STUDY PROTOCOL

MultiMal

Multi-Drug Combination-Therapies to Prevent the Development of Drug Resistance

Phase II Controlled Clinical Trial Assessing Candidate Regimens of Multiple-Antimalarial Combinations for the Treatment of Uncomplicated Malaria in Africa

Study protocol number	
Investigational product	
Version	1
Date	29 November 2019
Sponsor	

This study will be conducted in compliance with the protocol, Good Clinical Practice, and all other applicable regulatory requirements, including the archiving of essential documents.

1 Confidentiality statement

This document contains information that is privileged or confidential. As such, it may not be disclosed unless specific prior permission is granted in writing by Prof. Michael Ramharter. Persons to whom any of this information is to be disclosed must first be informed that the information is confidential. These restrictions on disclosure will apply equally to all future information supplied, which is indicated as privileged or confidential

2 Study protocol agreement form

I, undersigned, ……, hereby certify that I have examined the protocol of the above referenced study for PI to provide study site institutional name and that I have thoroughly discussed the objectives of this study as well as the contents of this protocol with the Sponsor's representatives.

I agree to keep the contents of this protocol confidential and not to disclose it to a third party and to use it only for the purposes of this study.

I agree to perform this study according to this protocol and to meet the objectives, to comply with the ethical rules and to ensure patient safety.

I have well understood that in case the Sponsor may decide to prematurely end or to suspend this study at any time and for any reason, I would be informed of this decision in writing. Conversely, in case I would decide to prematurely end or to suspend this study, I commit to immediately inform the Sponsor of this decision in writing.

INVESTIGATOR FOR (Country/Study site):

_____ _____

Name Date

Signature: _____

3 Statement of compliance

3.1 Declaration of the Sponsor

This clinical study protocol was subject to critical review and has been approved by the sponsor. The information it contains is was written in accordance with the World Medical Association Declaration of Helsinki, Regulation (EU) No 536/2014 of the European Parliament and of the council of 16 April 2014, and the regulative authorities of Gabon and Ghana.

_____ _____

Name Date

3.2 Declaration of Investigator

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined herein and will make a reasonable effort to complete the study within the time designated.

The study will be conducted in accordance with the following:
World Medical Association Declaration of Helsinki,
Regulation (EU) No 536/2014 of the European Parliament and of the council of 16 April 2014.

I agree to personally conduct or supervise the described Study.

I agree to inform all participants that the study drug is being used for investigational purposes and I will ensure that the requirements related to obtaining informed consent are in accordance with ICH Guidelines for Good Clinical Practices (GCP) section 4.8 and local requirements.
I agree to report adverse events that occur in the course of the Study to the sponsor in accordance with ICH Guidelines for GCP section 4.11 and local requirements.
I have read and understand the information in the Investigator's Brochure, including the potential risks and side effects of the study drug.
I agree to promptly report to the Ethics Committee (EC) all changes in the research activity and all unanticipated problems involving risk to participants. I will not make any changes to the conduct of the study without EC and sponsor approval, except when necessary to eliminate apparent immediate harm to participants.
I agree to maintain adequate and accurate records and make those records available in accordance with ICH Guidelines for GCP section 4.11 and local requirements.
I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
I understand that the Study may be terminated, or enrolment suspended at any time by the sponsor, with or without cause, or by me if it becomes necessary to protect the best interest of the participants.

_____ _____

Name Date

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I, undersigned, Ayoca A. Adegnika, hereby certify that I have examined the protocol of the above referenced study for PI to provide study site institutional name and that I have thoroughly discussed the objectives of this study as well as the contents of this protocol with the Sponsor's representatives.

I agree to keep the contents of this protocol confidential and not to disclose it to a third party and to use it only for the purposes of this study.

I agree to perform this study according to this protocol and to meet the objectives, to comply with the ethical rules and to ensure patient safety.

