differentiation of the strain of parasites using PCR. A reinfection with a new genetic strain of parasites was not judged as treatment failure. A recrudescence on the other hand of the same initial genetic strain of parasites was judged as treatment failure (section 2.3.2.2.2). For the definition and calculation of the other efficacy outcome measures see section 2.5.1. For the definition of PP and ITT see section 2.5.3.

# 2.2 Study setting

## 2.2.1 Study sites

### 2.2.1.1 Gabon

All the study sites involved in the clinical phase of the study are located in Gabon, a central African country situated along the equator bordering on the Gulf of Guinea, Cameroon, Equatorial Guinea and the Republic of the Congo as you can see in Figure 1.


*Figure 1: Map of Gabon* [81]

The former francophone colony became independent in 1960 [82]. Politically, Gabon is organized as a presidential republic with multiparty elections in place since 1990 [83]. Its estimated per capita gross domestic product is very high with 9.720$ in comparison to other sub-Saharan African countries. Nonetheless due to unequal distribution 32.7% of the population is poor by national poverty standards [84]. Most of the national per capita wealth is due to extraction of natural goods like oil or wood and the low population density with estimated 2,230,908 residents [82]. The climate is equatorial with a mean

average temperature of 24.9 °C, two rainy seasons alternating with two dry seasons and an average humidity level of around 80% [85, 86].

As one of the first countries in Sub-Saharan Africa Gabon introduced a national state-funded health insurance in 2008 called the *Caisse national d´Assurance Maladie et de Garantie Sociale* (CNAMGS). The provided health care still remains at the level of low to low-middle-income countries as stated in a World Bank Study in 2014 [87]. The burden of disease is majorly due to infectious diseases and maternal as well as neonatal health issues. According to the WHO country profile of health [88] the top three causes of death were HIV/AIDS with 2,300 deaths, infections of the lower respiratory tract with 1,300 deaths and malaria with 1,100 deaths in 2012; The top three causes of death among children below 5 years were malaria, prematurity and acute respiratory infections.

#### 2.2.1.2 CERMEL - Lambaréné

The study was conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL) in Lambaréné. Lambaréné has around 38,700 inhabitants and is situated next to Gabon's biggest river Ogooué in the Moyen Ogooué Province (Figure 1) [89]. The city has come to global prominence because of the Albert Schweitzer Hospital that was founded by the same-named doctor next to a catholic missionary in 1913. The CERMEL is a research institution located on the compound of the Albert Schweitzer Hospital. The following information on the history and perspectives of the CERMEL refer to the report by Ramharter et al. [90]. It was founded in 1981 and comprises several laboratory departments including a clinical, parasitological, microbiological and immunological laboratory as well as a laboratory specialized on tuberculosis and HIV. Many studies on the pathogenesis, prevention and treatment of tropical diseases have been performed here with a focus on malaria. One cornerstone of the scientific activities is the development of new antimalarial chemotherapies. Among many other activities the CERMEL conducted key studies in the development of the combination therapies atovaquone-proguanil, and artesunate-amodiaquine.

The following study procedures were performed at the CERMEL:

- study design
- coordination / organization
- screening

- treatment / hospitalization
- follow-up
- laboratory analyses
- data entry / data management

FOSPIP and several other studies were collectively conducted by the OZ working group. The OZ group consists out of two fieldworkers, four nurses and a changing number of study clinicians as well as medical students.

#### 2.2.1.3 CRMN – Fougamou

Many of the follow-up visits were performed either directly at the participants' residence or in the Centre de Recherches Médicales de la Ngounie (CRMN), a small laboratory associated with the regional governmental hospital in Fougamou, Ngounie Province in about 90 km distance from Lambaréné (Figure 1). The CRMN performs routine laboratory assessments for the regional hospital and functions as a satellite research site of the CERMEL.

### 2.2.2 Study population

The study population consisted of children aged from 1 to 5 years with acute uncomplicated malaria. Most of the participants came from rural areas in and around Fougamou due to the high prevalence of malaria in that region. They lived mostly in simple wooden houses with roofs made of corrugated iron. Often the houses had no individual water and sparse electric supply. Their livelihood mostly depended on agricultural activity, small businesses and hand crafts. For most of them it was difficult to reach health facilities due to low income and far distances.

## 2.3 Study procedures

The study flow with an overview of the respective clinical and laboratory assessment are presented in Figure 2.

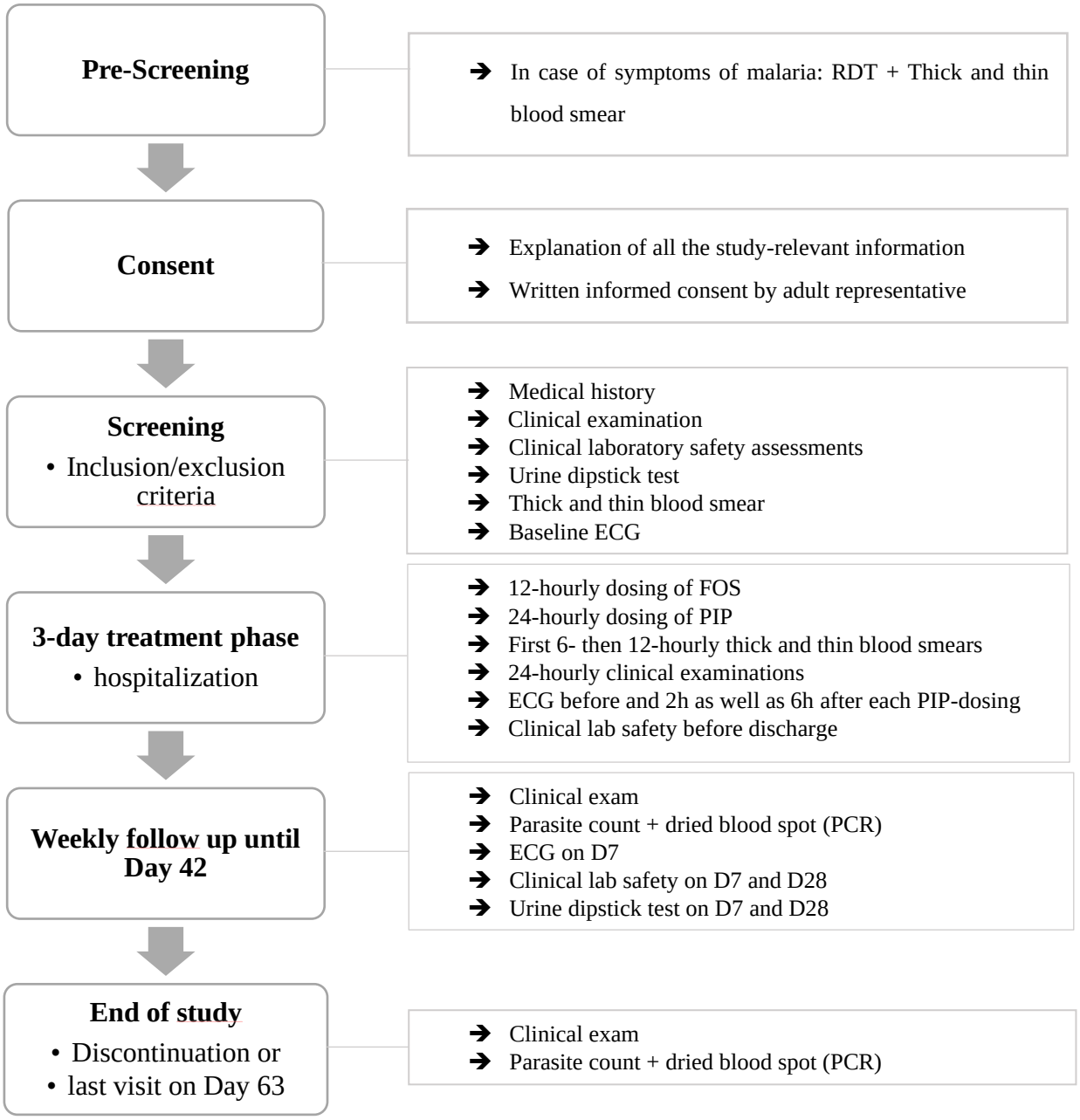



*Figure 2: Study flow + Overview of clinical and laboratory assessments*

RDT: rapid diagnostic test for malaria, Clinical lab safety: complete blood count and clinical blood chemistry, FOS: Fosmidomycin, PIP: Piperaquine, ECG: electrocardiogram, PCR: polymerase chain reaction

## 2.3.1 Clinical procedures

### 2.3.1.1 Pre-Screening

It was necessary to implement a pre-screening for a pre-selection of the study population since space and material facilities in the CERMEL was limited. The fieldworkers visited the villages around Fougamou and interviewed the residents for the presence of current or recent fever. If fever had occurred within the previous days, the fieldworkers would

explain about the CERMEL and the FOSPIP study. After guaranteeing an oral consent for the pre-screening diagnostic they performed a thick and thin smear and a rapid diagnostic test for Plasmodium falciparum malaria (PARACHECK Pf® by Orchid Biomedical Systems). The thick and thin smear were then analyzed in the CERMEL. Among the children that were positive for malaria parasites, those with a Plasmodium falciparum mono-infection with ≥1000 parasites/µl were transferred to the CERMEL and clinically screened for eligibility to the study. The children with a lower parasitemia received Artemether/Lumefantrine for three days twice daily as an antimalarial treatment given to them by the fieldworkers.

#### 2.3.1.2 Informed Consent

A study investigator explained the FOSPIP study to the patient's legal representative in detail, before initiating other screening procedures. This explanation included:

- the scientific context of malaria globally as well as locally
- the scientific background of the study drug
- the requirements for the participant and his/her parents
- the sample collection, storage and analysis procedures
- the voluntary nature of all study procedures
- the risks and benefits of the participation in the study

To ensure the voluntary consent cost-free treatment with the conventional treatment Artemether/Lumefantrine was offered alternatively. The potential cardiac and liver toxicity of the study drugs were specifically highlighted. The legal representatives of the possible participants were invited to ask questions and express concerns at any point. A copy of the consent form in French for further information was handed out.

After ensuring the legal representative had understood all relevant pieces of information, he or she was asked to sign a written declaration of consent form if agreeing for the protégé to partake. In case of illiteracy, a thumb print functioned as signature while the act of thumb printing had to be witnessed by an independent literate observer who then signed as a witness.

#### 2.3.1.3 Screening

If the consent was given a study investigator recorded the participant's demographic data and medical history. Afterwards the patient was examined thoroughly and two thick and thin blood smears, two dried blood spots for PCR analysis, one EDTA for hematology

and one Lithium-Heparin blood tube for clinical chemistry were collected (section 2.3.2.1). A urine dipstick test (Combur10 Test® by Roche AG) was performed and evaluated for possible urogenital pathologies. To weigh the participant a SECA 761 scale (quality assured by SECA GmbH until 2016) was used. The clinical laboratory then performed a full blood count plus the most important biochemical parameters such as liver and kidney functioning and electrolytes. If all the clinical and laboratory parameters aligned with the inclusion and exclusion criteria an ECG was performed with Ambu® Blue Sensor M ECG electrodes and a Welch Allyn® PC-Based Resting ECG device. The diagnostic software to display and auto-interpret the ECGs were included in the CardioPerfect® Workstation. A qualified study clinician analyzed the ECG for any relevant abnormalities. If there were no relevant abnormalities the patient was included, and an anonymous study ID was assigned in chronological order.

The **inclusion criteria** were as follows:

- Ability to swallow oral medication
- Signed informed consent from the parents or legal guardians or, if the patient/parent/guardian was unable to write, witnessed consent in accordance with local ethical guidelines
- Male and female subjects aged 1 to 5 years inclusive
- Body weight between 5kg and 25kg inclusive
- Mono-infection with Plasmodium falciparum determined by a thick and thin blood smear with asexual parasitemia between 1000 and 150,000 parasites/µL
- Fever with axillary temperature of ≥ 37.5°C OR history of fever during the previous 72 hours

The **exclusion criteria** were set to:

- Signs of severe/complicated malaria according to WHO criteria
- Mixed Plasmodium infection
- History of cardiovascular disease including symptomatic arrhythmias and clinically relevant bradycardia
- Family history of sudden death or of congenital or acquired conditions known to predispose to prolongation of the QT duration corrected for heart rate (QTc)

interval

- QTcB or QTcF >450ms at screening or prior to dosing
- Electrolyte disturbances as manifested by hypokalemia
- History of respiratory disease including active tuberculosis, gastrointestinal, hepatic and renal diseases, malignancy, neurological disorders including convulsions, psychiatric disturbances
- Severe vomiting on three or more occasions in the previous 24 hours
- Severe diarrhea on four or more occasions in the previous 24 hours
- Concomitant disease masking assessment of response including abnormal liver function tests with bilirubin >40 µmol/l, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels >2x upper limit of normal
- impaired renal function with creatinine level >2x upper limit of normal
- hemoglobin level <7.5g/dl
- white cell count >12000/µl
- History of immunological disease including Hepatitis A, B and C and human immunodeficiency virus (HIV) infection
- Severe malnutrition
- History of hypersensitivity or adverse reactions to fosmidomycin or piperaquine
- Any treatment which can induce prolongation of the QT duration
- Treatment with any antimalarial agent within the previous 63 days

#### 2.3.1.4 Treatment phase

The treatment phase took three days during which all participants were hospitalized to assure proper drug administration and monitoring of the participant's safety. The participants received six doses of Fosmidomycin sodium and three doses of piperaquine tetraphosphate. Fosmidomycin was administered every twelve hours and piperaquine every 24 hours as shown in Table 1.

**Table 1: Dosing schedule**

| Hours after treatment initiation | Dose N° | Drug(s) administered | |
|---|---|---|---|
| 0 | Dose 1 | Fosmidomycin | Piperaquine |
| 12 | Dose 2 | Fosmidomycin | |
| 24 | Dose 3 | Fosmidomycin | Piperaquine |
| 36 | Dose 4 | Fosmidomycin | |
| 48 | Dose 5 | Fosmidomycin | Piperaquine |
| 60 | Dose 6 | Fosmidomycin | |

The study medication was manufactured by Allphamed PHARBIL Arzneimittel GmbH, Germany. This included packaging and quality control. It was dispensed in accordance with a certificate of compliance and release. As FOSPIP was a single-arm Phase IIa proof of concept study there was no scheduled dosage variation, no blinding procedures nor control group. The target dose per administration was 30mg/kg for fosmidomycin and 16mg/kg for piperaquine. Fosmidomycin was formulated as sodium in capsules of either 75mg or 225mg. Piperaquine tetraphosphate was available as 320mg cross-scored tablets that could be halved or quartered if necessary. After inclusion an individual dispenser was assigned to each patient. The dispensers contained the weight-adapted study drugs to prevent confusion between different patients. This was performed following the dosing table shown in Table 2.

**Table 2: Dose per weight**

Fos: Fosmidomycin, Pip: Piperaquine

| Body weight | Target Fos dose 30mg/kg | N° of capsules | | Actual Fos dose | | Target Pip dose 16mg/kg | N° of tablets | Actual Pip dose | |
|---|---|---|---|---|---|---|---|---|---|
| kg | mg/kg | 75mg | 225mg | Total (mg) | mg/kg | mg/kg | 320mg | Total (mg) | mg/kg |
| 5 | 150 | 2 | | 150 | 30 | 80 | 0.25 | 80 | 16 |
| 6 | 180 | 2 | | 150 | 25 | 96 | 0.25 | 80 | 13 |
| 7 | 210 | | 1 | 225 | 32 | 112 | 0.5 | 160 | 23 |
| 8 | 240 | | 1 | 225 | 28 | 128 | 0.5 | 160 | 20 |
| 9 | 270 | 1 | 1 | 300 | 33 | 144 | 0.5 | 160 | 18 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 300 | 1 | 1 | 300 | 30 | 160 | 0.5 | 160 | 16 |
| 11 | 330 | 1 | 1 | 300 | 27 | 176 | 0.5 | 160 | 15 |
| 12 | 360 | 2 | 1 | 375 | 31 | 192 | 0.75 | 240 | 20 |
| 13 | 390 | 2 | 1 | 375 | 29 | 208 | 0.75 | 240 | 18 |
| 14 | 420 | | 2 | 450 | 32 | 224 | 0.75 | 240 | 17 |
| 15 | 450 | | 2 | 450 | 30 | 240 | 0.75 | 240 | 16 |
| 16 | 480 | | 2 | 450 | 28 | 256 | 0.75 | 320 | 20 |
| 17 | 510 | 1 | 2 | 525 | 31 | 272 | 1 | 320 | 19 |
| 18 | 540 | 1 | 2 | 525 | 29 | 288 | 1 | 320 | 18 |
| 19 | 570 | 1 | 2 | 525 | 28 | 304 | 1 | 320 | 17 |
| 20 | 600 | 2 | 2 | 600 | 30 | 320 | 1 | 320 | 16 |
| 21 | 630 | 2 | 2 | 600 | 29 | 336 | 1 | 400 | 19 |
| 22 | 660 | | 3 | 675 | 31 | 352 | 1 | 400 | 18 |
| 23 | 690 | | 3 | 675 | 29 | 368 | 1 | 400 | 17 |
| 24 | 720 | 1 | 3 | 750 | 31 | 384 | 1.25 | 400 | 17 |
| 25 | 750 | 1 | 3 | 750 | 30 | 400 | 1.25 | 400 | 16 |

Three hours before and after each drug administration the participants fasted if possible. The drugs were administered with water. If the participant could not swallow the capsules and/or tablets due to size, the fosmidomycin sodium and piperaquine tetraphosphate was administered as solution or suspension, respectively. Canadou sugar cane syrup by Bardinet S.A.S was added if the participant was not capable of swallowing the treatment due to bitter taste.

Following the first dose, thick and thin blood smears were performed after six and twelve hours and then 12-hourly until 72 hours after the first dosing to monitor the parasitological clearance during the course of treatment (section 2.3.2.1). Each time throughout the study when thick and thin blood smears were performed, the investigator or nurse simultaneously made two dried blood spots on a filter paper for potential genetic analysis of the parasites (section 2.3.2.2).

Clinical examinations including measurement of vital signs such as axillary body temperature, heart rate and blood pressure were performed every 24 hours. Any adverse events or administration of concomitant medication were carefully documented. Throughout treatment phase and follow up adverse events were determined by obtaining medical history and physical examination. The Common Terminology Criteria for

Adverse Events (CTCAE) [91] was used to grade severity. Adverse events were classified by primary organ system(s) affected. Terms were chosen according to the Medical Dictionary for Regulatory Activities (MedDRA), version 18.0 [92].

To observe and prevent potential arrhythmic effects an ECG was performed shortly before as well as two and six hours after each piperaquine dose. In the ECG the QT interval was of special interest to monitor the QT-prolonging effects of Piperaquine. Since the QT interval is dependent on pulse rate all QT measurements were corrected by Frederica (QTcF) and Bazett (QTcB) formula. The following conditions were defined es adverse events of special interest (AESI): 1. absolute QTcF and/or QTcB >500ms, 2. prolongation of ≥60ms comparing initial (baseline) QTcF and/or QTcB to any other time point during treatment.

Before discharge, scheduled 72 hours after treatment initiation, a clinical examination and blood sampling for clinical laboratory safety (section 2.3.2.1) was performed. Conditions for discharge of a patient were: stable clinical status and two consecutive thick blood smears negative for asexual parasites. Before discharge the participant's representative was given a schedule with follow up visits. Also, the telephone number of the responsible study investigator was handed out with the request to get in touch in case of any health issues.

#### 2.3.1.5 Follow up

Follow up visits were scheduled at D7, day 14 (D14), day 21 (D21), D28, day 35 (D35), day 42 (D42) and D63 after treatment initiation. Special assessments and events are highlighted in Figure 3.

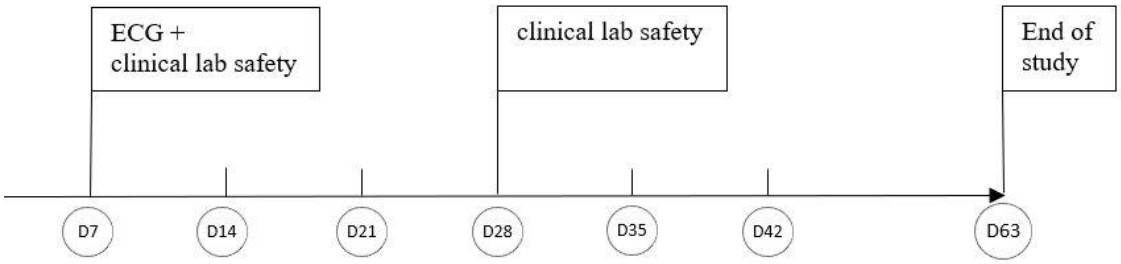



*Figure 3: Timeline of follow up visits with special assessments and events depicted in flags*

D: Day, ECG: Electrocardiography, Clinical lab safety: complete blood count and clinical blood chemistry

At each visit two thick and thin smears, two dried blood spots and a clinical examination including vital signs were performed. Any adverse events and concomitant medication

were documented. At D7 a single ECG was done to monitor late potential abnormalities. Clinical laboratory safety was monitored at D7 and D28.

#### 2.3.1.6 End of study

The end of study was defined as either completion of the study after 63 days of follow up or discontinuation from the study. Criteria for discontinuation during the treatment phase were defined as:

- Withdrawal of consent
- Deterioration in clinical condition
- QTcF and/or QTcB prolongation >500ms
- Prolongation of >60ms comparing initial QTcF and/or QTcB measurement and any further measurement during of the study
- Early treatment failure defined as:
    - parasitemia being greater or equal to that at pre-dosing 48 hours after treatment initiation or
    - positive parasitemia with axillary temperature $\geq 37.5C°$ 72 hours after treatment initiation or
    - parasitemia being $\geq 25\%$ of the pre-dosing count 72 hours after treatment initiation irrespective of axillary temperature
- Onset of severe malaria
- Onset of severe adverse events
- Vomiting within one hour of re-dosing
- Re-dosing required on more than two occasions

After completion of treatment, the discontinuation criteria were defined as:

- Persistent parasitemia 168 hours after initiation of treatment
- Parasitemia during follow-up period after initial clearance
- Onset of severe adverse events
- Lost to follow-up
- Withdrawal of consent
- Protocol violation

## 2.3.2 Laboratory procedures

### 2.3.2.1 Clinical laboratory

The clinical laboratory assessments encompassed hematology, biochemistry and electrolyte analysis as well as the thick and thin smears' lecture. The clinical laboratory safety comprised hematology, biochemistry, electrolyte and urine dipstick analysis. For hematology a full blood count was performed with the *Pentra 60* by Horiba ABX. Biochemical analysis was either performed with the *Cobas® Mira Plus* by AxonLab, *BA Mindray 88A* by Mindray or the *Reflotron® Plus* by Roche. The parameters were mostly selected to monitor the liver and kidney functioning as well as homeostasis of electrolytes, glucose and water. If the *Cobas® Mira Plus* was not available, the *SpotChem EL SE-1520* by Arkray was used for electrolyte analysis.

For the thick and thin blood smears capillary or venous blood was used. Thick and thin smears for screening were stained using a rapid stain (10% *Giemsa* solution over 20 minutes). All the other smears were stained with 3% *Giemsa* solution over 60 minutes. The smear preparation technique and staining procedures are more thoroughly described in the publication on the *Lambaréné method* by Planche et al. [93] and in the WHO training guide for basic malaria microscopy [94]. For each visit two thick and thin smears were made. Both were clearly marked with an identifier for the respective patient and the time point of the visit. One slide (containing one thick and one thin smear) was then transferred to the clinical laboratory for examination, the other was safely stored in a slide box as backup. All the microscopists were qualified by the WHO training course for basic malaria microscopy and certified by a final standardized examination provided by the WHO. To determine the parasitemia (number of parasites/µl of blood) via microscope the counting procedure *Lambaréné method* was applied. For more detailed information on the counting procedure see also Planche et al. [93]. Each slide was analyzed by two independent microscopists.

### 2.3.2.2 Molecular biology

In case of recurrent infection genotyping analysis was performed to differentiate between recrudescence and reinfection. The first half of samples (FOS052-FOS075) was analyzed in the molecular biology department of the CERMEL in Lambaréné, the second half (FOS078-FOS098) in the institute of tropical medicine of the Eberhard Karls Universität in Tübingen. The laboratory facilities differed between the two sites. The laboratory

facilities in Lambaréné only allowed for basic molecular assessments whereas in Tübingen the assessments were more elaborate. The quality of the molecular analysis therefore differs between the first and the second half of samples. In the CERMEL only the *merozoite surface protein 2* (*MSP2*) was genotyped using nested PCR (nPCR) and fluorescent agarose gel electrophoresis. In Tübingen next to *MSP2* also *merozoite surface protein 1* (*MSP1*) was genotyped. If the *MSP1* and *MSP2* genotyping was inconclusive unlinked microsatellite markers were additionally analyzed using capillary electrophoresis.

2.3.2.2.1 Dried blood spots and DNA extraction

As described in section 2.3.1 two dried blood spots were collected at each visit. To preserve the dried blood spots *Whatman 903TM Protein Saver Cards* by GE Healthcare were used. 10µl of capillary blood, EDTA-full blood or heparin-full blood were pipetted onto the filter paper and dried in the *Incubator 40°C Typ 405* by MELAG before storage. The dried blood spots were then stored at room temperature in a plastic bag containing desiccant. During follow up participants who had recurrent infections were identified. The DNA of the pre-treatment dried blood spot collected in the screening examination and of the post-treatment dried blood spot collected on the day of recurrence was extracted. The DNA extraction was conducted with the *QIAamp® DNA Blood Mini Kit*, for details see the *QIAamp® DNA Mini Handbook*, section *DNA Extraction from Dried Blood Spots* [95].

2.3.2.2.2 Genotyping procedures

The nPCR of the MSP1 and MSP2 gene, gel electrophoresis as well as the microsatellite genotyping and capillary electrophoresis was performed according to the respective standard operating procedures (SOP) of the CERMEL or the institute of tropical medicine in Tübingen. For more detailed information on the procedures of nPCR it is referred to the WHO genotyping recommendations [96] and the methods described by Snounou [97]. For microsatellite genotyping and capillary electrophoresis it is referred to the methods described by Su and Ferdig [98].

## 2.4 Data management

### 2.4.1 Data collection and Storage

All data collected in this study was primarily written on a source document. One individual source document was assigned to each participant, in which all data – clinical

and laboratory – was collected. Only investigators or specifically designated personal such as microscopists wrote in the source document. After all participants had ended the study the source documents were revised by an investigator and the clinical monitor for mistakes and completeness. Then the data from the source was transcribed onto a paper case report form (CRF) by an investigator. The paper CRF was provided by the sponsor Jomaa Pharma GmbH and delivered to the same after completion of transcription. The filled out CRFs were then revised again by the clinical monitor and data was queried if inconclusive or incomplete. The investigators would then solve the queries by comparing the data with the source documents.

All the source documents plus copies of each CRF remained in the archive of the CERMEL. All the participant-identifiable or study relevant information were kept safe from unauthorized access within the CERMEL.

### 2.4.2 Data entry

Depending on practicability and time of data entry three different kind of software were used. All microscopy and molecular biology documentation was entered into a Microsoft® Excel (Microsoft® Office Professional Plus 2016) worksheet directly from the source. All clinical data was entered from the CRF into Microsoft Access (Microsoft® Office Professional Plus 2016) or REDCap™ (Research Electronic Data Capture) – a secure web application for building and managing databases with a focus on clinical surveys and trials. The data entered in Microsoft® Excel and Access was stored on confidential hard drives. The REDCap™ data was stored on an especially secured CERMEL server and extracted to an Excel worksheet for analysis.

### 2.4.3 Data cleansing

While entering the data all mistakes, contradictions and gaps left in the source were corrected or filled in by querying the source and the investigators. The data entry software or applications had some data validation mechanisms that prevented wrong or missing data. Aside, the completeness of participants and variables in the databases as well as consistency of information was thoroughly checked by the author before undertaking any analysis. Missing data was marked as not available. Free text entries were harmonized and standardized by allocating suitable categories.

## 2.5 Statistical analysis

For statistical analysis data was extracted from Microsoft® Excel and Microsoft®

Access. Analytical and descriptive statistics were performed using *IBM SPSS Statistics 25.* Before further analysis data was controlled for normal distribution and homogeneity of variance. The normal distribution was controlled using several methods. Descriptive parameters like skewness and kurtosis for each of the variables were checked. Probability-probability plots in which the expected z-scores of an ideal normal distributed sample is plotted against the actual z-scores were used to graphically identify non-normal distribution. Also, the Kolmogorov-Smirnov test as well as the Shapiro-Wilk test were used to check if the sample distribution were significantly differently distributed than the normal distribution. The homogeneity of variance was controlled using Levene's test.

### 2.5.1 Efficacy analysis

Kaplan-Meier survival analysis was used to assess per protocol ACPR at D28 with and without PCR-correction, intention to treat ACPR at D63 without PCR-correction, time to gametocyte clearance as well as the time and rate of gametocyte appearance. For the Kaplan-Meier analysis of the PCR-corrected ACPR rate at D28 the data entry and analysis tool provided by the WHO [99] was utilized. The efficacy endpoints 0- to 48-hour parasite reduction ratio was calculated by dividing the mean parasitemia 48 hours after the first dose of treatment (corresponding to one asexual parasite life cycle) by the mean pre-dose parasitemia and expressed as the logarithm to the base 10. For calculating parasite clearance time, the first time point with a negative parasite count was identified for each participant with parasites at baseline and the median timepoint of all participants was expressed. The fever clearance time was calculated analogously whereas only patients that showed fever at baseline or during the treatment phase were included. Parasite elimination half-life was defined as the time span from inclusion to the first thick smear assessment that continually showed a parasitemia ≤ half of the initial parasitemia.

To estimate the clearance rate constant, the slope half-life, the duration of lag phases and the times to clear 50%, 90%, 95% and 99% of parasites the Parasite Clearance Estimator tool developed by the WorldWide Antimalarial Resistance Network (WWARN) was used. The Parasite Clearance Estimator contains the statistical models to estimate parasite clearance measures, lag phase duration and to identify tails in the parasite clearing curve. Lag phases are theoretically due to the parasitic life cycle and independent of anti-malarial treatment. Tails are the terminal part of the individual parasite clearance curve where parasitemia remains close to the detection limit and does not decrease over several

measurement time points. Values in the tail are therefore unreliable. To estimate the parasite clearance parameters lag phases and tails are excluded from analysis. The part of the parasite clearance slope left follows a first order process and is constant. The clearance rate constant then equals the minus slope of the $log_e$ parasitemia–time linear relationship. The estimated slope half-life is the estimated time in hours in which the parasitemia decreases by half following the fitted model. Patients with a baseline parasitemia <1000 p.f./µl were excluded from the analysis conducted by the Parasite Clearance Estimator. For more information it is referred to the WWARN website [100].

### 2.5.2 Safety analysis

The correlation between QTc duration and cumulative treatment exposure was assessed using univariate linear regression. Since there were no pharmacokinetic assessments included in the study the time of treatment exposure was used as correlating parameter instead of plasma levels of the study medication. Fit lines were fitted using the method of least squares. As correlation coefficient Pearson's r was calculated. To estimate the effect size, the coefficient of determination ($R^2$) was calculated. The F-ratio was calculated to determine the goodness of fit of the regression model. Anemia was defined according to the WHO cut-off ranges [101]. Neutropenia severity was classified as follows: Neutropenia Grade I: 1000 – 1500/µl, Grade II: 500 – 1000/µl and Grade III: 200 – 500/ µl according to the definition by Newburger and Dale [102]. Reference ranges of other clinical laboratory parameters refer to the reference ranges indicated by the clinical laboratory of the CERMEL.

### 2.5.3 Study populations

The ITT included patients who received any amount of the study medication and had confirmed positive parasitemia at baseline. The PP consisted of all ITT patients who had completed a full course of study medication. None of the PP patients received rescue treatment before the end or discontinuation of study. Patients with no evaluable efficacy endpoints were excluded from efficacy analysis. The safety population included patients who received any amount of study medication.

## 2.6 Ethical aspects

The study "Fosmidomycin and Piperaquine: A Phase IIa proof of concept study to explore the efficacy, tolerability and safety of fosmidomycin sodium when administered with piperaquine tetraphosphate to children aged 1 to 5 years with acute uncomplicated

Plasmodium falciparum malaria" was an amendment to the previous FOSPIP study in adults and children of six years and above. Both the original study and the amendment were approved by the National ethics committee of Gabon (Comité National d'Ethique pour la Recherche). Copies of the official documents of approval can be found in the attachments. All the procedures performed were aligned with the good clinical practice (GCP) and good clinical laboratory practice (GCLP) guidelines as defined by the International Council for Harmonisation and WHO.

Many provisions were taken to assure safety of participants. An age step down enrollment procedure was applied. FOSPIP Part 1 evaluated the drug efficacy and safety in adults and older children and justified the amendment in smaller children. In FOSPIP Part 2 the first ten children were 3 to 5 years. Only after an interim review of the safety data in those ten children followed up unto D7 children <3 years were enrolled.
The exclusion criteria were set to exclude the patients most at danger. Those were treated conventionally with Artemether/Lumefantrine or, in case of severe illness hospitalized at the Albert Schweitzer Hospital to be treated intravenously with quinine. During the treatment phase patients were strictly monitored for worsening of their parasitological or clinical condition with discontinuation criteria in place to assure early reaction. At any time point one nurse and one physician were on site and approachable. To monitor the potential toxic cardiac effects of piperaquine ECGs were performed before and after piperaquine intake (section 2.3.1.4). To avoid the risk of Torsade de pointes arrhythmia pathological QTc changes were defined as discontinuation criteria. Also, a defibrillator was installed and available to the attending clinician.

The participation in the FOSPIP study was voluntary and written consent was obtained from all the participant´s representatives (section 2.3.1.2). If patients resigned participation, they were treated with Artemether/Lumefantrine cost-free. All the patient-identifiable information was handled strictly confidential (section 2.4). Samples were labelled with the anonymous study ID. Important clinical or laboratory findings were communicated in private.
The participants' benefitted from costless transport between the CERMEL and their respective residence either performed by fieldworkers or by public transport; in the latter

case, transport costs were compensated. As many participants lived in remote areas and in families with poor income the transport to an adequate health facility would often have presented a financial challenge. During hospitalization the participants and their companions received free meals thrice daily and drinks as required. Any medical concern of the participant arising throughout the course of study was taken care of either by the study clinicians or financially compensated if outward health facilities were involved. Many of the participants received a cost-free mosquito net sponsored by another formerly conducted clinical trial. Apart from that no other financial incentives were offered to the participants.

## 2.7 Funding

The FOSPIP study was funded by Jomaa Pharma GmbH and Medicines for Malaria Venture (MMV).

# 3 Results

## 3.1 Conduct of the study

### 3.1.1 Milestones

The FOSPIP study Part 2 was conducted from October 2015 until May 2016 in Gabon. Active screening was performed from 25/10/2015 until 17/03/2016. Following the age step-down procedure, the first 10 patients enrolled were 3-5 years old. On the 08/12/2015 the first 10 patients had reached D7 assessment and an interim analysis was performed by the study investigators, clinical monitor and scientific consultant (section 2.1). It was collectively decided that it was safe to continue. From now on patients aged 1-5 years were enrolled. In the following conduct two patients showed relevant QTcF changes as defined in the discontinuation criteria (see section 2.3.1.6), namely FOS065 and FOS066. They were discontinued from the study. The clinical monitor was concerned that the ECG device was not well calibrated so that until recalibration the enrollment was suspended starting the 15/12/2015. Recalibration of the ECG device was performed. Enrollment was continued the 04/01/2016. The last participant ended the study on 20/05/2016.

### 3.1.2 Flow of patients

The flow of patients is shown in Figure 4.

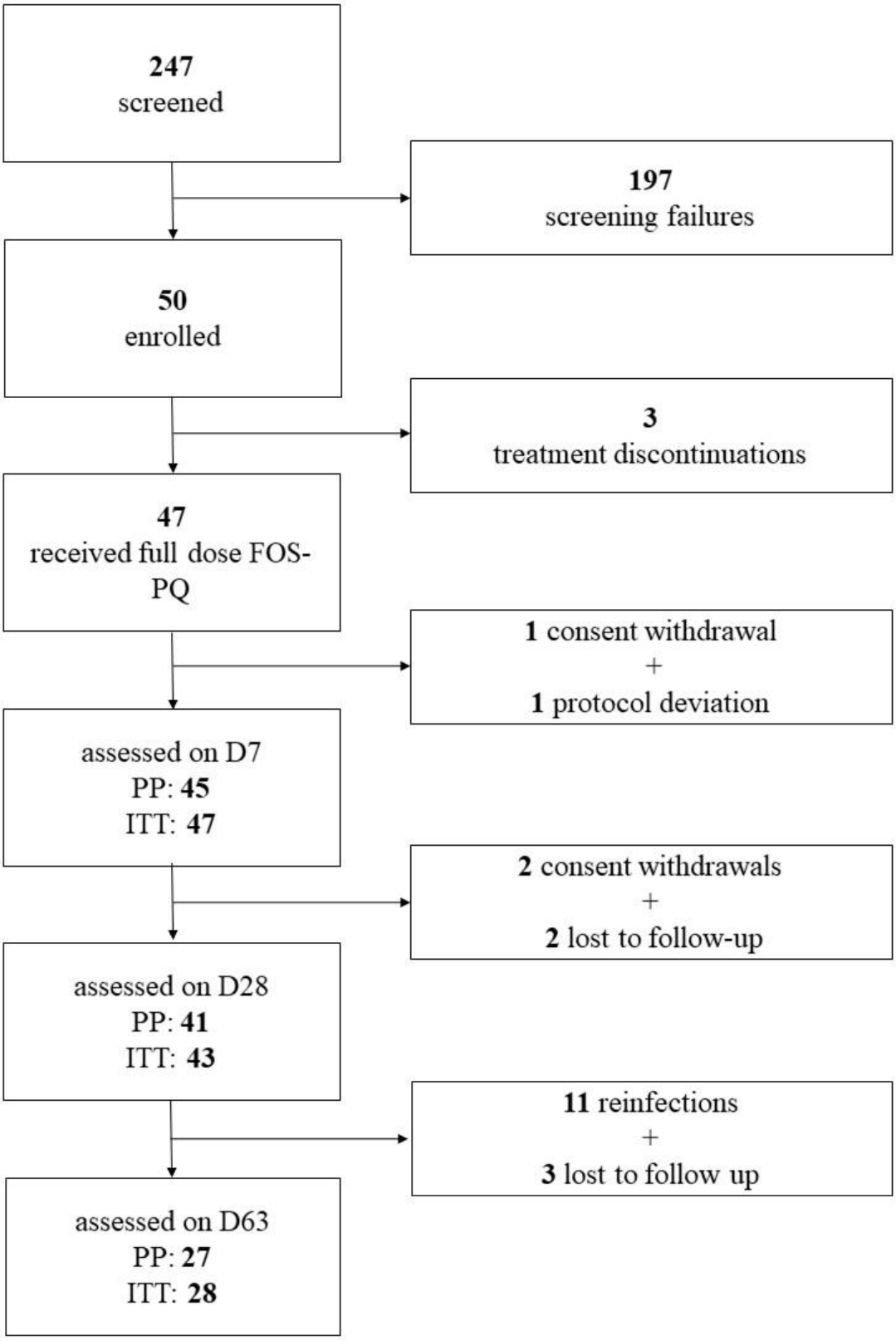



*Figure 4: Flow of patients*

Fos-PQ: Fosmidomycin sodium and piperaquine tetraphosphate combination therapy, D: Day, PP: per protocol population, ITT: intention to treat population

#### 3.1.2.1 Screening

247 potential participants that showed signs of acute malaria were screened from October 2015 to March 2016. Most patients screened came from Fougamou and surrounding villages. A list of the results for rapid diagnostic test and thick smear analysis are shown in Table 3. Defining blood smear microscopy as the gold standard sensitivity of the PARACHECK Pf® RDT was 93.7% (133/142; 95%CI: 88.4-96.6), specificity was 50.6% (41/81; 95%CI: 40-61.2), the positive predictive value was 76.9% (133/173; 95%CI: 70.1-82.5) and the negative predictive value was 82% (41/50; 95%CI: 69.2-90.2). In some cases, thick smear microscopy was not feasible due to missing slides or bad staining quality. Frequent reasons for exclusion were parasitemia <1,000 or >150,000 p.f./µl, mixed infections e.g. with P. ovale or P. malariae, severe anemia, severe vomiting and age <1 or >5 years. 50 patients were included into the study.

**Table 3: Screening results, rapid diagnostic test and blood smear microscopy**

RDT: rapid diagnostic test

| **RDT** | **Blood smear microscopy** | | | |
|---|---|---|---|---|
| | Positive, n | Negative, n | Not available, n | Total, N |
| Positive | 133 | 40 | 13 | 186 |
| Negative | 9 | 41 | 6 | 56 |
| Not available | 0 | 0 | 5 | 5 |
| Total | 142 | 81 | 24 | 247 |

#### 3.1.2.2 Flow of patients after inclusion

During the treatment phase 3 participants were early discontinued so that 47 patients received the full treatment regimen. Of these 3 participants 2 showed QTcF changes >60ms compared to baseline QTcF, both after the second dose of piperaquine (FOS065, FOS066). FOS065 received rescue medication. FOS066 was already negative for parasites in 2 consecutive thick smear assessments by the time of discontinuation and therefore did not receive rescue medication. One participant was discontinued due to inability to swallow the medication after the second dose of fosmidomycin and received rescue medication (FOS093). These 3 participants were included in the ITT efficacy analysis.