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INVESTIGATOR FOR (Country/Study site): GABON

Ayoca Akim ADEGNIKA — Name

06/12/2019 — Date

Signature: _____

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This clinical study protocol was subject to critical review and has been approved by the sponsor. The information it contains is was written in accordance with the World Medical Association Declaration of Helsinki, Regulation (EU) No 536/2014 of the European Parliament and of the council of 16 April 2014, and the regulative authorities of Gabon and Ghana.

[signature] 05.12.19

Name Date

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Ayoga A. Adégnika 06/12/2019.

Name Date

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INVESTIGATOR FOR (Country/Study site):

Ovidiu Maicca
Name

10.12.2019
Date

Signature: Maicca

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Name

05. 12. 19
Date

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Name

10.12.2019
Date

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Date

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I understand that the Study may be terminated, or enrolment suspended at any time by the sponsor, with or without cause, or by me if it becomes necessary to protect the best interest of the participants.

Name
Prof. Dr. Sebastian G. Wicha

6.12.2019
Date

2 Study protocol agreement form

I, undersigned, Prof. Dr. Sebastian G. Wicha, hereby certify that I have examined the protocol of the above referenced study for PI to provide study site institutional name and that I have thoroughly discussed the objectives of this study as well as the contents of this protocol with the Sponsor's representatives.

I agree to keep the contents of this protocol confidential and not to disclose it to a third party and to use it only for the purposes of this study.

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INVESTIGATOR FOR (Country/Study site): Hamburg, Germany

Prof. Dr. Sebastian G. Wicha
Name

6.12.2019
Date

Signature:

4 Clinical trial institutions

Clinical trial study centres	Institution: Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) Address: South-end Asuogya Road, KNUST, Kumasi, Ghana Institution: Centre de Recherches Médicales de Lambaréné (CERMEL) Address: B.P. 242 Lambaréné, Gabon
Principal investigator	Name: Prof. Dr. Michael Ramharter Institution: Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine (BNITM) Address: Bernhard-Nocht-Str. 74; D-20359 Hamburg, Germany Phone: +49 40 42818 511 email: ramharter@bnitm.de
Coordinating Investigator	Name: Dr. Johannes Mischlinger Institution: Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine (BNITM) Address: Bernhard-Nocht-Str. 74; D-20359 Hamburg, Germany Phone: +49 40 42818 480 email: mischlinger@bnitm.de
Co-investigator(s)	Local Principal Investigator Name: Dr. Oumou Maiga Institution: Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) Address: South-end Asuogya Road, KNUST, Kumasi, Ghana email: maiga@bnitm.de
	Local Principal Investigator Name: Prof. Ayôla A. Adegnika Institution: Centre de Recherches Médicales de Lambaréné (CERMEL) Address: B.P. 242 Lambaréné, Gabon Phone: +24107406464 email: aadegnika@cermel.org
	Name: Prof. Sebastian Wicha Institution: University of Hamburg, Institute of Pharmacy, Dept. of Clinical Pharmacy Address: Bundesstraße 45, 20146 Hamburg, Germany Phone: +49 40 42838 3487 email: Sebastian.wicha@uni.hamburg.de
Study/Trial Sponsor	Institution: Department of Tropical Medicine, BNITM Contact person: Prof. Dr. Michael Ramharter Phone: +49 40 42818 511 email: ramharter@bnitm.de
Study management	Name: Mareike Kallweit Institution: Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine (BNITM) Address: Bernhard-Nocht-Str. 74; D-20359 Hamburg, Germany mareike.kallweit@bnitm.de

	Phone: +49 40 42818 624 email: Mareike.kallweit@bnitm.de
Central Laboratory	Institution: Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine (BNITM) Contact person: Mareike Kallweit Shipping Address: Bernhard-Nocht-Str. 74; D-20359 Hamburg, Germany Phone: +49 40 42818 624 email: Mareike.kallweit@bnitm.de Institution: University of Hamburg Contact person: Prof. Sebastian Wicha Shipping Address: Bundesstraße 45, 20146 Hamburg, Germany Phone: +49 40 42838 3487 email: Sebastian.wicha@uni.hamburg.de