For the clinical and parasitological assessment on D7 45 patients were evaluable in the PP and 47 in the ITT. One participant withdrew consent after finishing the treatment course on D3 (FOS082). FOS069 was only positive for parasites in the screening thick

smear assessment and negative in all consecutive assessments including the pre-dosing thick smear. FOS069 was therefore excluded from the efficacy analysis and only included into safety evaluation.

For the main endpoint, namely ACPR on D28, 41 patients were evaluable in the PP and 43 patients in the ITT. 2 participants withdrew consent (FOS055, FOS097) and 2 were lost to follow up before reaching D28 (FOS054, FOS060).

For the final clinical and parasitological assessment on D63 27 patients were assessed in the PP and 28 in the ITT. 11 participants were discontinued because of recurrence of parasites (FOS052, FOS053, FOS056, FOS059, FOS070, FOS071, FOS075, FOS079, FOS080, FOS081, FOS95) and 3 participants were lost to follow up before reaching D63 (FOS062, FOS086, FOS094).

#### 3.1.2.3 Protocol deviation and missing data

As mentioned in 3.1.2.2 some patients were not evaluable at different time points and could therefore not be included in the efficacy and/or safety evaluation. Reasons for missing data with the respective subject number are shown in Table 4. As the fieldworkers would find the patients in their homes only patients that changed their residency were lost to follow up. Common reasons for relocation reported by relatives or neighbors were new employment of the patients' representatives in a different region and holidays. Regarding the consent withdrawals most legal representatives stated the amount of unpleasant examinations like blood sampling or ECG measurements to be the motivation for their withdrawal. Patients that lacked essential data were excluded from analysis.

In case of relevant violation of protocol procedures, mainly incomplete intake of treatment regimen, patients were excluded from PP but included in ITT or safety analysis. In case of mild protocol deviation, that were not judged relevant by the author, patients were included into PP analysis. Protocol deviations and allocation into PP, ITT or safety population per patient are shown in Table 5.

There are 5 patients that developed QTcF and/or QTcB prolongation after the suspension of the study in December 2015, namely FOS080, FOS084, FOS091, FOS092 and FOS098. FOS080 showed prolongation of QTcF only after the 3$^{rd}$ dose of piperaquine. The other 4 patients affected showed prolongation already after the 2$^{nd}$ dose of piperaquine. Even though prolongation of QTcF and/or QTcB was set as a

discontinuation criterion, the investigators, the clinical monitor and the study consultant decided in each of these 4 cases to continue the treatment. The decision was justified by the WHO recommendation of dihydroartemisinin-piperaquine combination therapy for acute malaria [79, 103].

All patients that are not listed in Table 4 and/or Table 5 had no relevant protocol deviations nor missing data to declare and followed all protocol procedures.

### Table 4: Missing data

PCR: polymerase chain reaction, D: Day

| Subject No. | Reason for missing data |
|---|---|
| 53 | PCR genotyping of the screening sample did not show conclusive results due to missing amplification of the MSP2 gene |
| 54 | Lost to follow up after D14 |
| 55 | Withdrawal of consent after D7 |
| 60 | Lost to follow up after D21 |
| 62 | Lost to follow up after D35 |
| 69 | Lost to follow up after D21 |
| 82 | Withdrawal of consent after D3 |
| 86 | Visit at D14, D21, D28, D35 and D63 was not performed |
| 94 | Lost to follow up after D42 |
| 97 | Withdrawal of consent after D21 |

### Table 5: List of protocol deviations and allocation of population

p.f./µl: Number of Plasmodium falciparum parasites per µl of blood, ETF: Early treatment failure, D: Day, QTcF: QT interval in ECG corrected for pulse rate by the Frederica formula, QTcB: QT interval in ECG corrected for pulse rate by the Bazett formula, ΔQTcF: Difference of QTcF measurement compared to baseline QTcF measurement, PQ: piperaquine tetraphosphate, PP: per protocol population, ITT: intention to treat population, Safety: safety population

[a]Inclusion criterion "*fever with axillary temperature of ≥37.5°C or history of fever during the previous 72 hours"* and exclusion criterion "*white cell count >12000/mm³"* was judged as not relevant by the author and is not mentioned; for other in- and exclusion criteria see section 2.3.1.3

[b]Participant was not discontinued even though this presented a protocol deviation

| Subject No. | Protocol deviation | | Population |
|---|---|---|---|
| | In- or exclusion criteria not met[a] | Discontinuation before D63 or other | |
| 53 | | PCR results not evaluable | PP |
| 55 | Screening parasitemia: 931 p.f./µl, Pre-dose Parasitemia: 665 p.f./µl | | PP |
| 61 | Screening parasitemia: 779 p.f./µl, Pre-dose Parasitemia: 368 p.f./µl | | PP |
| 65 | | ETF: ΔQTcF >60ms after 2nd dose of PQ[b] | Safety |

| | | | |
|---|---|---|---|
| 66 | | ETF: ΔQTcF >60ms after 2nd dose of PQ[b] | ITT |
| 69 | Screening parasitemia: 735 p.f./µl, Pre-dose parasitemia and all following assessments: 0 p.f./µl | | Safety |
| 72 | Screening parasitemia: 7714 p.f./µl, Pre-dose Parasitemia: 185 p.f./µl | | PP |
| 74 | Hypokalaemia, Hemoglobin <7,5g/dl | | PP |
| 79 | QTcF > 450ms at screening, Hypokalaemia | | PP |
| 80 | | ΔQTcF prolongation >60ms after 3rd dose of PQ[b] | PP |
| 82 | Treatment with antimalarial agent in previous 63 days | | ITT |
| 84 | | ΔQTcF >60ms after 2nd dose of PQ[b] | PP |
| 86 | Haemoglobin < 7.5 g/dl | | PP |
| 87 | Hypokalaemia | | PP |
| 91 | | ΔQTcF >60ms after 2nd dose of PQ, QTcB >500ms after 3rd dose of PQ[b] | PP |
| 92 | Screening parasitemia: 177800 p.f./µl, Pre-dose Parasitemia: 175996 p.f./µl | ΔQTcF >60ms after 2nd dose of PQ[b] | PP |
| 93 | | ETF: Inability to swallow medication | Safety |
| 94 | Hypokalaemia | | PP |
| 96 | Treatment with antimalarial agent in previous 63 days | | PP |

# 3.2 Demographic characteristics

## 3.2.1 Baseline demographics of the study population

50 children between 1 to 5 years were included. 11 children (22%) were aged below 3 years. 16 of 50 patients (32%) were female. Baseline demographic data including sex, age and body measurements are shown in Table 6. The age distribution is shown in Figure 5.

**Table 6: Baseline demographics of study population**

IQR: interquartile range (25$^{th}$ percentile – 75$^{th}$ percentile), SD: standard deviation
*available for 48 patients

| | |
|---|---|
| **Total cohort**<br>n | 50 |
| **Gender Male/Female**<br>n (%) | 16/34 (32/68) |
| **Age**, years<br>Median (IQR) | 4.2 (3.3-5.2) |
| **Weight**, kg | |

| | |
|---|---|
| Median (IQR) | 15 (12.8-16.9) |
| **Height**, cm<br>Mean (SD)* | 100 (95;105) |
| **BMI**, kg/m²<br>Mean (SD)* | 15.1 (14.5;15.9) |

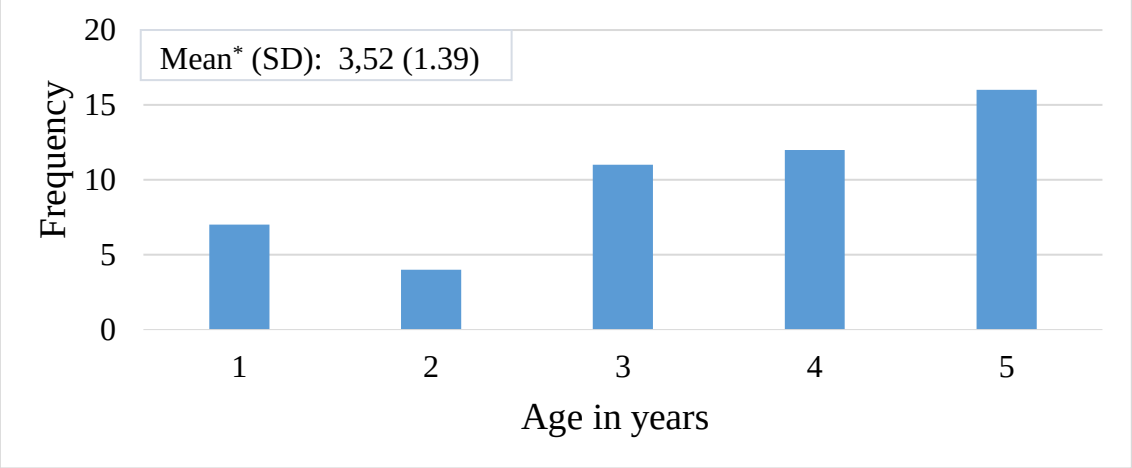



*Figure 5: Age distribution*

SD: Standard deviation, *arithmetic mean

### 3.2.2 Clinical baseline characteristics related to malaria

Many of the patients included showed clinical signs of malaria infection. The clinical and laboratory parameters possibly related to malaria infection are shown in Table 7 and Table 8 respectively. 12 patients (26%) showed fever at baseline defined as an axillary temperature ≥37.5°C. 46 patients (92%) were anemic at baseline with a mean hemoglobin value of 8.9 g/dl. 7 patients (14%) showed platelet counts <100,000/ $mm^3$. 6, 1 and 3 patients had raised Bilirubin, ASAT and ALAT values respectively. 7 patients had blood urea values raised over reference range. Most of the patients' potassium level was at the lower end of the reference range with mean potassium of 3.9mmol/l. None of the patients were hypoglycemic but 9 (18%) were hyperglycemic. Patients were not fasting before measurement. All patients were hemodynamically stable. The pre-dose parasite counts are visualized in Figure 6.

**Table 7: Clinical baseline characteristics related to malaria infection**

SD: standard deviation, CI: Confidence interval

| | |
|---|---|
| **Headache**<br>n/N (%) | 6/50 (12) |
| **Nausea** | |

| | |
|---|---|
| n/N (%) | 14/50 (28) |
| **Fever**<br>n/N (%)<br>**Axillary temperature**, °C<br>Median (range) | <br>12/50 (26)<br><br>36.6 (34.7 – 39.1) |
| **Paleness**<br>n/N (%) | <br>16/50 (32) |
| **Dehydration**<br>n/N (%) | <br>1/50 (2) |
| **Jaundice**<br>n/N (%) | <br>1/50 (2) |
| **Hepatomegaly**<br>n/N (%)<br>**Hepatomegaly quantified in cm below rib cage**<br>Mean (SD) | <br>11/50 (32)<br><br>3.5 (1.4) |
| **Splenomegaly**<br>n/N (%)<br>**Splenomegaly quantified in cm below rib cage**<br>Mean (SD) | <br>26/50 (52)<br><br>3.0 (1.7) |
| **Lymphadenopathy**<br>n/N (%) | <br>9/50 (18) |
| **Pulse rate,** bpm<br>Mean (SD) | <br>120 (21) |
| **Systolic blood pressure,** mmHg<br>Mean (SD) | <br>99 (10) |

## Table 8: Laboratory parameters related to malaria at baseline

p.f.: Plasmodium falciparum, IQR: interquartile range (25th percentile – 75th percentile), CI: Confidence interval, SD: Standard deviation, ASAT: aspartate amino transferase, ALAT: alanine transaminase

| | |
|---|---|
| **Pre-dose parasite count**, p.f./µl<br>Median (IQR) | <br>5450 (1474-20320) |
| **Proportion with gametocytes**<br>n/N (%; 95% CI) | <br>4/50 (8; 2.2–19.2) |
| **Hemoglobin value**, g/dl<br>Mean (SD) | <br>8.9 (1.0) |
| **White blood cell count**, $\times 10^3/mm^3$<br>Mean (SD) | <br>8.8 (2.2) |
| **Platelet count**, $\times 10^3/mm^3$<br>Mean (SD) | <br>195 (81.7) |
| **Bilirubin**, µmol/l<br>Mean (range) | <br>11.9 (0.9 – 37.7) |

| | |
|---|---|
| **ASAT**, U/l<br>Median (range) | <br>27 (5 – 93) |
| **ALAT**, U/l<br>Median (range) | <br>15 (5 – 86) |
| **Creatinine**, µmol/l<br>Mean (range) | <br>36.3 (7.0 – 68.0) |
| **Urea**, mmol/l<br>Median (range) | <br>3.3 (1 – 33) |
| **Potassium**, mmol/l<br>Mean (SD) | <br>3.9 (0.5) |
| **Glucose**, mmol/l<br>Mean (Range) | <br>5.4 (3.3 – 10.2) |

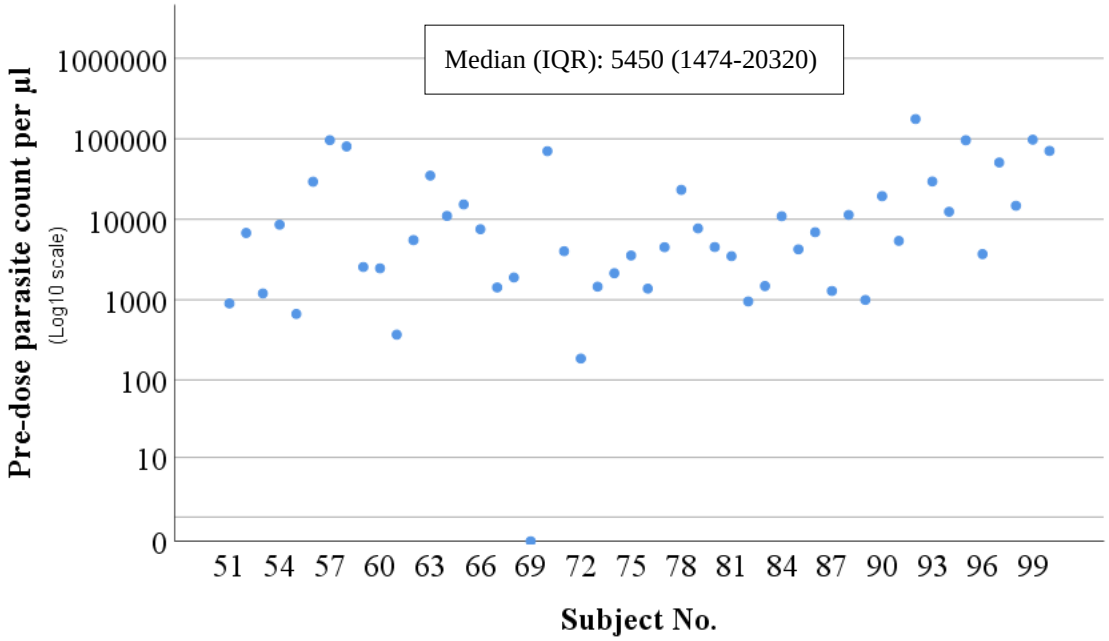



*Figure 6: Scatter plot of pre-dose parasite counts per patient on a Log10 scale*

IQR: Interquartile range (25$^{th}$ percentile – 75$^{th}$ percentile)

## 3.3 Efficacy analysis

### 3.3.1 Efficacy outcome regarding asexual parasitemia

39 out of 39 (100%) patients in the PP showed PCR-corrected ACPR on D28. There were two recurrences of parasites on D28 that were identified as reinfections by genotyping analysis (section 0). During the 63-day follow up parasites recurred in 14 patients. Twelve of these recurrences of parasites were judged to be due to reinfection by genotyping analysis. In two cases recurrence of parasites were not be judged as reinfections, namely for FOS053 on D38 and FOS070 on D42. FOS053's genotyping assessment showed no

conclusive results and was therefore excluded from the D63 per protocol analysis. FOS070's genotyping assessment of the MSP2 gene indicated a recrudescent infection on D42. The median parasite clearance time (PCT) was 48 hours and the 0- to 48-hour parasite reduction ratio equaled $10^{3.7}$. The median fever clearance time (FCT) was 12 hours in 14 patients who showed febrile temperatures at any given time point during the treatment phase. 8 of these received antipyretic treatment. The results for the efficacy endpoints regarding asexual parasites are shown in Table 9 and Table 10 for the PP and ITT respectively. Figure 7 shows the output given by the data entry and analysis tool for antimalarial drug efficacy studies provided by the WHO [99] for the per protocol analysis of ACPR on D28. Figure 8 shows the Kaplan-Meier survival plot for patients that had no recurrence of parasites during the time of follow up in the ITT. The event in the Kaplan-Meier analysis was defined as recurrence of parasites. The Kaplan-Meier estimate for the mean survival time without recurrence of parasites is 55.7 days (95% CI: 51.8-59.6 days).

**Table 9: Efficacy outcomes in the per protocol population**

CI: Confidence interval, ACPR: Adequate clinical and parasitological response, PCR: Polymerase chain reaction
[a]1 protocol deviation, 3 early discontinuations, 1 consent withdrawal
[b]1 protocol deviation, 3 early discontinuations, 3 consent withdrawals, 2 lost to follow up
[c]1 protocol deviation, 3 early discontinuations, 3 consent withdrawals, 2 lost to follow up, 2 reinfections
[d]1 protocol deviation, 3 early discontinuations, 3 consent withdrawals, 5 lost to follow up
[e]1 protocol deviation, 3 early discontinuations, 3 consent withdrawals, 5 lost to follow up, 12 reinfections, 1 participant's PCR result was not evaluable

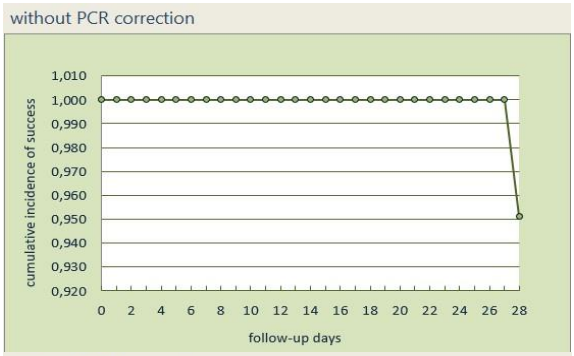
| Efficacy outcome parameter | Result |
|---|---|
| **Day 7 cure rate** | |
| n/N (%; 95% CI) | 45/45[a] (100; 92.1-100) |
| **Day 28 PCR-uncorrected** | |
| ACPR, n/N | 39/41[b] |
| Treatment failure, n | 2 |
| Cumulative incidence of success, % (95% CI) | 95.1 (84.9-98.7) |
| **Day 28 PCR-corrected** | |
| ACPR, n/N | 39/39[c] |
| Treatment failure, n | 0 |
| Cumulative incidence of success, % (95% CI) | 100 (91.4-100) |
| **Day 63 PCR-uncorrected** | |
| ACPR, n/N | 24/38[d] |
| Treatment failure, n | 14 |
| Cumulative incidence of success, % (95% CI) | 63.2 (47.3-76.6) |
| **Day 63 PCR-corrected** | |

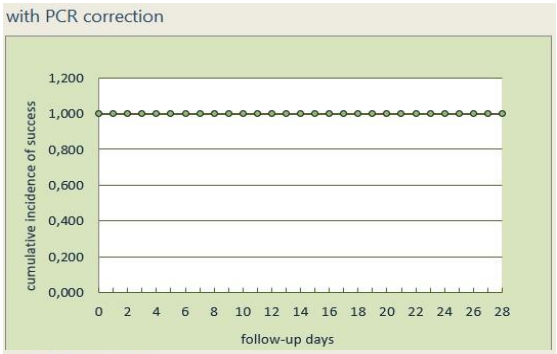
| | |
|---|---|
| ACPR, n/N | 24/25[e] |
| Treatment failure, n | 1 |
| Cumulative incidence of success, % (95% CI) | 96 (80.5-99.3) |