5 Table of Contents

6 List of abbreviations

ACT	Artemisinin-based Combination Therapy
AE	Adverse Event
AESI	Adverse Event of Special Interest
AL	Artemether-lumefantrine
ALT	Alanine aminotransferase
An	Anopheles
AP	Atovaquone-proguanil
ASAQ	Artesunate-amodiaquine
AST	Aspartate aminotransferase
BUN	Blood Urea Nitrogen
CEM	Cohort Event Monitoring
CHW	Community Health Worker
CMV	Cytomegalovirus
CPK	Creatinine phosphokinase
CRF	Case Report Form
DILI	Drug Induced Liver Injury
DOT	Direct Observational Treatment
DSMB	Data Safety Monitoring Board
DTM	Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine
eCRF	Electronic Case Report Form
EBV	Epstein Barr Virus
EC	Ethics committee
HAV	Hepatitis A Virus
Hb	Haemoglobin
HBc	Hepatitis B core antigen
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
IgM	Immunoglobulin M
IMP	Investigational medical product
IPTp	Intermittent Preventive Treatment in pregnancy
ITNs/LLINs	Insecticide Treated Nets / Long Lasting Insecticidal Nets
IRS	Indoor Residual Spraying
LDH	Lactate Dehydrogenase
LFTs	Liver function tests
MedDRA	Medical dictionary for Regulatory Activities
NMCP	National Malaria Control Programme
PCR	Polymerase chain reaction
P. falciparum	Plasmodium falciparum
PI	Principal Investigator
QTcB	QTc corrected by Bazett's
QTcF	QTc corrected by Fridericia
RDT	Rapid Diagnostic Test
RNA	Ribonucleic acid

SAE	Serious Adverse Event
SDV	Source Data Verification
SmPC	Summary of Product Characteristics
SP	Sulfadoxine-Pyrimethamine
STM	Study Team Member
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
WHO	World Health Organisation

7 List of figures

8 List of tables

9 Document History

Review Date	Version Number	Brief description of changes	Signature
		First release	

10 Project summary

Table 1 Summary of study

Title	Multi-Drug Combination-Therapies to Prevent the Development of Drug Resistance: Phase II Controlled Clinical Trial Assessing Candidate Regimens of Multiple-Antimalarial Combinations for the Treatment of Uncomplicated Malaria in Africa
Clinical Phase	Proof of concept clinical phase 2 trial
Investigational Product/Study Agent	The combination of antimalarial treatments: artesunate-pyronaridine-atovaquone/proguanil (APAP) and artesunate-fosmidomycin-clindamycin (AFC)
Objectives	**Primary:** To describe the pharmacokinetic properties of each partner drug and their principal active metabolites in the two antimalarial combination treatments artesunate-pyronaridine-atovaquone/proguanil (APAP) and artesunate-fosmidomycin-clindamycin (AFC), respectively in patients with uncomplicated malaria.
	Secondary: • To determine the PCR corrected adequate clinical and parasitological response on Day 42 in per protocol population • To determine the PCR corrected cure rate on day 28 in per protocol population • To determine the PCR uncorrected cure rates on days 28 and 42 in intention to treat population • To determine the safety and tolerability of combination therapies in intention to treat population • To determine the parasite clearance dynamics of combination therapies • To determine the proportion of patients with sexual stage parasitaemia during follow up
	Exploratory: None
Location of the study	Field work will be performed at St. Francis Xavier Hospital, Assin Fosu, Ghana which is affiliated with the Kumasi Center for Clinical Research (KCCR) and in Lambaréné, Gabon at the Centre de Recherches Médicales de Lambaréné (CERMEL). These sites have the unique advantage of established infrastructure for clinical trials and proven access to appropriate study populations which had been well characterized during previous studies. Both centres are well equipped to provide adequate health care and have trained staff and adequate infrastructure to execute clinical trials up to phase III in a sound and efficient manner. Finally, successful and longstanding collaborations exist with the applicants of this study.
Timeline and duration of the study	01.10.2019 - 30.09.2021