## Table 10: Efficacy outcomes in the intention-to-treat population

ACPR: Adequate clinical and parasitological response
[a]1 patient with negative parasitemia was excluded from the intention to treat population

| **Efficacy outcome parameter** | **Result** |
|---|---|
| **Intention-to-treat population,** total N | 49[a] |
| **Day 7** | |
| cured, n | 47 |
| Treatment failures (Lost to follow up, withdrawals, recurrence of parasites) | 2 |
| Cumulative incidence of success, % (95% CI) | 95.9 (86.3-98.9) |
| **Day 28** | |
| ACPR, n | 41 |
| Treatment failures (Lost to follow up, withdrawals, recurrence of parasites) | 8 |
| Cumulative incidence of success, % (95% CI) | 83.7 (71.0-91.5) |
| **Day 63** | |
| ACPR, n | 25 |
| Treatment failures (Lost to follow up, withdrawals, recurrence of parasites) | 24 |
| Cumulative incidence of success, % (95% CI) | 51.0 (37.5-64.4) |
| **0- to 48-hour parasite reduction ratio** | |
| log10 | 3.7 |
| **Parasite clearance time** in hours | |
| Median (range) | 48 (24-72) |
| **Fever clearance time** in hours | |
| Median (range) | 12 (6-36) |
| **Parasite elimination half-life** in hours | |
| Median (range) | 12 (6-36) |




without PCR correction

| | number | % | lower 95%CI | upper 95% CI |
|---|---|---|---|---|
| ETF | 0 | 0,0 | 0,0 | 8,6 |
| LCF | 1 | 2,4 | 0,1 | 12,9 |
| LPF | 1 | 2,4 | 0,1 | 12,9 |
| ACPR | 39 | 95,1 | 83,5 | 99,4 |
| Total patients per protocol | 41 | | | |
| WTH | 7 | | | |
| LFU | 2 | | | |
| Total patients LFU/WTH | 9 | 18,0 | | |
| Total patients at baseline | 50 | | | |

with PCR correction

| | number | % | lower 95%CI | upper 95% CI |
|---|---|---|---|---|
| ETF | 0 | 0,0 | 0,0 | 9,0 |
| LCF | 0 | 0,0 | 0,0 | 9,0 |
| LPF | 0 | 0,0 | 0,0 | 9,0 |
| ACPR | 39 | 100,0 | 91,0 | 100,0 |
| Total patients per protocol | 39 | | | |
| WTH | 9 | | | |
| LFU | 2 | | | |
| Total patients LFU/WTH | 11 | 22,0 | | |
| Total patients at baseline | 50 | | | |

*Figure 7: Per protocol efficacy analysis of ACPR on D28 and Kaplan-Meier analysis without and with PCR-correction. Output from the data entry and analysis tool for antimalarial drug efficacy studies provided by the WHO**

PCR: polymerase chain reaction, ACPR: adequate clinical and parasitological response, CI: confidence interval, ETF: early treatment failure, LCF: late clinical failure, LPF: late parasitological failure, WTH: withdrawal of consent/protocol deviation or early discontinuation, LFU: lost to follow up
*available online at http://www.who.int/malaria/areas/drug_resistance/efficacy-monitoring-tools/en/ [last accessed: September 13, 2018]

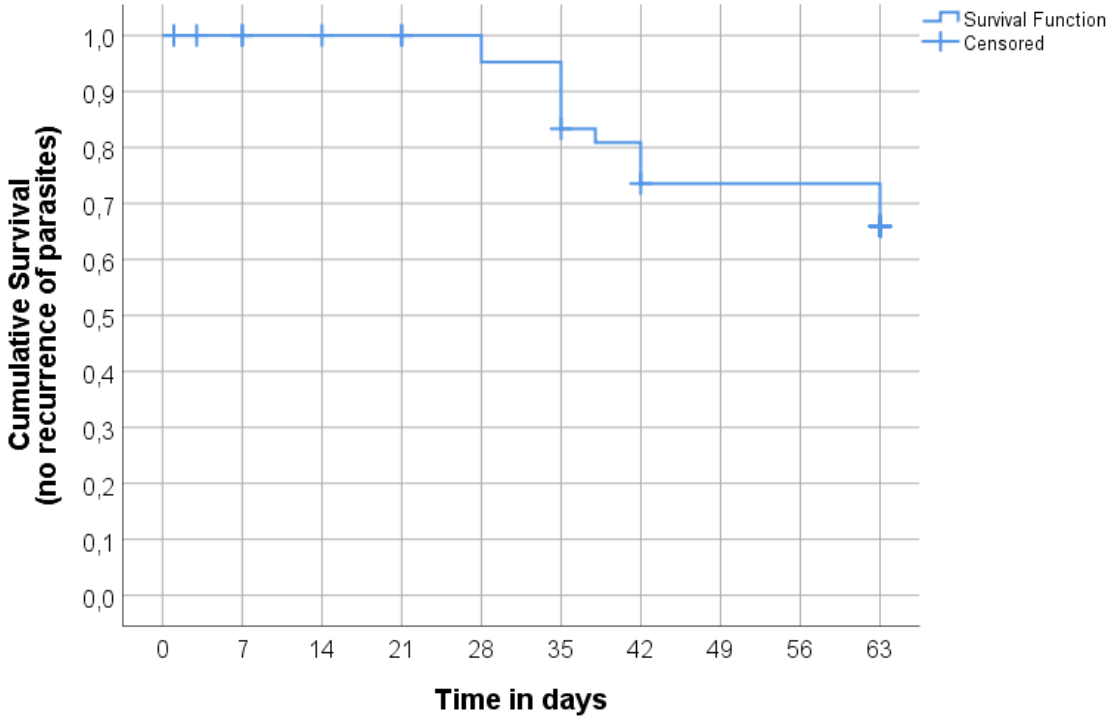



*Figure 8: Kaplan-Meier survival plot for patients without recurrence of parasites during follow up in the intention to treat population (N=49)*

The median clearance rate constant and estimated median parasite clearance half-life was estimated by the Parasite Clearance Estimator tool to be 0.19 per hour and 3.71 hours respectively. All the outcome variables calculated by the Parasite Clearance Estimator are depicted in Table 11.

**Table 11: Results calculated with the Parasite Clearance Estimator developed by the WorldWide Antimalarial Resistance Network (WWARN)**

IQR: interquartile range (25$^{th}$ percentile - 75$^{th}$ percentile)
[a]FOS093 was excluded from testing due to missing data
[b]Parasitemia below 1000 p.f./µl (FOS051,055,061,069,072,082,089)

| | |
|---|---|
| Total number of patients tested, n/N | 49[a]/50 |
| Number of patients excluded due to initial parasitemia[b], n/N | 7/49 |
| Number of patients included into parasite clearance estimation, n/N | 42/49 |
| Number of patients with lag phase detected, n/N | 11/42 |
| Number of patients with tail detected, n/N | 0/42 |
| Clearance rate constant, /hour<br>Median (IQR) | 0.19 (0.14-0.27) |
| Estimated parasite clearance half-life, hours<br>Median (IQR) | 3.71 (2.62-5.03) |
| Duration of lag phase, hours<br>Median (Range) | 6 (6-24) |
| Time to clear 50% of parasitemia, hours<br>Median (IQR) | 6.91 (3.71-10.44) |
| Time to clear 90% of parasitemia, hours<br>Median (IQR) | 15.64 (12.17-21.53) |
| Time to clear 95% of parasitemia, hours<br>Median (IQR) | 19.66 (14.60-27.22) |
| Time to clear 99% of parasitemia, hours<br>Median (IQR) | 29.38 (21.42-37.87) |

The development of parasite counts during the treatment phase is visualized in Figure 9. Figure 9 shows the box plotted parasite counts per measurement time point during the treatment phase on a logarithmic scale to the base 10. The star signs each mark individual cases that were identified as outliers in the distribution. One can clearly see a decline of parasite counts over time. Median parasitemia starts at around 6,000 p.f./µl. After 72 hours of treatment all the patients were aparasitemic.

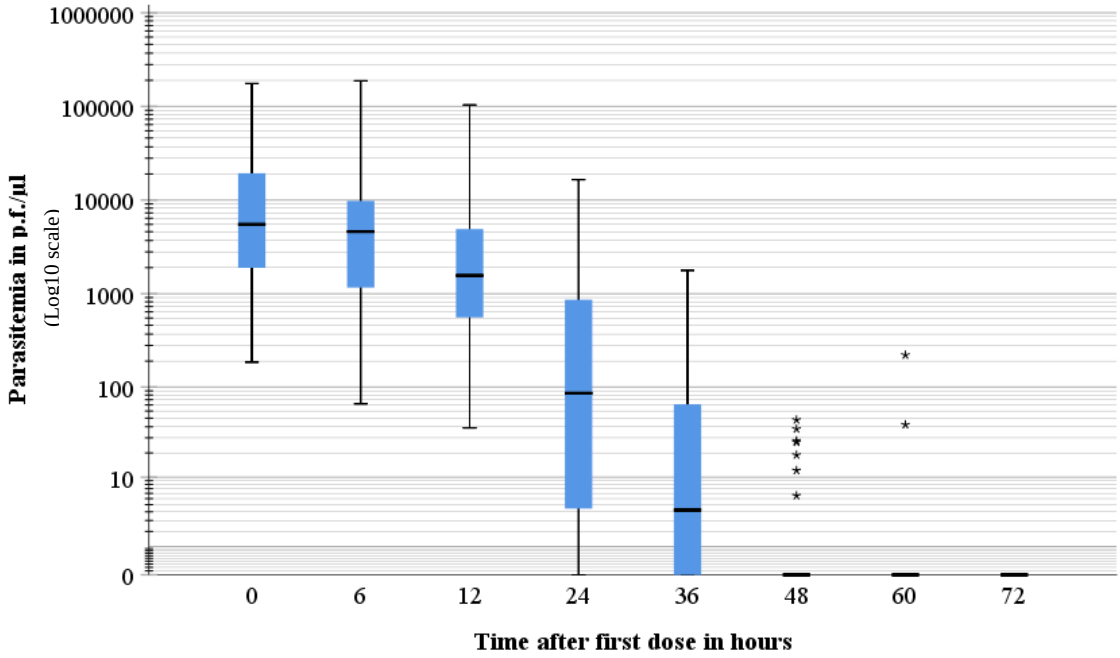



*Figure 9: Development of parasite counts in box plots during the first 72 hours of treatment on a log10 scale*

p.f.: Plasmodium falciparum, Log10: Logarithm to the base 10, ⋆: individual parasitemias marked as outliers

#### 3.3.1.1 PCR results

As mentioned in section 2.3.2.2 the first half of samples was analyzed in the CERMEL in Lambaréné and the second half of samples in the institute of tropical medicine in Tübingen. Since the methodology differed between the two sites the results are presented separately.

##### 3.3.1.1.1 First half of samples analyzed in the CERMEL

The pictures from the MSP2 genotyping analysis of the gene loci FC27 and 3D7 for the first half of samples (FOS052 – FOS075) analyzed in the CERMEL are shown in Figure 10. In each of the pictures the screening sample (marked as *Patient ID Scr*) is paired with the sample collected at the timepoint of P. falciparum recurrence (marked as *Patient ID Day of recurrence of parasites*). One can see that the screening sample of FOS053 (FOS053 Scr) did not show any amplification neither of the FC27 locus nor of the 3D7 locus (yellow rectangle). In this case differentiation between recrudescence and reinfection was not possible. The sample FOS070 D42 also shows no amplification of the FC27 locus. The visible band (blue ellipse) does not represent an FC27 amplificon but the whole MSP2 gene amplificon leftover from the first step of the nPCR. However,

looking at the 3D7 locus the pre- and post-treatment samples of FOS070 have one band representing an allele in common (red ellipse) which indicates a recrudescent infection. All the other samples show at least for one locus that all alleles differ when the coupled samples are compared (green rectangles). These cases of recurrent infections were therefore judged to be due to reinfection and not recrudescence.

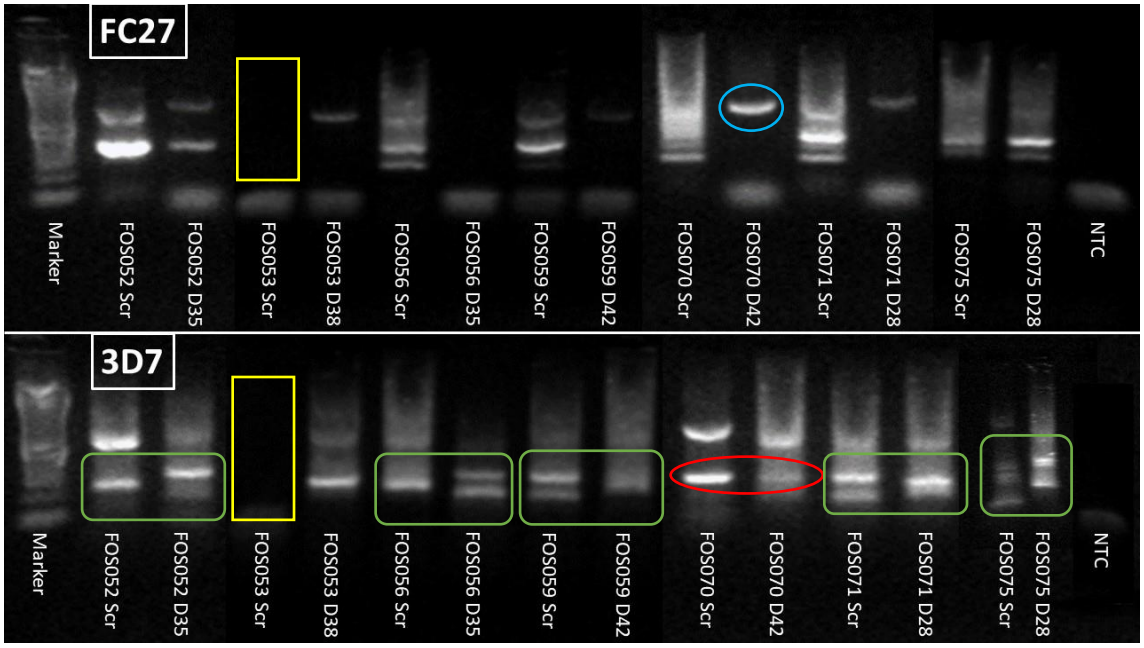



*Figure 10: Genotyping results for the first half of samples analyzed in the CERMEL. Gel electrophoresis: FC27 (upper half) and 3D7 (lower half) loci of the MSP2 gene with respective sample IDs*

Scr: Screening, D: Day, FC27: gene locus FC27 of the merozoite surface protein 2 gene, 3D7: gene locus 3D7 of the merozoite surface protein 2 gene, NTC: negative template control

3.3.1.1.2 Second half of samples analyzed in the institute of tropical medicine in Tübingen

The *MSP1* and *MSP2* genotyping results of the second half of samples (FOS078 – FOS098) analyzed in the institute of tropical medicine in Tübingen are presented in Table 12. For each respective gene locus of *MSP1* and *MSP2* the band's width of the alleles was quantified in base pairs. Alleles that match in size when comparing the pre- and post-treatment sample indicating recrudescence are highlighted in orange.

**Table 12: Genotyping results for the second half of samples analyzed in the institute of tropical medicine in Tübingen**

ID: Identifier, Scr: Screening, D: Day, PCR: polymerase chain reaction, bp: base pairs, MSP1: merozoite surface protein 1, MSP2: merozoite surface protein 2
* Alleles that match in size when comparing the pre- and post-treatment sample indicating recrudescence are highlighted in orange

| Sample ID | PCR Product Sizes (bp)* | | | | | Results |
|---|---|---|---|---|---|---|
| | *MSP1* | | | *MSP2* | | |
| | **K1** | **MAD20** | **RO33** | **FC27** | **3D7** | |
| FOS078 Scr | 250 | 220 | 190 | 400 | 300, 350 | **Recrudescence** |
| FOS078 D63 | 250 | 220 | 190 | 400 | 280, 350, 400 | |
| FOS079 Scr | 250 | | 180 | | 300, 350 | **Recrudescence** |
| FOS079 D42 | 200 | | 180 | | 300 | |
| FOS080 Scr | 250 | 220 | | 400 | 280, 300 | **Recrudescence** |
| FOS080 D35 | 250 | 220 | 180 | 400 | 300, 350 | |
| FOS081 Scr | 250 | 220 | 180 | | 300 | **New infection** |
| FOS081 D35 | 250 | | | | 280, 350 | |
| FOS090 Scr | 250 | 220 | | 400 | 300 | **New infection** |
| FOS090 D63 | 250 | | 180 | 380, 400 | 300, 350 | |
| FOS095 Scr | | 220 | | 400 | | **Recrudescence** |
| FOS095 D42 | 250 | 220 | | 400 | 300 | |
| FOS098 Scr | 250 | 220 | | 420 | 280, 300 | **New infection** |
| FOS098 D63 | | | 180 | | 350 | |
| **3D7 Control** | 250 | | | | 280, 450 | Correct sizes |

The samples that had been identified as possible recrudescent infections were further analyzed via microsatellite genotyping and capillary electrophoresis. All potential recrudescent infections could be shown to be new infections. The results are shown in Table 13.

**Table 13: Microsatellite genotyping results quantified by capillary electrophoresis**

Chr.: Chromosome, ID: Identifier, Scr: Screening, D: Day, bp: base pairs

| Microsatellite markers | | Poly-α (Chr. 4) | PFPK2 (Chr. 12) | TA81 (Chr. 5) | ARA2 (Chr. 11) | TA87 (Chr. 6) | TA40 (Chr. 10) | |
|---|---|---|---|---|---|---|---|---|
| Genbank accession nr. | | L18785, AF166139–43 | X63648, AF166134–8 | AF010510, AF166147–51 | X17484, AF166128–130 | AF010571, AF166152–6 | AF010542, AF286888–91 | |
| Repeats of trinucleotides | | (AAT)11–20 | (TTA)8–13 | (AAT)7–15 | (AAT)9–12 | (AAC)7–11/ (AAT)9–12 | (AAT)5–8 | |
| Size range | | 114-201 | 159-192 | 112-142 | 63-90 | 90-126 | 217 | |
| Pair | Sample ID | Allele sizes of Plasmodium falciparum microsatellite markers in bp | | | | | | Results |
| 1 | FOS078 Scr | 152-176 | 168-174-186 | 118-121 | 63-66-69-72 | 90-93-96 | 220 | New infection |
| | FOS078 D63 | 152-164-173 | 162-165-171 | 121-127-142 | 63-66-69-78 | 90-99-102 | 226-229 | |
| 2 | FOS079 Scr | 149-155 | 165-168-171 | 121-124-127 | 63-66-72-75 | 93-99 | 217-220 | New infection |
| | FOS079 D42 | 158 | 159-162 | NA | NA | 99-102-105 | 217-220 | |
| 3 | FOS080 Scr | 114-135-141-161 | 159-162-165 | 121-124-130 | 63-66 | 93-96 | 220 | New infection |
| | FOS080 D35 | 129-167-170 | 165-168-171 | 118-121-124-127 | 63 | 102-105 | NA | |
| 6 | FOS095 Scr | 164-170-173 | 159 | 115-118-121 | 63-66-69-72 | 90-93 | NA | New infection |
| | FOS095 D42 | 143-155-158-164 | 162-168-171 | 118-121-124 | 63 | 90-93-96 | 220-229 | |
| Positive controls | 3D7 | 151 | 162-165 | 115-118 | 66-69 | 90-93 | 217-220 | Expected allele size |
| | HB3 | 179 | 180-186-189 | 121-127-130 | 63-66 | 96-99-102 | NA | |

### 3.3.2 Analysis of gametocyte dynamics

A total of 17 (34%; 95%CI: 22.4-47.9) patients carried gametocytes at least at one timepoint during the study including the baseline assessment. The proportion of patients in the ITT with gametocytes at baseline was 4 out of 49 (8.2%; 95%CI: 3.2-19.2) and 2 out of 47 (4.3%; 95%CI: 1.2-14.3) on D7. Of the 46 patients, that had not carried gametocytes at baseline, 13 (28.3%; 95%CI: 17.3-42.6) developed gametocytemia during the study. The cumulative gametocyte carrier rate (patients with gametocytemia on at least one timepoint of follow up) was 16 out of 50 (32%; 95%CI: 20.8-45.8). Except for FOS068, who showed gametocytes only at day 63 of follow up, all gametocytemias first appeared during the 72 hours treatment phase. Between D7 and D28, 2 out of 43 (4.7%; 95%CI: 1.3-15.5) patients had gametocytes in the thick smear analysis. One of them had a simultaneous reinfection (FOS071). If one corrects for this case the rate of gametocytemia between D7 and D28 was 1 out of 42 (2.4%; 95% CI: 0.4-1.2).

Figure 11 shows a Kaplan-Meier plot for the time to gametocyte clearing in patients with gametocytes at baseline (N=4). The median Kaplan-Meier estimate for gametocyte clearing was 7.0 days (95% CI: 0-19.7 days). Figure 12 shows a Kaplan-Meier plot for the survival of patients in which gametocytes did not appear during the first 72 hours. All patients were included into the analysis (N=50) but patients with gametocytes at baseline were marked as censored. The median Kaplan Meier estimate for the time to gametocyte appearance was 24.0 hours (95% CI: 10.2-37.8 hours). Figure 13 depicts the development of individual gametocyte counts during the first 28 days of follow up in a multiple line diagram. Each line represents one patient that presented gametocytemia at any given time point.