Study Design	A **randomised-controlled clinical trial** (RCT) will be conducted comprising three treatment arms: **Group A**: Oral artesunate/pyronaridin standard treatment **(AP)** **Group B:** Oral artesunate-pyronaridine-atovaquone/proguanil **(APAP)** **Group C:** Oral artesunate-fosmidomycin-clindamycin **(AFC)** There are 3 age groups in the step-down procedure: 18-65y --> 11-17y --> 6 months - 10 y For **Group A**: It will be **0 patients** for 18-65y, **10 patients** for 11-17y and **10 patients** for 6 months - 10y For **Group B**: It will be **10 patients** for 18-65y, **10 patients** for 11-17y and **20 patients** for 6 months - 10y For **Group C:** It will be **10 patients** for 18-65y, **10 patients** for 11-17y and **20 patients** for 6 months - 10y **Randomization and group allocation** A 1:2:2 randomization will be performed for each age group using computer generated random permuted blocks. Allocation will be concealed until the randomization is performed by the investigator. No blinding/masking will be performed for clinical investigations in this open label clinical trial. Genotyping of reappearing parasitaemia will be performed in a single-blinded way by concealing treatment groups to the molecular biologist. Treatment schedules for treatment groups are summarized below: **Group A (AP):** Artesunate-pyronaridine (Pyramax): Once daily oral dosing for three days independent of food: Paediatric dosing regimen: 5 <-8 kg: 1 sachet daily 8 -<15 kg: 2 sachets daily 15-20 kg: 3 sachets daily 1 sachet contains 20 mg artesunate and 60 mg pyronaridine Adult dosing regimen: 20-<24 kg: 1 tablet daily 24-45 kg: 2 tablets daily 45-<65 kg: 3 tablets daily >65 kg: 4 tablets daily 1 tablet contains 60 mg artesunate and 180 mg pyronaridine **Group B (APAP):**

	Atovaquone-proguanil (Malarone or generic): Once daily oral dosing for three days with food/milk: 5-8 kg: Atovaquone/proguanil 125 mg/50 mg 9-10 kg: Atovaquone/proguanil 187.5 mg/75 mg 11-20 kg: Atovaquone/proguanil 250 mg/100 mg 21-30 kg: Atovaquone/proguanil 500 mg/200 mg 31-40 kg: Atovaquone/proguanil 750 mg/300 mg >40 kg: Atovaquone/proguanil 1000 mg/400 mg **Group C (AFC):** Artesunate: 2 mg/kg twice daily oral dosing for 3 days independent of food as calculated closest to the capsule strength Fosmidomycin: 30 mg/kg twice daily oral dosing for 3 days independent of food as calculated closest to the capsule strength Clindamycin hydrochloride: 10 mg/kg twice daily oral dosing for 3 days independent of food as calculated closest to the capsule strength (150 mg, 300 mg, 600 mg)
Study Drug or Study Agent Administration	AP & APAP: Oral once daily over three days AFC: Twice daily over three days
Number of Patients and Randomizations/ per Site	Total of 100 patients, 50 per site
Study Population	The clinical trial will be conducted using an age-step-down procedure involving both adult and paediatric participants.
Selection criteria	**Inclusion Criteria** 1. Male or female patient age >6 months <66 years. 2. Body weight >5 kg <90 kg 3. Presence of mono-infection of P. falciparum with: a. Fever, as defined by axillary temperature ≥ 37.5°C or oral/rectal/tympanic temperature ≥ 38°C, or history of fever in the previous 24 hours b. Microscopically confirmed parasite infection, in range 1,000 to 100,000 asexual parasites /µL of blood. 4. Written informed consent provided by the adult patient, or parent or legally acceptable representative (LAR) of the minor patient or by an impartial witness (if the patient or patient's LAR is illiterate), stating that the information has been read and/or is understood, and by the medically qualified Investigator. Children will be asked to provide assent where appropriate. The age from which this will be sought will be defined by local legislation.
	Exclusion Criteria 1. Presence of severe malaria (according to WHO definition – WHO 2013) 2. Anti-malarial treatment in the last 6 weeks.