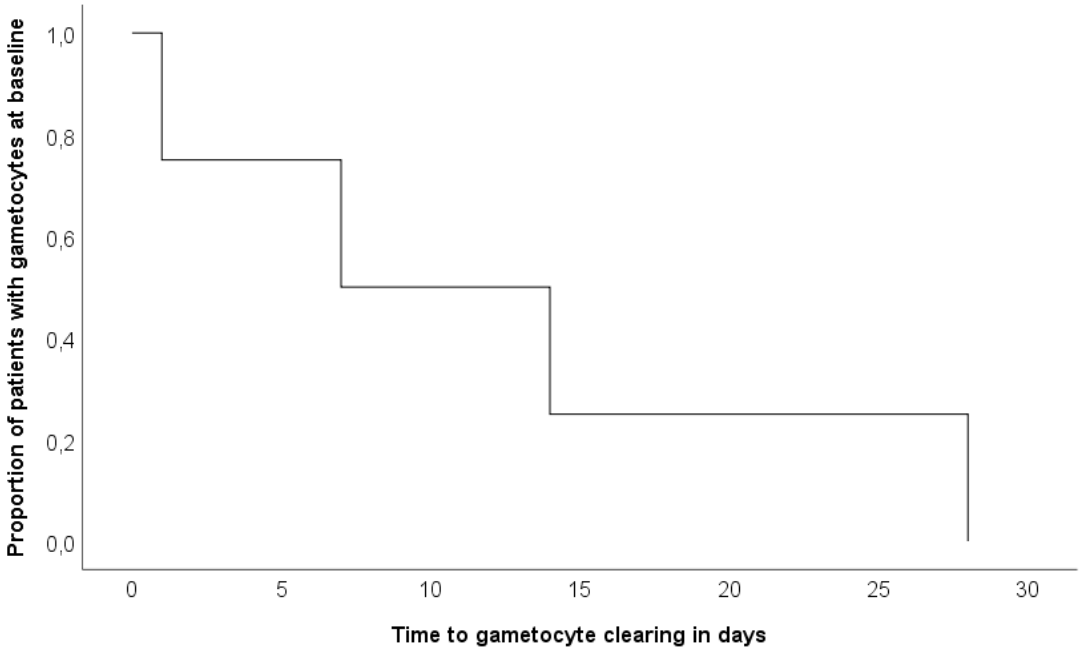



*Figure 11: Kaplan-Meier plot for the time to gametocyte clearing in patients with gametocytemia at baseline (N=4)*

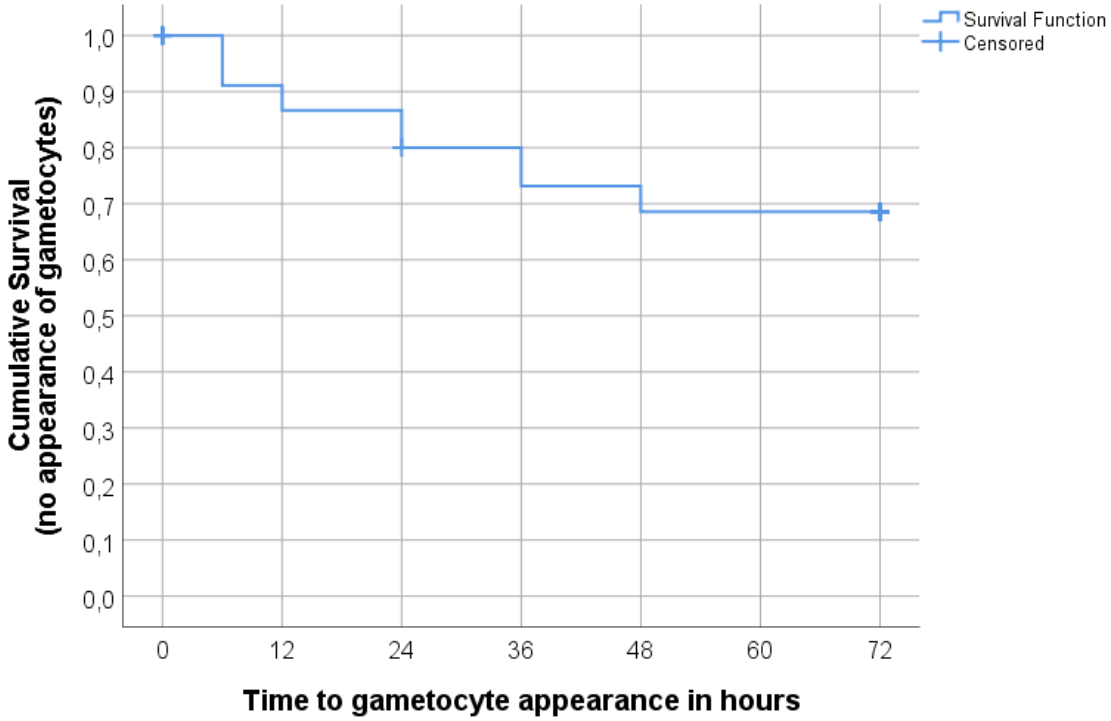



*Figure 12: Kaplan-Meier plot for gametocyte appearance in the first 72 hours* (N=50; Patients with gametocytes at baseline are censored)*

*There were no new gametocyte appearances observed after the first 72 hours, except for FOS068 who showed gametocytes at day 63 of follow up for the first time. The latter was judged as an outlier and not included into the analysis.

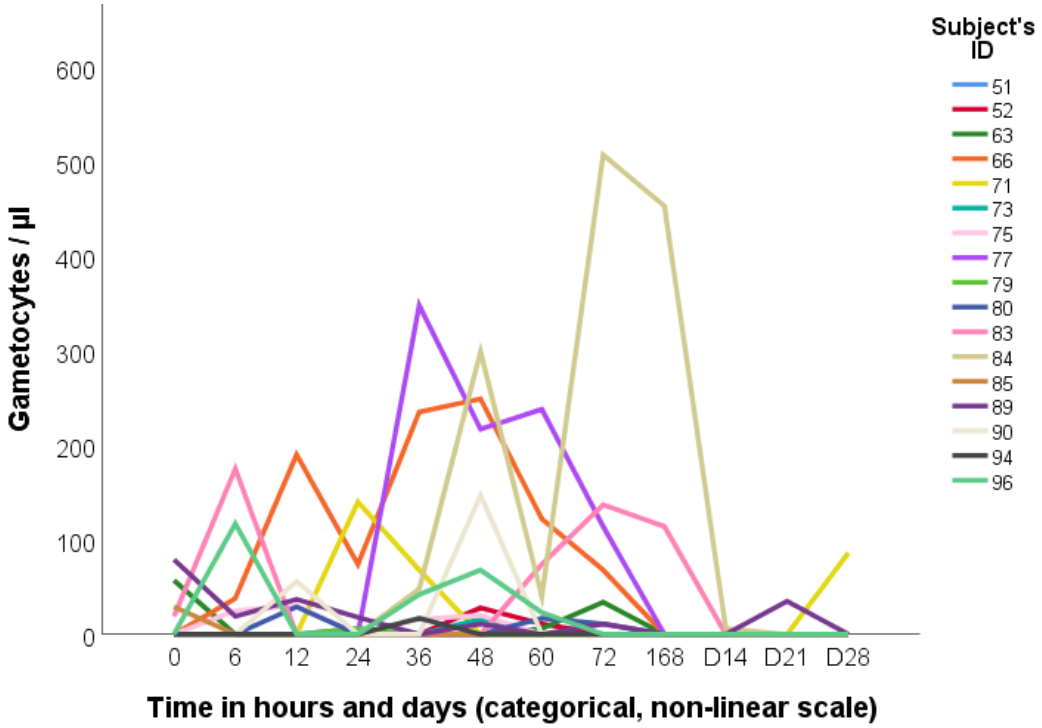



*Figure 13: Development of gametocytemia over time per patient, including only patients presenting gametocytemia at any given timepoint (N=17)**

D: Days

*FOS068 showed gametocytes only on D63. This was judged as an outlier and was therefore not included in the analysis.

## 3.4 Safety analysis

### 3.4.1 Clinical laboratory parameters

Clinical laboratory safety was assessed on D0, D3, D7 and D28. Table 14 shows the proportion of patients with clinical laboratory parameter values differing from the respective reference range and the development of mean values during the follow up period. At screening most patients (46/50; 92%) presented with anemia. Mean hemoglobin (Hb) was 8.9g/dl at screening and dropped to 8.2g/dl on D3. After the antimalarial treatment most of the patients' Hb values rose with a mean of 9.3 and 10.9g/dl on D7 and D28 respectively. The proportion of patients being anemic on D28 was 10/37 (27%). 2 patients had a drop of Hb >2mg/dl (section 3.4.2.1).

Neutropenia was also observed in a few patients at each timepoint. There was one case of neutropenia grade II (neutrophil count: 500 - 1000/µl) during treatment: FOS094 had a neutrophil count of 1496, 2387, **646** and 2387/µl on D0, D3, **D7** and D28 respectively. The patient did not show any clinical symptoms of immunodeficiency. The other cases

of neutropenia during treatment presented as neutropenia grade I (neutrophil counts: 1000 - 1500/µl). The development of the individual neutrophil counts of the patients affected by neutropenia are depicted in Figure 14. Both the majority of individual neutrophil counts as well as the mean neutrophil counts dropped from D0 to D3, rose from D3 to D7 and dropped again on D28.

7/50 (14%) patients presented with a platelet count <100,000/µl at baseline. On D7 all these 7 patients presented with platelet counts >200,000/µl. Regarding liver parameters, bilirubin was elevated in 6 patients at baseline. All of these had recovered on D7. ALAT and ASAT were elevated consistently in only one patient (FOS080). ALAT values in this patient were 86, 102, 99U/l (reference: >50U/l) and ASAT values were 93, 84, 96U/l (reference: >50U/l) at D0, D3 and D7 respectively. At D28 ALAT as well as ASAT values had normalized in this patient, ALAT being 28U/l and ASAT being 30U/l. All creatinine values measured were within the reference range. A few patients showed hypoglycemia during follow up.

**Table 14: Proportion of patients with abnormal values and mean values in the clinical laboratory assessment**

Hb: Hemoglobin concentration, WBC: White blood cell count, ASAT: aspartate aminotransferase, ALAT: alanine transaminase, SD: standard deviation, IQR: interquartile range
[a]In the assessment of clinical laboratory parameters all patients were included (N=50). If N differs from 50, values are missing
[b]Neutropenia Grade I was defined as neutrophil counts: 1000 – 1,500/µl
[c]Neutropenia Grade II was defined as neutrophil counts: 500 – 1000/µl

| Clinical laboratory parameters[a] | Day 0 | Day 3 | Day 7 | Day 28 |
|---|---|---|---|---|
| Hb <10.2 g/dl, n/N (%)<br>Mean Hb (g/dl) (SD) | 46/50 (92)<br>8.9 (1.0) | 40/43 (93)<br>8.2 (1.2) | 39/46 (85)<br>9.3 (1.1) | 10/37 (27)<br>10.9 (1.0) |
| WBC ≠ 5.1-13.4×$10^3$/µl, n/N (%)<br>Mean WBC (×$10^3$/µl) (SD) | 3/50 (6)<br>8.8 (2.3) | 4/43 (9)<br>9.5 (2.5) | 8/46 (17)<br>9.6 (2.8) | 4/37 (11)<br>9.3 (2.6) |
| Neutropenia Grade I[b], n/N (%)<br>Neutropenia Grade II[c], n/N (%)<br>Mean neutrophil count (×$10^3$/µl) (SD) | 3/49 (6)<br>1/49 (2)<br>3.1 (0.2) | 6/45 (13)<br>0/45 (0)<br>2.5 (0.2) | 1/42 (2)<br>1/42 (2)<br>2.9 (0.2) | 6/34 (18)<br>0/34 (0)<br>2.6 (0.2) |
| Platelet count <100×$10^3$/µl, n/N (%)<br>Mean platelet count ($10^3$/µl) (SD) | 7/50 (14)<br>198 (82) | 2/43 (5)<br>259 (108) | 0/46 (0)<br>340 (125) | 3/37 (8)<br>245 (94) |
| Bilirubin <17.1µmol/l, n/N (%)<br>Median bilirubin level (µmol/l) (IQR) | 6/48 (13)<br>11.9 (6.2) | 1/39 (3)<br>8.6 (3.3) | 0/44 (0)<br>7.4 (4.1) | 0/26 (0)<br>6.2 (4.7) |
| ASAT >50U/l, n/N (%)<br>Mean ASAT level (U/l) (IQR) | 1/50 (2)<br>27 (8) | 1/41 (2)<br>26 (12) | 1/44 (2)<br>29 (8) | 0/29 (0)<br>31 (12) |
| ALAT >50U/l, n/N (%) | 3/50 (6) | 2/41 (4) | 1/44 (2) | 0/29 (0) |

| | | | | |
|---|---|---|---|---|
| Mean ALAT level (U/l) (IQR) | 15 (7) | 13 (8) | 15 (7) | 13 (11) |
| Urea >7.1mmol/l, n/N (%)<br>Median urea level (mmol/l) (IQR) | 7/42 (17)<br>3.3 (1.0) | 4/41 (10)<br>3.7 (2.0) | 3/44 (7)<br>3.3 (1.3) | 0/27 (0)<br>3.3 (0.4) |
| Median creatinine level (µmol/l) (IQR) | 44 (22) | 39 (8) | 38 (15) | 35 (19) |
| Glucose <3.5mmol/l, n/N (%)<br>Mean glucose level (mmol/l) (SD) | 0/46 (0)<br>5.4 (1.4) | 1/45 (2)<br>5.0 (0.8) | 6/42 (14)<br>4.7 (1.0) | 2/22 (14)<br>4.4 (0.9) |

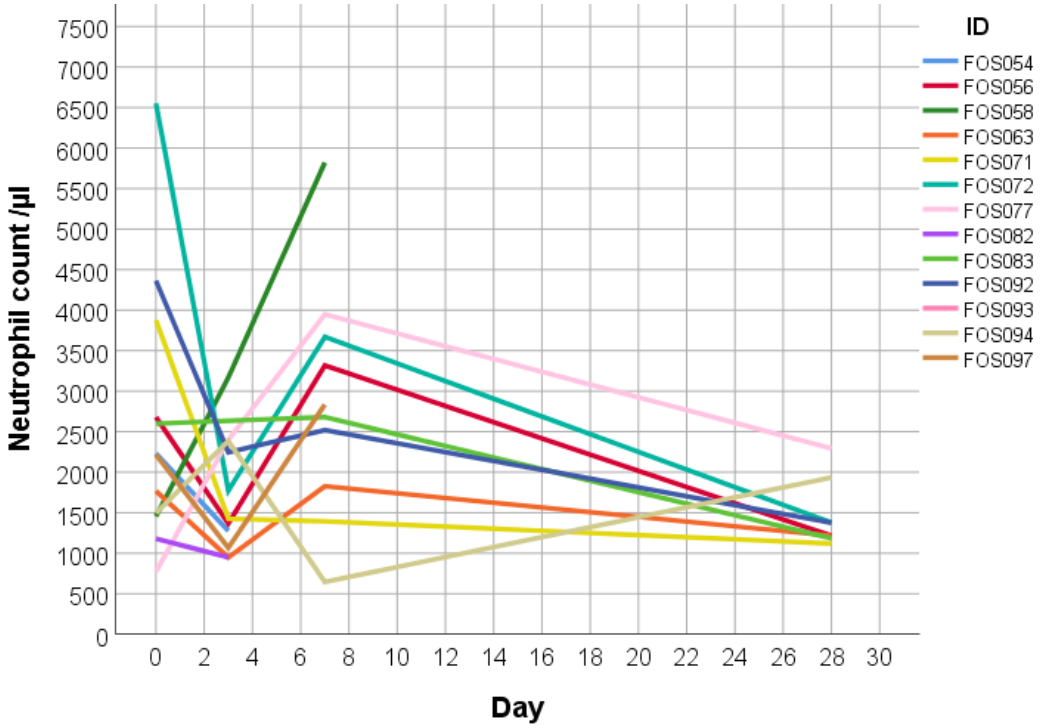



*Figure 14: Development of individual neutrophil counts of the patients affected by neutropenia (N=13)*

### 3.4.2 Adverse events

In the safety population (N=50) 44 patients (88%) reported to have at least one AE within the 63-day follow up period. A total of 146 AEs was reported. Most of them affected the gastrointestinal or respiratory system and were transient. Based on the CTCAE grading system 94 (64%) were judged as mild, 50 (35%) as moderate and 2 (1%) as severe. The two severe AEs presented as a drop of hemoglobin >2mg/dl in patients that were already anemic at baseline. No blood transfusion, hospitalization or discontinuation was necessary. 10 AEs of special interest were reported: 2 events with a drop of hemoglobin >2mg/dl as mentioned above and 8 events with a prolongation of QT duration. Since the safety population consisted of 50 patients that equaled a 4% rate of significant Hb drops and a 16% rate of cases with significant QT-prolongation. 2 patients (FOS065 and FOS066) were discontinued from treatment on D2 due to QT-prolongation according to

the protocol discontinuation criterion (section 3.1.2.2). Both patients showed no clinical symptoms, in particular no arrythmia and were clinically stable. None of the other AEs were judged to be treatment-limiting (section 3.1.2.3). 42 (29%) AEs required therapeutic measures such as antibiotic treatment, iron supplementation or antipyretics.

Regarding causal relation to study drug intake, 77 (53%) AEs were judged as not related, 40 (27%) as unlikely related, 16 (11%) as possibly related, 1 (1%) as likely related and 12 (9%) as most likely related. The AEs that were judged as possibly, likely and most likely related to study drug intake consisted of gastrointestinal symptoms such as diarrhea (N=4) and vomiting (N=5), cardiac abnormalities presented as prolongation of the QT-duration (N=8), fever (N=1), headache (N=1), papulous rash (N=2) as well as blood and lymphatic disorders presented as neutropenia (N=9) and a drop of hemoglobin of >2mg/dl (N=2). Table 15 shows all the specific AEs sorted by System Organ Class according to the MedDRA [92].

**Table 15: Adverse Events sorted by System Organ according to the Medical Dictionary of Regulatory Activities (MedDRA)**

QTcF: QT interval duration in the ECG corrected for heart rate by the Frederica formula
[a]Total N of AEs = 146
[b]Change in the QT interval duration corrected for heart rate by the Frederica formula of any measured time point compared to the baseline ECG measurement

| System organ class | n[a] (%) | Specific clinical feature, n (%) | n[a] (%) |
|---|---|---|---|
| Blood and lymphatic disorders | 9 (7) | Lymphadenopathy | 7 (5) |
| | | Drop of hemoglobin >2g/dl | 2 (1) |
| | | Neutropenia | 9 (6) |
| Cardiac disorders | 8 (6) | ΔQTcF[b] > 60ms | 6 (4) |
| | | QTcF ≥ 500ms | 2 (1) |
| Ear and labyrinth disorders | 5 (4) | Ear pain | 2 (1) |
| | | Otitis media | 3 (2) |
| Gastrointestinal disorders | 27 (20) | Vomiting | 9 (6) |
| | | Diarrhea | 14 (10) |
| | | Abdominal pain | 4 (3) |
| General disorders | 9 (7) | Fever | 9 (6) |
| Infections and infestations | 16 (12) | Malaria | 14 (10) |
| | | Mumps | 1 (1) |

| | | | |
|---|---|---|---|
| | | Skin abscess | 1 (1) |
| Nervous system disorders | 3 (2) | Headache | 3 (2) |
| Respiratory disorders | 40 (29) | Infection of upper respiratory tract | 12 (8) |
| | | Cough | 11 (8) |
| | | Rhinorrhea | 14 (10) |
| | | Rhonchus | 3 (2) |
| Skin and subcutaneous disorders | 18 (13) | Mycosis | 7 (5) |
| | | Rash maculo-papular | 11 (8) |
| Urinary disorders | 1 (1) | Hematuria | 1 (1) |

#### 3.4.2.1 AEs of special interest (AESI)

There were two patients with a drop of hemoglobin >2mg/dl (FOS058 and FOS070). FOS058 had a hemoglobin concentration (Hb) of 8.8g/dl at baseline and an Hb of 6.7g/dl on D3. FOS070 had an initial Hb of 9.6g/dl that decreased to 7.5g/dl on D3 and 7.3g/dl on D7. The patients were 3 and 5 years old. Both presented with high fever (axillary temperature: 38.9°C and 38.6°C) and very high parasite counts (105,070 p.f./µl and 77,805 p.f./µl) at baseline. They responded well to antimalarial treatment, PCT being 48 and 60 hours. After the treatment phase iron was supplemented. On D28 the Hb values were measured at 10.5g/dl and 11.5g/dl.

The other event defined as AESI per protocol was the prolongation of the QT interval corrected for heart rate by either the Frederica or the Bazett formula (section 2.3.1.4). 7 patients presented significant prolongation of QTc as defined per protocol (section 2.1). 2 of these patients presented absolute QTcB values >500ms. In the other 5 cases QTcF changed >60ms from baseline. FOS091 both presented the highest absolute QTcB (504ms) and the highest change in QTcF from baseline (104ms). The individual QTcF and QTcB measurements are visualized as individual lines in Figure 15 and Figure 16 respectively. Three out of these 7 patients had fever and 2 patients presented high heart rates at baseline. The baseline characteristics possibly relevant for QTc duration are shown in Table 16.