	3. Known history or evidence of clinically significant disorders such as, cardiovascular, respiratory (including active tuberculosis), hepatic, renal, gastrointestinal, immunological (including active HIV-AIDS), neurological (including auditory), endocrine, infectious, malignancy, psychiatric, history of convulsions or other abnormality (including head trauma). 4. Mixed Plasmodium infection 5. Severe vomiting, defined as more than three times in the 24 hours prior to enrolment in the study or inability to tolerate oral treatment, or severe diarrhoea defined as 3 or more watery stools per day 6. Severe malnutrition (defined for subjects aged ten years or less as the weight-for- height being below -3 standard deviation or less than 70% of median of the NCHS/WHO normalised reference values, and for subjects aged greater than ten years, a body mass index (BMI) of less than 16 (WFP Manual, Chapter 1)). 7. Known history of hypersensitivity, allergic or adverse reactions to any of the study drugs 8. Known active Hepatitis A IgM (HAV-IgM), Hepatitis B surface antigen (HBsAg) or Hepatitis C antibody (HCV Ab). 9. Haemoglobin level below 8 g/dL. 10. Serum creatinine levels ≥2 x ULN 11. Female patients of child bearing potential must be neither pregnant (as demonstrated by a negative pregnancy test) nor lactating, and must be willing to take measures not to become pregnant during the study period and safety follow-up period. 12. Have received an investigational drug within the past 4 weeks. 13. Previous participation in any malaria vaccine study or received malaria vaccine in any other circumstance. 14. Refusal to participate and to provide written or witnessed informed consent or assent.
Study procedures	Written informed consent will be obtained prior to inclusion. Baseline patient information will be collected on standardized case record forms. To ensure anonymity, identifiers will be allocated to each participant and their case report forms. Treatment and follow-up Treatment will be administered under direct supervision over a three-day period at the study centre. Patients will be followed up on days 0, 1, 2, 3, 7, 14, 21, 28, 35 and 42. Blood sampling and analysis Blood samples will be taken and stored at -80° C until shipped on dried ice for further analysis by HLPC and LC-MS analysis. The bioanalysis will be performed at the Dept. of Clinical Pharmacy, Institute of Pharmacy, University of Hamburg, Germany.

	Pharmacokinetic analyses Pharmacokinetics will be performed using peripheral blood samples. Pharmacokinetic sampling will be performed following a predefined sampling schedule (0, 0.25, 0.5, 0.75, 1.5, 3, 5, 8, 48, 168, 336, 672 and 1008 h) [see Appendix 4]. Sampling times for the paediatric part of the study will be re-evaluated and the number of sampling timepoints will potentially be reduced during the age-step-down procedure. Pharmacokinetic modelling will be performed for all individual drugs using non-linear mixed effects modelling in NONMEM®. Patient characteristics (e.g. body composition, organ function, maturation) will be assessed as covariates of the pharmacokinetics of each drug. Potential drug-drug interactions of atovaquone-proguanil on artesunate-pyronaridine pharmacokinetics will be evaluated. The time-courses of the pharmacodynamic markers (PCR corrected antimalarial efficacy) will be modelled by multi-state pharmacometric modelling. The pharmacokinetics will be linked to pharmacodynamic models to derive the exposure-response relationships of each regimen. Patient covariates correlating with treatment success and/or failure will be evaluated. Time-to-event modelling will be employed on the recrudescence data from the follow-up period together with the linked pharmacokinetic-pharmacodynamic model to potentially identify predictive factors for treatment failure. Parasitological assessment Blood samples for parasitological assessment will be obtained using capillary or venous blood by applying finger pricks and venipunctures, respectively. Standardized light microscopy samples and filter paper samples will be prepared twice daily until parasite clearance and subsequently once per visit. Parasitological assessment will be conducted at day 0 (dosing), at days 1, 2, 3, 7, 14, 21, 28, 35, 42 post-dose and in case of an unforeseen visit (See Appendix 4). Antimalarial efficacy will be assessed using standard microscopy (Method of the WHO) and molecular genotyping for distinction between recrudescence and reinfection. Blood for biochemical and haematological safety and tolerability will be sampled at days 0, 2, 3, 7, 14 and 28. Biochemistry and haematology analyses will be performed using automated systems according to local standard operating procedures. 12-lead ECG Patients should rest supine for 10 minutes prior to taking an ECG. All ECG measurements will be taken prior to any blood sampling. A single 12-lead ECG will be obtained each time before dosing and on day 7 post-dose.
	Primary:

Endpoints/ Evaluation criteria	E1: Descriptive analysis of pharmacokinetics of each partner drug and their principal active metabolites
	Secondary: E2.1: PCR corrected adequate clinical and parasitological response on Day 42 in per protocol population E2.2: PCR corrected cure rate on day 28 in per protocol population E2.3: PCR uncorrected cure rates on days 28 and 42 in intention to treat population E2.4: Safety and tolerability of combination therapies in intention to treat population E2.5: Parasite clearance dynamics of combination therapies E2.4: Proportion of patients with sexual stage parasitaemia during follow up
	Exploratory: None
Statistical analysis/Analysis Plan	Each group will consist of 20 evaluable patients (with 10 patients in the semi-immune sub-group allocated to the age groups 18-65 years and 11-17 years, respectively) and a consecutive group of 20 patients aged 6 months to 10 years, to allow for adequate pharmacokinetic characterization of study drugs. The total number of evaluable participants will be 100. The sample size was corroborated by a clinical trial simulation using NONMEM® (version 7.4, ICON development solutions, Hanover, USA). Simulation of 'virtual' clinical trials using the present trial design and literature information on the PK of the drugs indicated that the design supports estimation of structural pharmacokinetic parameters with high precision and accuracy (mean absolute bias: 5.9%, mean rRMSE: 12.1%). The design is suitable to detect differences in drug clearance of 20% between the groups B or C vs. A with adequate statistical power (> 81.8%).
Safety reporting	A structured safety reporting system will be established to protect the safety of the study participants. It is the investigator's obligation to document any untoward medical occurrence in the respective section of the source document. In case of fulfilment of a seriousness criterion, AESI definition or any other relevant event following study drug administration the investigator should report the event within 24 hours of knowledge of the event to a designated safety contact. A Medical Monitor will be available for the trial sites to resolve arising safety related questions and uncertainties.
Ethical issues	The clinical study will be conducted in accordance with the principles laid down by the International Conference of Harmonization guidelines for good clinical practice (ICH-GCP) respectively the country specific applicable laws and regulations.