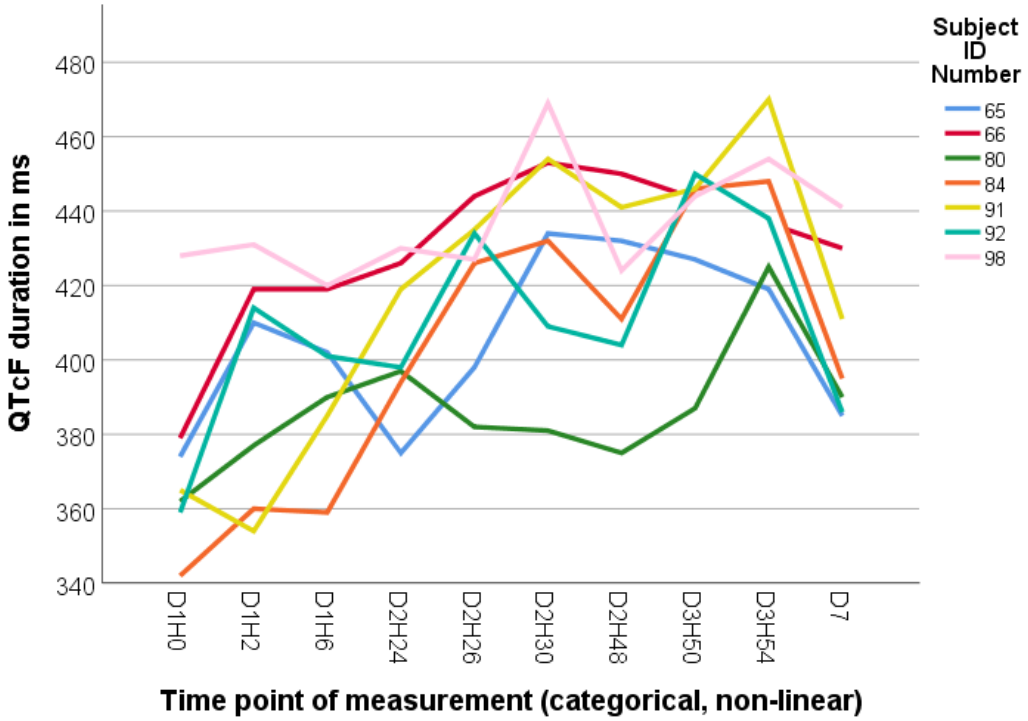



*Figure 15: Individual QTcF durations per measurement point in a multiple line diagram*

QTcF: QT interval corrected for heart rate by the Frederica formula, D: Day, H: Hour

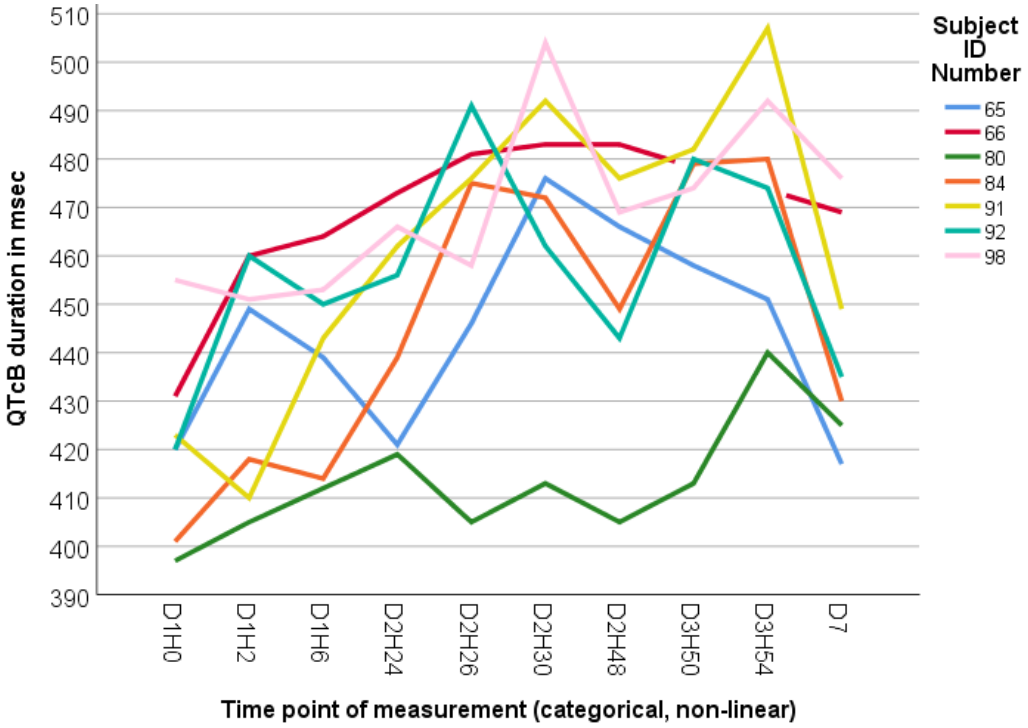



*Figure 16: Individual QTcB durations per measurement point in a multiple line diagram*

QTcF: QT interval corrected for heart rate by the Bazett formula, D: Day, H: Hour

**Table 16: Potentially relevant baseline characteristics for patients with significant QTc prolongation**

QTc: QT interval duration corrected for heart rate, F: female, M: male, NA: not available

| Subject ID | Age | Gender | Temperature, °C | Heart rate, bpm | Potassium level, mmol/l | Pip dosage, mg/kg |
|---|---|---|---|---|---|---|
| FOS065 | 5 | F | 38,1 | 113 | 3,5 | 18.6 |
| FOS066 | 3 | F | 36,6 | 113 | 5,1 | 17.5 |
| FOS080 | 5 | M | 37,4 | 113 | 3,7 | 16.8 |
| FOS084 | 5 | M | 38,9 | 116 | 3,5 | 19.0 |
| FOS091 | 2 | F | 37,8 | 155 | 4,3 | 18.6 |
| FOS092 | 2 | M | 38,6 | 177 | NA | 20.2 |
| FOS098 | 5 | M | 34,7 | 104 | 3,3 | 17.8 |

To further assess the relation between study drug intake and QT-prolongation the mean values with standard deviation of the QTcF and QTcB values at the time points of measurement are shown in Table 17. The development of mean QTcF and QTcB measurements during the treatment phase is visualized in Figure 17. Both in the table and the graph one can see that before each piperaquine drug intake at H0, H24 and H48 (each marked with an arrow in Figure 17) the mean QTcF and QTcB values are lowest, 2 hours after each drug intake they are higher and after 6 hours they are highest. One can also see in Table 17 that in the last ECG measurement at D7 the mean QTcF (396ms) and QTcB (432ms) values are only slightly higher than the QTcF (385ms) and QTcB (427ms) values at baseline. The mean change in QTcF and QTcB from baseline to the timepoint of the highest expected cumulative dose was 33ms and 19ms respectively.

Despite the absence of pharmacokinetic data, a significant positive correlation between QTc duration and the time of treatment with increasing cumulative piperaquine dosage could be statistically fitted. The correlation was especially strong for QTcF, the coefficient of determination ($R^2$) being 12% and the F-Ratio being 58.19 ($p<0.001$). The other parameters of the linear regression model fitting QTcB and QTcF for the time spent being treated are shown in Table 18.

**Table 17: Mean QTcF and QTcB measurements for each time point**

QTcF: QT interval corrected for heart rate by the Frederica formula, QTcB: QT interval corrected for heart rate by the Bazett formula, H: Hour, D: Day, SD: standard deviation

| | QTcF, Mean (SD) in ms | QTcB, Mean (SD) in ms |
|---|---|---|
| H0 | 386 (19) | 427 (16) |
| H2 | 394 (22) | 432 (19) |
| H6 | 403 (19) | 440 (23) |
| H24 | 395 (18) | 435 (21) |
| H26 | 403 (28) | 440 (28) |
| H30 | 415 (23) | 449 (23) |
| H48 | 405 (20) | 438 (23) |
| H50 | 413 (20) | 445 (22) |
| H54 | 419 (21) | 446 (25) |
| D7 | 396 (20) | 432 (20) |

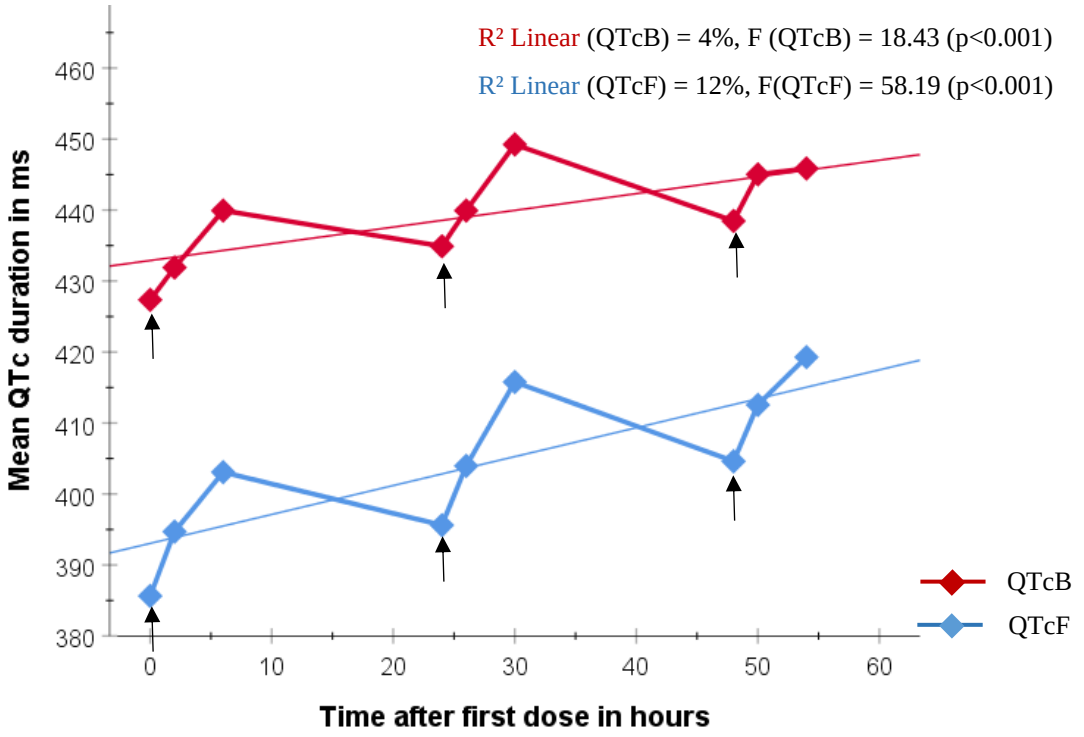



*Figure 17: Changes of mean QT interval corrected for heart rate by Frederica and Bazett formula during the treatment phase with regression lines*

QTc: QT interval in ECG corrected for heart rate, QTcB: QT interval corrected for heart rate by the Bazett formula, QTcF: QT interval corrected for heart rate by the Frederica formula, $R^2$: Pearson's r squared, F: F-ratio

**Table 18: Parameters of the linear regression model of QTc duration and time in the treatment phase**

QTcF: QT interval corrected for heart rate by the Frederica formula, QTcB: QT interval corrected for heart rate by the Bazett formula

| Statistical parameters | QTcF | QTcB |
|---|---|---|
| Pearson's $R^2$ | 12% | 4% |
| F-ratio | 58.19 ($p<0.001$) | 18.43 ($p<0.001$) |
| $B_0$ | 393.1 | 432.9 |
| $t(B_0)$ | 223.56 ($p<0.001$) | 239.1 ($p<0.001$) |
| $B_1$-value | 0.41 | 0.24 |
| $t(B_1)$ | 7.63 ($p<0.001$) | 4.3 ($p<0.001$) |

# 4 Discussion

The second part of the proof of concept study FOSPIP aimed to investigate the efficacy and safety of the potential NACT fosmidomycin-piperaquine in 50 children between 1 to 5 years with acute uncomplicated P. falciparum malaria. In this study the fosmidomycin-piperaquine combination therapy as a twice daily 3-day regimen showed very good efficacy and good safety as well as tolerability. Since the present first-line ACTs still perform very well in most of malaria endemic regions FOSPIP was designed as a non-inferiority study. The primary endpoint of the study was the per protocol PCR-corrected cure rate on D28. To evaluate this endpoint the hypothesis of this dissertation was formulated as follows: The combination FOS-PQ is effective at ≥95% for the treatment of uncomplicated P. falciparum malaria in children aged ≤5 years. Based on the data collected the hypothesis could be confirmed.

In the following the results of the FOSPIP study Part 2 will be interpreted in the context of comparable studies and its clinical relevance will be discussed. The quality of data and the validity of evidence obtained in this study will be critically examined in the light of potential sources of bias created by the study design and applied methods.

## 4.1 Discussion of methods

### 4.1.1 Study design

FOSPIP was designed as a phase IIa open-label, uncontrolled, single arm proof of concept study. That means none of the people involved were blinded, there was no control group nor different treatment arms examining different treatment regimens.

The lack of blinding poses a very important source of bias on several levels. First, the participants who know that they receive a new drug could be influenced to be more aware of side effects. This might be relevant for subjective symptoms like headache or gastrointestinal pain. Since the study population consisted of very young children, of whom many probably did not understand the concept of a new drug, this effect can be neglected. More important though, is the lack of blinding of the investigators and laboratory personnel. Especially when it comes to the interpretation of blood smear results and the MSP2 genotyping results of the first half of samples this might have led to an overestimation of efficacy results (section 4.1.3). Lastly, the data collector and analyst might have been biased by knowing that all patients had received active treatment and by knowing the timepoints of the analyzed samples.

The lack of a control group makes it harder to compare the results with the standard first-line treatment. There are study site specific conditions that differ from the conditions in other sites and at other timepoints. Without a direct comparator group receiving the first-line treatment, the performance of the study drug can only be compared to an estimated performance of the first-line treatment by referring to other antimalarial drug studies from the same region. In future multiple site randomized controlled studies are needed to back up the evidence generated in this proof of concept study.

### 4.1.2 Clinical procedures

There are several factors in the design of the clinical procedures that were well-suited for a proof of concept study and still are an unrealistic representation of the real treatment conditions in malaria endemic areas.

The risk of *sampling bias* on the outcome introduced by pre-screening and screening can be rated as minor for many of the in- and exclusion criteria reflect the definition of acute uncomplicated malaria. Only the exclusion of patients with prolonged QTc duration might relevantly differ from treatment reality as not all malaria patients will receive an ECG before treatment. Another issue is the strict treatment supervision that secured each drug intake. This was vital to making any reliable assumptions about efficacy and safety of the drug combination, but it is unlikely that the real target population will stick to the regimen as strictly. Future studies will have to determine efficacy and safety under more realistic treatment conditions.

Another constraining issue is the dosing scheme applied in this study. Fosmidomycin was administered twice-daily as capsules and in high doses. Piperaquine was administered only once daily and as tablets. To account for the weight-adapted dosage a complicated dosing table was applied, and different sizes of capsules and tablets had to be individually selected for each weight group. In this study compliance was not a relevant problem as all patients were hospitalized during the treatment phase and each drug administration was monitored by an investigator. In real treatment conditions though, the complicated drug administration procedures might hinder compliance. Ideally, the study drug should be formulated as syrup or tablets containing both study drugs in one formulation to simplify weight-adaption and avoid the intake of too many tablets. Furthermore, future dose-optimization studies should assess the possibility of a once-daily treatment regimen and possibly a lower dosage of fosmidomycin to facilitate compliance.

Clinical procedures like the inquiry of the clinical history, performance of the clinical exam, collection of blood samples and ECG measurement were conducted in a standardized way defined by the protocol. Certain exam results depend on the examiner like estimation of liver size or on the patient's answer like intake of other antimalarials before or during study participation. Especially the latter could have influenced the study outcome if answered incorrectly. However, most of the important clinical procedures related to specific endpoints did not rely on subjective evaluation. Measurement of the axillary temperature and QT interval duration, preparation of the thick and thin smear as well as the dried blood spots were conducted strictly following protocol procedures independent of the investigator performing it. The performance of these clinical procedures should not have relevantly biased the study outcome.

### 4.1.3 Laboratory procedures

Procedures such as hematology and biochemistry assessment were automated, and machines were always calibrated correctly. The analysis of thick and thin smears as well as *MSP2* genotyping results were more prone to mistakes and subjective interpretation in the sense of *detection bias* or more specifically *observer bias*.

Thick and thin smear analysis is a reliable and well-proved method [104]. Nonetheless reliable quantification can be very difficult, especially when the smear's staining quality

is poor [42]. For this reason, each smear was analyzed by at least two well qualified, independent microscopists. In case of bad smear quality, the backup smear was analyzed instead. The issue remains that the microscopists were not blinded and knew the timepoint of the smear's preparation. So, depending on the time point of the smear's preparation the microscopist might have judged an artefact to be a parasite or vice versa. This could have influenced efficacy outcomes depending on quantification like the PCT and the parasite reduction ratio. The qualitative differentiation between infection or no infection is only difficult or unfeasible in very low parasitemias [42]. Since young children have little or no innate immunity against malaria they are more often symptomatic and the parasite densities are higher compared to older children and adults in endemic malaria regions [18]. The risk of overseeing a recrudescent infection in young children over a period of 63 days can therefore be judged unlikely.

The following aspects could have been optimized for higher validity of the genotyping results. According to the WHO genotyping recommendations [96] a digital gel documentation and analysis software should be used for sizing of fragments and setting cut-off intensities for spurious bands to ensure unbiased comparison of paired samples. In this study gel analysis was performed by two independent but unblinded investigators. Furthermore, the WHO recommends performing *MSP1* and/or *glurp* genotyping as well as higher resolution techniques like restriction fragment length polymorphism analysis or capillary electrophoresis if the MSP2 genotyping results are not conclusive. The techniques mentioned above were performed for the second half of samples analyzed in the institute of tropical medicine in Tübingen but not for the first half analyzed in Lambaréné. Concerning the first half of samples the lack of a digital gel analysis software as well as higher resolution techniques represent important sources for potentially inconsistent analysis.

### 4.1.4 Data management and analysis

The data collection was performed by different investigators and checked on several levels for mistakes and completeness. As one investigator and a clinical monitor revised the source documents before transcribing the data onto the CRFs and as the CRFs were revised again by the clinical monitor the risk for data collection mistakes was minimized. The data entry from the source into Microsoft® Excel and Access worksheets as well as REDCap™ and the data analysis for this thesis was solely performed by the author. The

author was also an investigator of this study and unblinded. In an ideal setting, data entry should have been performed by two independent investigators to prevent transcription mistakes. Data analysis should have been conducted by an independent, blinded data analyst to prevent biased interpretation.

### 4.1.5 Funding

The study was funded by Jomaa Pharma GmbH and Medicines for Malaria Venture (MMV). There was a strict separation between the tasks of the investigators and the tasks of the clinical monitor to minimize the influence of the sponsor on the study results. None of the investigators had financial affiliations with the sponsor except for the author of this thesis whose parents are shareholders of Jomaa Pharma GmbH. As the author of this thesis was involved in nearly all relevant study procedures this represents an important source of funding bias. However, the author of this thesis tried to respect good clinical and laboratory practice standards as any other investigator would and all data obtained was checked for validity and conclusiveness by the principle investigator of this study.

## 4.2 Discussion of results

### 4.2.1 Study population

The study population consisted of 50 children ≤5 years old. 11 of these children were <3 years and 16 were female. The small sample size especially of the very young children is an important limitation of this study. The uneven gender distribution of 32% female and 68% male children also limit the representative value of this study population for the whole target population of young children. However, the sample size and composition are appropriate for the proof of concept design of the study. A reason for the slight underrepresentation of very young children might be the age-step-down procedure applied and the higher rate of severe malaria symptoms in very young children leading to more exclusions [18, 105].

The clinical and laboratory baseline characteristics align well with the clinical presentation of acute and chronic malaria infection. The very high rate of anemia (92%), spleno- (52%) and hepatomegaly (32%) is typical for children living in high-transmission malaria regions as they suffer from frequent and sometimes chronic infection [5]. The clinical symptoms like headache, nausea and fever as well as thrombocytopenia, raised liver and kidney functioning parameters are also expected in acute malaria infections [37]. The wide spectrum of parasitemias from around 100 to 150,000 p.f./µl is a good

representation of the different degrees of severity in acute uncomplicated malaria infection and is therefore an adequate sample to examine the efficacy of the new drug combination therapy.

## 4.2.2 Flow of patients

### 4.2.2.1 Screening

Out of the 247 patients around one fifth, namely 50, were included. Frequent reasons for exclusion were parasitemia <1,000 or >150,000 p.f./µl, mixed infections, severe anemia and severe vomiting. The amount of patients with a parasitemia <1,000 p.f./µl is in line with the findings of Zoleko Manego et al. [11] that assessed malaria epidemiology in Tsamba-Magotsi, Ngounie province. The infection rate in 2-10-year-old asymptomatic children was 46% indicating a high burden of disease with many chronic infections. Mixed infections were found in 23% of cases. The high rate of patients with severe anemia and vomiting as well as parasitemias >150,000 p.f./µl might be due to the young age of the patients. Generally, in highly endemic areas children are more affected by malaria than adults and they are especially often affected by anemia due to frequent and chronic malaria infections [18, 106, 107].

Regarding the screening test results of the PARACHECK Pf® RDT when compared to blood smear microscopy the results are congruent with published data about the test's performance in areas with high malaria transmission [45]. The low specificity of 50.1% can be explained by the long half-life of *Histidine-rich protein 2* with ongoing test positivity even after parasite clearance and detection of submicroscopic parasitemias [46, 108]. Both factors are especially relevant in an area of frequent and chronic infections.

### 4.2.2.2 Follow up

An important limitation of this study in the sense of *attrition bias* is the high rate of patients that were lost to follow up, discontinued or withdrew consent during the study. On D28 and D63 out of 49 patients only 41 and 38 respectively were evaluable in the PP. That means 16% and 22% of the PP had dropped out of the study until reaching D28 and D63 respectively. This is a very high drop-out rate which might seriously undermine the validity of the study results [109, 110]. Three reasons for the high drop-out rate could be identified. First, intolerability and side effects of the medication: one patient was discontinued because he was unable to swallow the medication, probably due to the bitter taste of piperaquine. 2 other patients were discontinued due to QTc prolongation >60ms

when compared to the baseline ECG. 5 patients that showed relevant QTc prolongation were not discontinued to prevent even higher drop-out rates. As the QTc prolonging effects of piperaquine are to be expected [79] future studies will have to decide if they define QTc prolongation as a discontinuation criterion less strictly to prevent high drop-out rates. Second, high rate of migration: 5 children treated in this study changed their residency during the ongoing study. Third, unpleasant and frequent examinations: three legal representatives withdrew their consent for study continuation. Judging by their statements the legal representatives were not concerned with side effects of the medication but with the amount of unpleasant examinations like frequent blood sampling and ECG measurements performed on their small children. Summarized, the majority of lost-to-follow-up patients did not drop out of the study because of unregistered bad efficacy, tolerability or compliance with the study drug but because of reasons independent of the study medication. To minimize the effect of the high drop-out rates on the validity of the study results, the ITT with a lower drop-out rate was also analyzed and statistical methods correcting to some extent for drop-out like survival analysis were performed.

### 4.2.3 Efficacy

#### 4.2.3.1 Asexual parasite clearance

This was the first clinical trial assessing the efficacy of a FOS-PQ combination therapy. The new drug combination showed strong antimalarial activity and very good efficacy for the treatment of acute uncomplicated malaria in Gabonese children ≤5 years. The PCR-corrected and non-PCR-corrected ACPR on D28 in the PP was 100% (95%CI: 91.4-100) and 95.1% (95%CI: 84.9-98.7) respectively. The median PCT and FCT were 48 and 12 hours respectively. The results are generally in line with previous pediatric clinical trials assessing the efficacy of fosmidomycin in combination therapies. There is a total of 6 clinical trials that assessed the efficacy of fosmidomycin combination therapies: 5 of them in combination with clindamycin and one in combination with artesunate. The studies reported PCR-corrected cure rates on D28 for a 3-day treatment regimen of 100% [69], 90% [70], 100% [71], 89% [72], 94% [73] and 45.9% [74] with an overall cure rate of 85% (95% CI: 71-98%) as determined by Fernandes et al. [66]. Overall mean PCT and FCT were calculated to be 39 hours and 30 hours respectively by Fernandes et al. [66]. Non-PCR-corrected cure rates on D28 were reported as 91% [69], 80% [70], 90% [71],

83% [72] and 40.5% [74].

Interestingly, most of these studies were conducted in older children. Only the trials conducted by Borrmann et. al [72] and Lanaspa et. al [74] included children <3 years. An efficacy analysis in the sub-group of children <3 years in the study by Borrmann et al. [72] showed a PCR-corrected cure rate of only 62% on D28. This is much closer to the bad performance of 45.9% in the trial conducted by Lanaspa et al. [74]. The trial by Lanaspa et al. is the only pediatric clinical trial conducted outside of Gabon, namely in Mozambique, and it included only children <3 years. Specific genetic reasons that might explain the bad performance in the Mozambican children with a recrudescent infection were ruled out by a whole-genome sequencing study by Guggisberg et al. [111]. In conclusion, the FOS-PQ combination of this study performed much better in young children, including 11 children <3 years, than the FOS-CM combination did in both Gabonese as well as Mozambican children.

One reason for the bad performance of FOS-CM in Mozambique discussed by Fernandes et al. [66] was the formulation of the study medication as syrup which might have led to underdosing. To prevent potential underdosing in this study instead of syrup fosmidomycin capsules and piperaquine tablets were administered. However, the drug formulation cannot solely explain the very good performance of FOS-PQ in young children. In the study conducted by Borrmann et al. [72] the children <3 years also received FOS-CM as capsules but performance was bad with cure rates of 62% in this subgroup.

Another possible reason for the good performance could be a higher level of synergy of FOS-PQ compared to FOS-CM. The in vitro assessments conducted so far indicate otherwise. While FOS-CM showed a synergistic effect in vitro with a sum FIC of 0.43 [75], FOS-PQ showed an indifferent effect with sum FIC ranging from 1.1-1.5 [unpublished data referring to the study IB]. As these studies only assessed the in vitro effects in vivo performance might relevantly differ. Nonetheless the data obtained so far suggests that synergy of fosmidomycin and piperaquine cannot explain the excellent performance in this study.

It is more likely that the difference in performance can be explained by the higher antimalarial activity of piperaquine compared to clindamycin. In a review conducted by Lell and Kremsner in 2002 [112] the authors conclude that Clindamycin performed well

in monotherapy with mean cure rates of 98% but only if given for at least 5 days due to its relatively slow mode of action compared to other antimalarials. If given in a three-day-regimen cure rates ranged from 50 to 100%. In combination with quinine and chloroquine, clindamycin also showed mixed results with cure rates ranging from 50 to 100% in a 3-day regimen. Piperaquine on the other hand seems to perform well in monotherapy (even though evidence is very scarce) and very well in combination therapies. There is one small study conducted by Pasay et al. [113] that assessed the antimalarial activity of piperaquine alone in an induced blood stage malaria model reporting cure rates of up to 100% in a single-dose treatment. Such a high cure rate though was only achieved in the treatment arm with the highest dosage (960mg) and in a very small sample (N=3). Also, the parasitemias are much lower in an induced blood stage malaria model than in acute infections. The other outcome parameters of the study conducted by Pasay et al. [113] like parasite reduction rate and parasite clearance half-time indicated rapid parasite clearance in piperaquine monotherapy. There is more evidence for piperaquine's good performance in combination with dihydroartemisinin. In a review conducted by Gargano et al. [114] the authors conclude that DHA-PQ combination therapies consistently showed cure rates >95% throughout many different large stage III trials except for one trial in Papua New Guinea. The evidence presented indicates that piperaquine possesses stronger antimalarial activity than clindamycin, especially in a three-day regimen. This is probably the most important reason for the high cure rates in very young children observed in this study in comparison to the bad performance observed in the studies conducted by Borrmann et al. [72] and Lanaspa et al. [74].

As this trial was designed as a non-inferiority study one must look at these results in the context of the current first-line treatments. In a study conducted by the Four Artemisinin-Based Combinations (4ABC) Study Group [115] 4 different ACTs were tested for non-inferiority in 4,116 children aged 6-59 months out of seven Sub-Saharan countries. Only 3 of these ACTs are currently recommended as first-line treatment against P. falciparum malaria. In the following only the results of these 3 ACTs will be discussed. The PCR-corrected cure rates on D28 for AS-AQ, A-L and DHA-PQ were 96.8%, 95.5%, 97.6% respectively; The non-PCR-corrected cure rates on D28 were 90.5%, 72.7% and 80.8% respectively. In the context of these results FOS-PQ performed non-inferior to any of the

3 first-line treatment ACTs. The results of the 4ABC Study Group also demonstrate that piperaquine is a powerful drug partner in preventing recurring infections. The non-PCR-corrected cure rates on D28 were significantly higher in the DHA-PQ group than in all the other tested ACTs.

Regarding secondary efficacy outcomes, FOS-PQ also proved non-inferior to the approved ACTs. The PCR-corrected and non-PCR-corrected cure rate on D63 was 96% (95%CI: 80.5-99.3) and 63.2% (95%CI: 47.3-76.6) respectively compared to 95.8% and 67.7% (AS-AQ), 94.6% and 57.8% (A-L), 95.7% and 68.8% (DHA-PQ) [115]. The median PCT and FCT for FOS-PQ observed in this study was 48 hours and 12 hours respectively. Both is comparable to the performance of A-L when given as a six-dose regimen; In a review that assesses the efficacy of Coartem® Makanga and Krudsood [116] present PCTs and FCTs that range from 24-48 hours and 8-35 hours respectively. The parasite clearance half-life of FOS-PQ estimated by the *Parasite Clearance Estimator* provided by the *WWARN* was 3.71 hours (95%CI: 2.62-5.03) in this study. This is a little slower than the reference of artesunate and A-L therapy provided by the WWARN which is around 2.5 - 3 hours [117]. Nonetheless the estimate indicates rapid parasite clearance of the FOS-PQ combination therapy.

All the data presented so far suggests a very good efficacy of FOS-PQ. However, there are increasing reports of piperaquine resistance in southeast Asia that compromise these promising results. In several clinical studies treatment failure rates of DHA-PQ combination therapy increased from 25% in 2010 to 46% in 2014 in Western Cambodia since the adoption of DHA-PQ as first-line treatment [118]. The latest study tracking DHA-PQ resistance by van der Pluijm et al. [61] report mean treatment failure rates of DHA-PQ of even 50% in the Greater Mekong subregion. The lowest efficacy of DHA-PQ was observed in North-Eastern Thailand with a PCR-corrected cure rate of only 12.7% [61].

So far, DHA-PQ still remains very effective in the rest of the world [119]. In the light of recent developments though, PQ maybe does not present the ideal partner drug for a new NACT designed to tackle multi-drug-resistant parasites.

#### 4.2.3.2 Gametocytocidal activity

The proportion of gametocyte carriers was 8% at baseline and 4% on D7. This implies a

gametocytocidal effect of 50%. But as the sample size of patients with gametocytes at baseline was very small (N=4) no valid assumptions can be made in this aspect. 28.3% (13/46) of patients without gametocytes on admission developed gametocytemia during the study. The cumulative gametocyte carrier rate (gametocytes detected during at least one follow-up visit) was 32% (16/50). Between D7 and D28 2 out of 43 (4.7%) patients were detected carrying gametocytes.

Previous results from clinical trials investigating fosmidomycin alone or FOS-CM generally showed high cumulative gametocyte carrier rates ranging from 27% to 80% [67, 69, 70, 72] with the majority of the results being around 70-80% [66]. Only when fosmidomycin was combined with artesunate a low cumulative gametocyte carrier rate of 16% was observed [71]. Consequently, based on this limited data FOS-PQ seems to block gametocyte development slightly better than fosmidomycin alone and FOS-CM but worse than fosmidomycin-artesunate (FOS-AS) combination therapy.
In the study conducted by Borrmann et al. [69] high gametocyte carrier rates seemed to be associated with a failure of the treatments to radically eliminate asexual parasites though this association was not significant. Hence, the lower gametocyte carrier rates observed in this study might be associated with the higher efficacy in radically eradicating asexual parasites of FOS-PQ compared to fosmidomycin alone and FOS-CM. This might include submicroscopic infections. The aspect of submicroscopic infections remains subject to speculation though as no evaluable data is available in this regard.

Regarding the better performance of FOS-AS when compared to FOS-PQ the results are in line with other studies assessing the gametocytocidal effect of antimalarials. Out of the drugs mentioned above only artemisinin derivatives are known to have gametocytocidal activity [53]. Fosmidomycin does not seem to have relevant gametocytocidal activity [120]. The difference in gametocytocidal activity of fosmidomycin versus artemisinin derivatives is underlined by comparing the results of FOS-PQ to the performance of first-line ACTs. In a meta-analysis of individual patient data conducted by the WWARN Gametocyte Study Group [121] including data from 18,388 patients the rate of gametocyte appearance in patients with no gametocytes at baseline ranged from 0.5-6% in the different ACTs during a 63-day follow up. This is much lower than the 28%

observed in this study. In the meta-analysis [121] the risk of appearance of gametocytemia did correlate negatively with age, hemoglobin concentration and fever. If one assumes that the same confounding factors apply to NACTs like FOS-PQ the young age, low mean Hb and low rate of fever might partly explain the higher rate of gametocytemia observed in this study.

When comparing other gametocytemia outcome measures than cumulative gametocyte carrier rates the differences between ACTs and FOS-PQ are minor. The proportion of gametocyte carriers on D7 and the prevalence of gametocytemia between D7 and D28 was only 4% in both cases which is similar to observations made in first-line ACTs ranging from 2-7% on D7 and 2-5% between D7 and D28 [115]. Concluding from these results, FOS-PQ does not seem to block gametocyte appearance as effectively as ACTs during the acute infection phase.

### 4.2.4 Safety and tolerability

The FOS-PQ combination therapy demonstrated to be safe and well tolerated in the present study. Most AEs were mild or moderate and apart from a drop of Hb and neutrophil counts no relevant laboratory changes could be observed. The most important safety finding was a relevant QT-prolongation in 7 (14%) patients. One patient was discontinued due to incapability to swallow the medication.

#### 4.2.4.1 Clinical laboratory parameters

There was a drop in mean Hb and mean neutrophil counts from baseline to D3 of 0.7mg/dl and $0.6\times10^3$/µl respectively. There were 2 cases (4%) with a drop of Hb >2mg/dl and several cases of neutropenia throughout the study.

A significant drop of mean Hb from baseline to D2 or D3 has been repeatedly reported in previous pediatric trials assessing fosmidomycin in combination with clindamycin as well as artesunate [70–72]. The magnitude of mean Hb decline in the present study was in line with these studies that reported declines of -1.0mg/dl, -0.6mg/dl and -0.7mg/dl respectively. However, the incidence of cases in which Hb dropped >2mg/dl was generally higher in the trials assessing FOS-CM with 8% [70] and 14 % [72] and slightly lower in the trial assessing FOS-AS (2%) [71]. In this study both patients with a drop of Hb >2mg/dl presented with very high parasitemias at baseline (105,070 p.f./µl and 77,805 p.f./µl). No discontinuation nor blood transfusion was necessary since both patients did not present severe anemia at any timepoint. Both recovered well after completion of

treatment.

Anemia is a well-known feature of acute and chronic malaria infection and the pathophysiology is complex (section 1.1.3). A potential hemolytic effect of fosmidomycin has already been discussed by Borrmann et al. [72]. As fosmidomycin showed no hemolytic effect in healthy volunteers [115] or patients with asymptomatic malaria infections [69] Borrmann et al. concluded that fosmidomycin most likely has no relevant hemolytic effect. Piperaquine has never been associated with hemolysis as well [76, 78]. Based on the data generated so far, the drop of mean Hb as well as the individual Hb values was probably not drug-induced but can be attributed to the acute malaria infection. Other underlying factors like glucose-6-phosphate dehydrogenase deficiency cannot be ruled out as these were not assessed in this study. One reason for the higher rates of cases with a significant drop of Hb in the FOS-CM trials compared to FOS-AS and FOS-PQ might be the more rapid parasite clearance of the latter two combination therapies.

The issue of neutropenia in fosmidomycin combination therapies has also been thoroughly discussed by Borrmann et al. [72]. In previous trials assessing fosmidomycin alone or in combination a distinct immunosuppressive effect presenting in high rates of neutropenic patients has been repeatedly observed. Analogically to this study the fall in mean and individual neutrophil counts was strongest from baseline to D2 or D3. The rate of patients with neutropenia grade I or II (for definition see section 2.5.1) on D3 was lower in this study than in the combination therapies of FOS-CM and FOS-AS with 12% versus 24% and 16% respectively [71, 72]. Due to a change of scientific consensus over time classification of neutropenia slightly differs in this dissertation from the classification applied in earlier studies that assessed fosmidomycin combination therapies. As the results of this study do not change regardless of which classification is applied, this factor can be neglected. As the current criteria are stricter, they might if at all lead to a relative underestimation of the immunosuppressive effects of previous studies in direct comparison. Possible factors for neutropenia independent of study drugs cited by Borrmann et al. are vitamin B12 and folate deficiencies, immunosuppression caused by acute malaria infection [123] and sequestration of neutrophils [124]. The immunosuppressive effect of acute malaria on leucocyte counts is supported by several other studies that specifically evaluated hematological changes in children with acute

malaria [125–128]. Even though inhibition of neutrophil function could be demonstrated for some antimalarials like mefloquine in vitro [129] this effect could only be observed in concentrations much higher than the in vivo concentrations obtained in malaria therapy [130, 131]. For piperaquine no specific immunosuppressive effect has been described so far [76, 77]. Interestingly, the proportion of patients with neutropenia in this study was higher on D28 (18%) than on D3 (12%) which suggests also a long-term suppression of neutrophils by either the acute malaria infection, the study medication and/or other underlying factors.

As both anemia and neutropenia are common features of malaria the assessment of specific negative effects of the FOS-PQ combination therapy on hemolysis or immunosuppression is very difficult in a noncomparative study design. Future controlled trials will need to carefully examine the effect of fosmidomycin combination therapies on these hematological features.

#### 4.2.4.2 Adverse events

A total of 32 AEs was judged to be at least possibly related to the study drug. The majority of these either presented as gastrointestinal symptoms like diarrhea and vomiting, blood and lymphatic disorders as described above (section 4.2.4.1) or cardiac abnormalities presenting as QT-prolongation.

In previous studies with FOS alone or in combination with clindamycin or artesunate gastrointestinal symptoms also presented the most common AEs [67, 69–74]. When fosmidomycin alone was compared to FOS-CM or clindamycin alone gastrointestinal symptoms seemed to be more prevalent in the groups receiving clindamycin even though these differences were non-significant [69]. This might indicate that fosmidomycin plays a role in causing gastrointestinal symptoms but is still better tolerated than clindamycin. The rate of gastrointestinal AEs possibly related to study drug intake was 18% (9/50) in this study. Compared to other antimalarial drug studies conducted at the same study site this rate was rather low. It was similar or lower than the rates observed in previous fosmidomycin trials (18-47%) [69, 70, 72] and in the AS-AQ trial (29%) [132], and only slightly higher than the rate observed in the atovaquone-proguanil trial (13%) [133]. These numbers suggest that FOS-PQ affects the gastrointestinal system rather mildly in comparison to other antimalarials assessed at the same study site.

As fosmidomycin is an antibiotic the gastrointestinal symptoms might be caused by disturbance of the intestinal flora. Another possible reason cited by Borrmann et al. [69] is the release of parasite antigen that is being accelerated by rapidly acting antimalarials causing an intensified immune response and possibly malaria symptoms including gastrointestinal symptoms. None of the gastrointestinal AEs in this study led to discontinuation. Indeed, one patient was unable to swallow the medication and therefore discontinued. Since the patient did not vomit this case can rather be regarded as an issue of compliance than of tolerability. In the study conducted by Lanaspa et al. on the other hand 5 out of 52 patients had to be discontinued due to gastrointestinal AEs. 4 patients repeatedly vomited the study medication and one patient suffered from moderate dehydration due to persistent diarrhea [74]. Factors for this difference in gastrointestinal tolerability could be the younger mean age in the study conducted by Lanaspa et al. or local differences, e.g. in comorbidity. It might, however, also further indicate a better gastrointestinal tolerability of FOS-PQ than FOS-CM in children.

QT-prolongation is a well-known side effect of piperaquine. So far no actual arrhythmias have been observed neither in this study nor in any of the numerous large trials assessing DHA-PQ [79]. Since one review conducted by Vanachayangkul et al. [134] found a weak but significant correlation between food intake prior to administration of piperaquine and QT-prolongation patients were fasting in this study 3 hours before and after piperaquine administration if feasible. Apart from food intake no epidemiological risk factor for QT-prolongation could be identified in the review [134]. Similarly, no specific epidemiologic pattern in the collective of patients with QT-prolongation emerged in this study. Age, sex, body temperature, potassium levels as well as individual dosages appeared to be evenly distributed. Correction by the Frederica formula seemed to be more sensitive to change in QT duration than correction by the Bazett formula (6 versus 3 cases respectively). The Bazett formula on the other hand seemed more sensitive in detecting the transgression of absolute values >500ms (2 cases versus 0 cases when QT was only corrected by the Frederica formula).