Schematic study design

Day		0									1		2		3	7	14	21	28	35	42	Unscheduled
	Screen	0	15 m	30 m	45 m	90 m	3 h	5 h	8 h	12 h	24 h	36 h	48 h	60 h	72 h							
Informed consent	x																					
Inclusion/exclusion criteria	x																					
Physical exam, Malaria signs & symptoms	x									x	x	x	x	x	x	x	x	x	x	x	x	x
Demography, medical history	x																					
Prior and concomitant medication	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Pregnancy test	x																					x
Clinical laboratory safety	x												x		x	x	x		x			
Asexual & gametocyte parasite count (thick and thin blood films) ≥35 kg	x								x	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood spot for parasite genotyping (≥35 kg)	x								x	x	x	x	x	x	x	x	x	x	x	x	x	x
12-Lead ECG (single)		x								x	x	x	x	x	x	x						x
Vital signs (single)	x	x								x	x	x	x	x	x	x	x	x	x		x	x
Temperature (single)	x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PK sampling (≥35 kg)		x	x	x	x	x	x	x	x		x		x			x	x	x	x	x	x	x
Dosing (see table 2)		x								(x)	x	(x)	x	(x)								
AEs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Patient weight	PK samples necessary for AP, APAP and AFC	Additional PK samples necessary for AP and APAP[L]
>5 to < 10 kg	0h, 1h, 8h, 24h, 48h, Day 7	Day 14, Day 42
>10 to <20 kg	0h, 1h, 8h, 24h, 48h, Day 7	Day 14, Day 28, Day 42
>20 to < 35 kg	0h, 1h, 5h, 8h, 24h, 48h, Day 7	Day 14, Day 28, Day 35, Day 42

Dosing	Food/Milk	Baseline	12h	24h	36h	48h	60h
AP	independent	x		x		x	
APAP	with	x		x		x	
AFC	independent	x	x	x	x	x	x

AP: Artesunate/pyronaridin
APAP: Artesunate/pyronaridin/atovaquone-proguanil
AFC: Artesunate/fosmidomycin/clindamycin

Figure 1 Schematic study design (for details see Annex 4: Visit and sampling schedule)

11 Introduction

11.1 Background information

Antimalarial drug resistance is one of the most important challenges in the control and elimination of malaria. Artemisinin combination therapy (ACT) as bi-therapy is the standard of care in all malaria endemic countries (World Health Organization, 2018). However, the efficacy of ACTs as bi-therapy declined in the past decade in the Greater Mekong Region of South East Asia (SEA). Subsequently, epidemiological genomic studies confirmed that in fact artemisinin resistance developed much earlier in numerous foci, resulting in resistant parasites. Importantly, drug resistance against the partner drugs evolved simultaneously leading to decreasing cure rates of first line antimalarial treatments in SEA (Amato et al., 2017; 2018; Miotto et al., 2015).

One pharmacokinetic consideration, which may explain the failure of all currently employed ACTs to avoid the development of drug resistance, is their pharmacokinetic mismatch. Whereas the artemisinin derivative has a remarkably short half-life of < 3 hours, the partner drugs are characterized by intermediate (lumefantrine) to exceptionally long half-lives (mefloquine, piperaquine with half-lives of more than 4 weeks). This pharmacokinetic mismatch allows for a very short period where the two drugs protect each other and a long period of time when the slowly eliminated partner drug is unprotected from the rapidly eliminated artemisinin derivative in prolonged sub-therapeutic drug

levels, and therefore paves the way for the parasite to develop resistance to these drugs if reinfection occurs during the convalescence period. Multi-drug combination therapy is therefore particularly appealing to increase the barriers for resistance if partner drugs with matched half-lives are combined.

11.2 Rationale

Specific drugs were carefully considered during the design of this study. The outcome of this consideration was that the specific multi-therapeutic ACT combinations, discussed below, were decided on based on the following aspects: efficacy, potential for drug interactions, modes-of-action, half-life of the individual drugs, parasitological stages the drug acts on, dosing, availability of a paediatric formulation and cost.
The two drug combinations envisaged to investigate during this study address two particular aspects of treatment of uncomplicated malaria in the sub-Saharan African region. Firstly, **artesunate-pyronaridine-atovaquone/proguanil** uses a quadruple drug treatment with combinations of different modes of action to protect each other from the parasite developing resistance to either during the treatment. Secondly, the combination of **artesunate-fosmidomycin-clindamycin** as a matched-short-half-life combination additionally addresses the issue of bacterial co-infections which frequently occur in sub-Saharan Africa.