The rate of patients with a QT-prolongation of >60ms when corrected by the Frederica formula was 14% in this study. This is equivalent to the results of two studies assessing the Artekin® formulation of DHA-PQ [135, 136] with a mean rate of 14.3% as

determined by Mytton et al. [137]. Mean changes of QTcB and QTcF from baseline to the timepoint of highest expected cumulative dose were also similar: 19ms (QTcB) and 33ms (QTcF) in this study compared to 14 ms (QTcB) and 29ms (QTcF) in the Artekin® trials. When comparing the mean changes of QTcB observed in this study to other antimalarial treatments FOS-PQ seems to have a stronger QT-prolonging effect than A-L (7-11ms) and artemether-mefloquine (8ms) but a weaker effect than halofantrine (41-63ms) and quinine (22-60ms) [137].

The rate of patients with a QTcB >500ms observed in this study (2 out of 50; 4%) on the other hand is surprisingly high when compared to other trials with DHA-PQ. In a large stage III trial assessing the Eurartesim™ formulation of DHA-PQ in over 1,039 children conducted by Bassat et al. only 2 patients (0.2%) showed a QTcB >500ms [138]. The differing rates might be explained by the timepoints of ECG measurement. In the study conducted by Bassat et al. only one ECG on day 2 was performed. Also, the ECG was performed before drug administration. In the present study ECGs were performed on each day of hospitalization including day 3. ECGs were performed right before as well as 2 and 6 hours after each drug administration. Both patients with QTcB >500ms detected in the present study would not have been detected in the setting of the study conducted by Bassat et al. In both cases their QTcB only exceeded 500ms after the second dose of piperaquine on D2. Overdosage does not explain the higher rates since the target dosage in the present study (16mg/kg) was similar to the Eurartesim™ trial conducted by Bassat at al. (18mg/kg) [138]. The individual dosages of the 2 patients affected in this study were also not particularly high with 18.6 (FOS091) and 17.8mg/kg (FOS098). Finally, individual pharmacokinetic or genetic differences might also have played a role. Fosmidomycin has so far never shown any significant effect on the QT-duration.

Apart from the AESIs described above no SAEs were observed. None of the patients were discontinued due to side effects or clinical deterioration of malaria symptoms. That is a better safety outcome than in previous studies that assessed FOS-CM in children ≤5 years with a comparable sample size. In the study by Borrmann et al. [72] one patient developed severe anemia. In the study by Lanaspa et al. [74] one patient with severe anemia and one patient with moderate dehydration had to be discontinued.

## 4.3 Conclusions

This proof of concept study was the first clinical trial to assess the efficacy and safety of the potential NACT FOS-PQ in children ≤5 years. It showed very good efficacy and safety comparable to the first-line ACTs. In the face of surging artemisinin resistance FOS-PQ could present a viable alternative to failing ACTs.

The new combination treatment has certain limitations though. Increasing reports of upcoming resistance against piperaquine in south-east Asia are alarming. Fosmidomycin is indeed a new compound in antimalarial therapy with a unique mechanism of action but it also has a very short half-life and limited long-term efficacy in monotherapy. This might leave piperaquine vulnerable to further development of parasite resistance and hinder a once-daily dosing regimen. Also, FOS-PQ does not seem to have relevant gametocytocidal activity which might facilitate transmission of resistant alleles to the mosquito vector and thereby additionally accelerate the spread of parasite resistance.
Besides, the treatment with piperaquine can be associated with a relatively high rate of QT-prolongations. Even though no arrhythmias or sudden deaths have been observed so far, this side effect needs to be treated with caution.

Future work on a FOS-PQ combination therapy will have to confirm these preliminary results and address the open questions regarding potential hemolytic and immunosuppressive effects in a blinded and comparative setting with a larger sample size. Dose-optimization studies should ideally aim for a once-daily dosage in a 3-day regimen to facilitate compliance. Also, FOS-PQ should be co-formulated in a fixed-dose tablet or syrup that can easily be weight-adapted. For the limitations described above next to piperaquine other drug partners for fosmidomycin should be examined in the future development. Fosmidomycin might also be an interesting candidate for a triple combination therapy as it has shown excellent safety and tolerability with many different antimalarial agents so far.

# 5 Summary

**Background:** Malaria is the most important parasitic infection known to mankind with an estimated yearly incidence of around 228 million cases and 405,000 deaths. Due to

surging drug resistance the highly effective artemisinin-combination therapies (ACTs) are starting to fail as first-line treatment in Southeast Asia. To assure malaria control and to prevent a potential health crisis in case of worldwide spread of artemisinin resistance, non-artemisinin-based combination therapies (NACTs) that are as effective and safe need to be developed. The antibiotic fosmidomycin is one of the promising candidates in development as it demonstrated good efficacy and safety for the treatment of malaria in pre-clinical as well as clinical studies. As fosmidomycin failed to convince in combination with clindamycin, especially in very young children, the presented dissertation deals with the first clinical trial to combine fosmidomycin with piperaquine for the treatment of malaria in young children. The hypothesis was defined as: The combination FOS-PQ is effective at ≥95% for the treatment of uncomplicated P. falciparum malaria in children aged ≤5 years.

**Methods:** The study was designed as a Phase 2a, single arm, uncontrolled proof-of-concept study to assess the efficacy, tolerability and safety of a fosmidomycin-piperaquine combination therapy (FOS-PQ) in children aged 1 to 5 years with acute uncomplicated *plasmodium falciparum* monoinfection. The children had initial parasite counts between 1,000 and 150,000 p.f./µl and were treated orally for 3 days with 30mg/kg fosmidomycin sodium 12-hourly and 16mg/kg piperaquine tetraphosphate 24-hourly. The primary efficacy endpoint was defined as the per protocol polymerase chain reaction (PCR) -corrected adequate clinical and parasitological response (ACPR) on day 28. Patients were followed up for 63 days. Safety outcomes were assessed by regular clinical examinations, clinical laboratory assessments as well as parasitological and electrocardiographic monitoring.

**Results:** Out of 247 patients screened 50 children were enrolled, of which 16 (32%) were female. The median age was 4.2 years (IQR: 3.3-5.2) comprising 11 children aged <3 years. The per protocol PCR-corrected ACPR on D28 was 100% (95%CI: 91.4-100). The per protocol PCR-uncorrected ACPR was 95.1% (95%CI: 84.9-98.7) and 63.2% (95%CI: 47.3-76.6) on D28 and D63 respectively. In total 14 cases of recurrent parasitemia were observed between D28 and D63. All but one recurrence of parasites on day 42 could be identified as reinfections by molecular genotyping. The median parasite and fever

clearance time was 48 hours (range: 24-72) and 12 hours (range: 6-36) respectively. The cumulative gametocyte carrier rate (gametocytes detected during at least one follow-up visit) was 32%. The gametocyte carrier rate on day 7 was 4%.
Most adverse events observed were transient and mild to moderate in severity. Apart from a significant drop of hemoglobin in 2 patients no serious adverse events were observed. The most important safety finding was a significant QT-prolongation in 7 (14%) patients with 2 cases of a QT duration >500ms when corrected by the Bazett formula. All cases of QT-prolongation had resolved by day 7.

**Discussion:** The new treatment combination of fosmidomycin and piperaquine proved to be highly efficacious with rapid parasite clearance, safe and well tolerated in young and very young children. It showed little gametocytocidal activity though and a relatively high rate of QT-prolongation. The fosmidomycin-piperaquine combination as well as fosmidomycin in combination with yet another drug partner or other drug partners might present a viable alternative to failing ACTs.

# 6 Zusammenfassung auf Deutsch

**Hintergrund:** Malaria ist mit geschätzten 228 Millionen Erkrankungen und 405,000 Todesfällen pro Jahr die medizinisch bedeutsamste parasitäre Erkrankung des Menschen. Die Wirksamkeit der Artemisinin-basierten Erstlinientherapeutika ist insb. in Südostasien aufgrund zunehmender parasitärer Resistenzentwicklung zunehmend gefährdet. Im Falle einer weltweiten Ausbreitung dieser multiresistenten Erreger droht eine humanitäre Gesundheitskrise. Die Entwicklung neuer nicht-Artemisinin-basierter, effektiver Chemotherapeutika ist daher eines der Hauptziele im weltweiten Kampf gegen Malaria. Einer der vielversprechendsten Kandidaten ist das Antibiotikum Fosmidomycin. In den bisherigen prä-klinischen und klinischen Studien zur Malariatherapie zeigte es eine gute Wirksamkeit und Verträglichkeit. Da Fosmidomycin in Kombination mit Clindamycin vor allem bei sehr jungen Kindern nicht überzeugen konnte, befasst sich die vorliegende Dissertation mit der ersten klinischen Studie zur Kombination von Fosmidomycin mit Piperaquine zur Behandlung von Malaria bei jungen Kindern. Die Hypothese wurde wie folgt definiert: Die Kombination aus Fosmidomycin und Piperaquine als Therapie der unkomplizierten Plasmodium falciparum Malaria hat eine Heilungsrate ≥95% bei Kindern im Alter von ≤5 Jahren.

**Methoden:** Die Studie war als einarmige, unkontrollierte, unverblindete, Phase 2a, Proof of Concept Untersuchung konzipiert, um erste Aussagen über die Wirksamkeit und Verträglichkeit einer Fosmidomycin-Piperaquine Kombinationstherapie in der Therapie von Kindern zwischen 1 bis 5 Jahren mit akuter, unkomplizierter Plasmodium falciparum Monoinfektion treffen zu können. Die Kinder mit Parasitenzahlen von 1.000 bis 150.000 p.f./µl erhielten eine orale Medikation bestehend aus 30mg/kg Fosmidomycin-Natriumsalz 12-stündig und 16mg/kg Piperaquine-Tetraphosphat 24-stündig für insgesamt 3 Tage und wurden insgesamt 63 Tage nachverfolgt. Der primäre Wirksamkeitsendpunkt war definiert als die mittels Polymerase-Ketten-Reaktion überprüfte Heilungsrate an Tag 28 nach Therapiebeginn. Die Verträglichkeit wurde anhand regelmäßiger klinischer, parasitologischer und elektrokardiographischer Untersuchungen überwacht.

**Resultate:** Von 247 gescreenten Patienten wurden insgesamt 50 Patienten in die Studie eingeschlossen. 16 Probanden (32%) waren weiblich. Das mediane Alter des Patientenkollektivs war 4,2 Jahre (IQR: 3,3 - 5,2). 11 Patienten waren <3 Jahre alt. Die mittels Polymerase-Ketten-Reaktion überprüfte Heilungsrate an Tag 28 war 100% (95%CI: 91.4-100) in der Per-Protocol-Population. Ohne Überprüfung durch Polymerase-Ketten-Reaktion lag die Heilungsrate in der Per-Protocol-Population bei 95.1% (95%CI: 84.9-98.7) an Tag 28 und 63.2% (95%CI: 47.3-76.6) an Tag 63. Insgesamt wurden zwischen Tag 28 und Tag 63 14 Fälle mit rekurrierender Parasitämie beobachtet. In einem Fall kam es zur Rekrudeszenz an Tag 42. Alle anderen Fälle konnten mittels Genotypisierung als Reinfektionen identifiziert werden. Es dauerte im Median 48 Stunden (range: 24-72) bis keine Parasiten mehr im Blut nachgewiesen konnten und 12 Stunden (range: 6-36) bis die Patienten fieberfrei waren. Die kumulative Gametozyten-Träger-Rate (Anzahl der Patienten mit Gametozytämie während mind. einer Follow-up-Visite) betrug 32%. Die Gametozyten-Träger-Rate an Tag 7 betrug 4%.
Die meisten beobachteten unerwünschten Ereignisse waren vorübergehend und leicht bis mittelschwer. Abgesehen eines signifikanten Hämoglobinabfalls bei 2 Patienten wurden keine schwerwiegenden Nebenwirkungen beobachtet. Die wichtigste Nebenwirkung war eine signifikante QT-Verlängerung am letzten Behandlungstag bei insgesamt 7 (14%)

Patienten. Dabei kam es in 2 Fällen zu einer QT-Zeit >500ms, wenn diese nach Bazett frequenzkorrigiert wurde. An Tag 7 hatten sich alle QT-Veränderungen wieder normalisiert.

**Diskussion:** Die neue Kombination aus Fosmidomycin und Piperaquine erwies sich als hochwirksam, sicher und gut verträglich bei jungen und sehr jungen Kindern. Die Kombination zeigte allerdings geringe gametozide Aktivität und eine relativ hohe Rate an QT-Verlängerungen. Die Kombination aus Fosmidomycin und Piperaquine sowie Fosmidomycin in Kombination mit einem anderen Medikamentenpartner bzw. anderen Medikamentenpartnern könnte eine gute Alternative zu den Artemisinin-basierten Kombinationstherapien darstellen.

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# 8 Declaration of own contribution

The research for this thesis was conducted at the institute of tropical medicine, travel medicine and human parasitology at the university department of medicine of the Eberhard Karls Universität in Tübingen under supervision of P.G. Kremsner.

This dissertation deals with the second part of the results of the FOSPIP study. The study was designed by Ghyslain Mombo-Ngoma (Principal Investigator) in collaboration with David Hutchinson, Stephan Duparc, Joerg Moehrle, Thirumalaisamy P. Velavan, Bertrand Lell, Michael Ramharter, Ayola Akim Adegnika, Benjamin Mordmüller and P.G. Kremsner (Co-Investigators).

The clinical procedures (informed consent, screening, examinations, monitoring of the treatment, follow up) were performed by me and other investigators (Jonathan Remppis, Rella Zoleko Manego, Lilian Endamne, Johanna Kim, The Trong Nguyen, Johannes Mischlinger, Luzia Veletzky and Lena Flohr) as well as the nurses and fieldworkers of the OZ team under supervision of Ghyslain Mombo-Ngoma. The clinical laboratory procedures (slide preparation, microscopy, clinical laboratory safety) were performed by me, other investigators and the clinical laboratory personnel of the CERMEL. The genotyping analysis was partly conducted by Noemie Garcia-Tardon assisted by me and partly by Albert Lalremruata.

Data management and analysis including statistical analysis for this thesis was performed by me under supervision of Ghyslain Mombo-Ngoma. I assure that the manuscript of this thesis was written only by myself under supervision of Ghyslain Mombo-Ngoma. No other than the noted references were used.

The manuscript of the publication presenting the results of the entire FOSPIP study was written by Ghyslain Mombo-Ngoma under supervision of P.G. Kremsner.

# 9 Publications

Parts of the data presented in this thesis were already published in the following publication:

Mombo-Ngoma G, Remppis J, Sievers M, Zoleko Manego R, Endamne L, Kabwende L, Veletzky L, Nguyen TT, Groger M, Lötsch F, Mischlinger J, Flohr L, Kim J, Cattaneo C, Hutchinson D, Duparc S, Moehrle J, Velavan TP, Lell B, Ramharter M, Adegnika AA, Mordmüller B, Kremsner PG (2018) Efficacy and Safety of Fosmidomycin-Piperaquine as Nonartemisinin-Based Combination Therapy for Uncomplicated Falciparum Malaria: A Single-Arm, Age De-escalation Proof-of-Concept Study in Gabon Clin Infect Dis. 66:1823-1830.

# 10 Attachments

COMITE NATIONAL D'ETHIQUE

PRESIDENT

SECRETARIAT GENERAL

N°012/2015/SG/P

## ATTESTATION D'AVIS ETHIQUE

Le Comité National d'Ethique du Gabon, agréé et constitué par le décret n° 00732/PR/MRSDT du 15 septembre 2008 et dont le Bureau a été mis en place par arrêté n°00028 /MESRSDT/2009 du 30 septembre 2009 ;
En application de la loi n° 22/2000 du 10 janvier 2001 déterminant les principes fondamentaux de la Recherche Scientifique en République Gabonaise, du code de la santé publique en République Gabonaise et de la réglementation en vigueur relative à la recherche scientifique et biomédicale au Gabon ;

En application des principes et dispositions éthiques internationaux, notamment le Code de Nuremberg, la Déclaration d'Helsinki, la Déclaration de Manille et les lignes directrices internationales du CIOMS ;

Ayant été ressaisi, pour ce même protocole dans sa version amandé le 05 février 2015 suite à notre lettre N°_001/2015/CNE/SG/P du 08 Décembre 2014, par le **Docteur Ghyslain MOMBO NGOMA**, Investigateur Principal de l'étude, objet de la présente au Centre de Recherche Médicale de Lambaréné (CRMEL), agissant au nom et pour le compte de ladite structure: pour examen par le comité national d'éthique du protocole d'étude intitulé :

**« Une étude de phase IIa de preuve de concept à explorer l'efficacité, la tolérabilité et l'innocuité de fosmidomicine sodium lorsque administré avec pipéraquine tétraphosphate chez les enfants de 1 à 5 ans atteints de *plasmodium falciparum* sans complication »** ;

Référencée chez le promoteur sous l'identifiant interne protocole de recherche biomédicale, Amendement N°2 **numéro de protocole : JP017**, Version 7 du 16 février 2015, et dont l'Investigateurs Principal de cette étude au Gabon est le **Docteur Ghyslain MOMBO NGOMA** ;

Ayant reçu le 05 février 2015 le protocole d'étude constitué des éléments exigés au complet,

Ayant examiné le dossier de recherche ainsi constitué de :

- Un résumé de protocole d'étude en français intitule : « Une étude de phase IIa de preuve de concept à explorer l'efficacité, la tolérabilité et l'innocuité de fosmidomicine sodium lorsque administré avec pipéraquine tétraphosphate chez les enfants de 1 à 5 ans atteints de *plasmodium falciparum* sans complication »
- Un protocole de l'étude en Anglais ;
- Une note d'information aux participants à l'étude suscitée;
- Un formulaire de consentement éclairé pour les participants ;
- Curricula vitaux de l'Investigateur Principal et du consultant que sont respectivement : le **Docteur Ghyslain MOMBO NGOMA** et le **Professeur Peter. G. KREMSNER** ;

Ayant, après vérification de la conformité normative, enregistré ce dossier sous la référence interne **PROT N° 012/2015/SG/CNE ;**

Ayant examiné pour la deuxième fois et définitivement ce dossier de recherche lors de sa séance plénière du 28 Février 2015 en session extraordinaire au cours de laquelle les membres du CNE délibérants (cf. liste en annexe) ;

Après avoir entendu les explications, les réponses aux nombreuses questions sur le fond et sur la forme ; et éclaircissements supplémentaires fournies pars l'Investigateur de ladite étude ;

Ayant pris acte des avis des experts commis spécialement à cet effet, les membres du comité d'éthique délibérants de la présente session, ont reconnu :

- la pertinence du sujet, au vue de l'importance de la pandémie et de la nécessité de rechercher d'autres molécules curatives contre le paludisme avec la garantie d'une bonne efficacité sur le parasite, d'une part et d'avoir une bonne tolérance et une meilleur observance d'autre part;
- l'autonomie des participants dans cette recherche est respectée, au vue d'un recrutement basé sur la signature par les parents des malades d'un formulaire de consentement libre et éclairé ;

Comité National d'Ethique pour la Recherche B.P. 2217 Libreville * Le Président *

- les membres du CNE ont admis que les bénéfices individuels des patients/participants sont garanti car ce traitement vise la guérison des patients ;
- Ils admettent que dans ce projet, il n'y a pas des éléments de malfaisance ;
- En prenant en compte ce qui précède, le CNE considèrent que les principes de justice et d'équité sont respectés pour chaque patient ;
- L'équipe de recherche mise en place par le CERMEL et Jomaa Pharma GmbH, dans ce projet est jugé compétente, et disposeront d'un plateau technique et des moyens humains, financiers et matériaux suffisants pour mener à bien cette étude ;
- En conséquence, les membres du Comité National d'Ethique du Gabon ont délibéré à l'unanimité et validé sans réserve **« l'étude de phase IIa de preuve de concept à explorer l'efficacité, la tolérabilité et l'innocuité de fosmidomicine sodium lorsque administré avec pipéraquine tétraphosphate chez les enfants de 1 à 5 ans atteints de *plasmodium falciparum* sans complication »**, et ont émis un

**AVIS FAVORABLE**

Pour la mise en œuvre à Lambaréné, au Gabon, de l'étude n° 012/2015/SG/CNE, Intitulé : **« Une étude de phase IIa de preuve de concept à explorer l'efficacité, la tolérabilité et l'innocuité de fosmidomicine sodium lorsque administré avec pipéraquine tétraphosphate chez les enfants de 1 à 5 ans atteints de *plasmodium falciparum* sans complication »**

Fait à Libreville, le 07 Mars 2015

**Le président du CNE du Gabon**

**Pierre Blaise MATSIEGUI, MD, MSc, PhD**

# 11 Acknowledgements

First, I thank all the children and their representatives to have participated. They were both reason and foundation for this thesis.

Furthermore, I thank:

Peter Kremsner, for giving me the opportunity of researching in such a relevant and interesting field and for his supervision of my thesis.

The whole OZ team and all my colleagues at the CERMEL

Ghyslain Mombo-Ngoma and Jonathan Remppis, both were scientifically as well as personally very inspiring and helpful to me.

Jelka

And I thank my parents from the depths of my heart for all the support and opportunities they have offered me.