11.2.1 Artesunate-pyronaridine-atovaquone/proguanil (APAP)

The combination of artesunate-pyronaridine-atovaquone/proguanil holds promise as it combines two highly efficacious antimalarial treatments which have a proven exceptionally favourable safety and tolerability profile. Based on known drug metabolism there is no indication for clinically relevant drug-drug interaction between the partner drugs. The independent mode of action of artesunate, pyronaridine and atovaquone-proguanil is anticipated to provide a strong barrier for the development of drug resistant mutants.

Although the exact mechanism of action of **artesunate** is not entirely clear, it is known that this drug has a significant role to play in the inhibition of the parasite's calcium adenosine triphosphatase enzyme. As is the case for the other artemisinin drugs, artesunate is especially known for its very fast action, but also short half-life. Artesunate kills all erythrocytic stages of the malaria parasite, including early gametocyte stages which transmit malaria.

Pyronaridine has been proven to elicit high potency against *P. falciparum*, notably also chloroquine-resistant strains (Croft et al., 2012).. General toxicity of this drug is known to be less than e.g. that of chloroquine although embryotoxicity in rodents may call to be careful applying this treatment during pregnancy (Shao et al., 1990).
Pyronaridine has been developed as a fix dose treatment with artesunate in a 3:1 ratio to treat acute uncomplicated *P. falciparum* malaria as well as the blood stage *P. vivax* malaria. In combination, artesunate-pyronaridine is particularly appealing as anti-malarial therapy as it combines the advantages of high efficacy, highly favourable tolerability and safety, low cost, long shelf life, once daily dosing, absence of clinically relevant food effect, and availability of paediatric drug formulations. It has been developed by Medicines for Malaria Venture and has been favourably assessed under §58 by the European Medicine Agency (Sagara et al., 2016; West African Network for Clinical Trials of Antimalarial Drugs (WANECAM), 2018).

This already appealing ACT is to be further supplemented by two more drugs (proguanil and atovaquone) on account of their ability to protect artesunate-pyronaridine from development of drug resistant mutants.

Proguanil is a prophylactic drug which is known to be inhibiting reproduction in both *P. falciparum* and *P. vivax* once it gets to the red blood cells by inhibiting dihydrofolate reductase in the parasite, the lack of which in turn prevents DNA- and amino acid synthesis as well as methylation in the parasite. To date, it is exclusively used in treatment of malaria in combination with atovaquone – an analogue of ubiquinone. Atovaquone is an antimicrobial which in the case of *Plasmodium spp*. acts on the cytochrome bc1 complex whereby it indirectly inhibits several metabolic enzymes, rendering the parasite inactive (Stickles et al., 2016; Winter et al., 2008).

Atovaquone-proguanil is a well-established antimalarial combination that has been extensively used over more than two decades mostly in the treatment of returning travellers and in chemoprophylaxis. In combination, atovaquone-proguanil requires once-daily dosing and is characterized by high efficacy, favourable tolerability and safety profile, lack of epidemiologically relevant drug resistance, and availability of paediatric drug formulations. Its use in Africa was limited due to its high costs. However due to off-patent generic drug producers the cost of this drug is now cut by more than 90 %, which makes it attractive for its use in sub-Saharan Africa. Importantly, atovaquone-proguanil has been shown to exert not only a chemoprophylactic effect but also an anti-transmission effect by acting against the sexual development of *P. falciparum* (Enosse et al., 2000)..

The combination of rapidly eliminated drugs artesunate and proguanil (half-life < 1 day) with the intermediate half-life of pyronaridine (~7-10 days) and atovaquone (~3 days) lead to a faster drug elimination than the conventional partner drugs mefloquine or piperaquine and therefore shorter exposure of sub-therapeutic drug levels in regions of high malaria transmission. In addition, the combination of artesunate – which eliminates young stage gametocytes, with atovaquone-proguanil, which has been demonstrated to exert a delayed effect on the sexual development of *Plasmodium spp*., will most likely lead to an enhanced anti-transmission property of this drug combination. This is considered a crucial feature to avoid the selection of drug resistance in high transmission settings. Importantly this drug combination allows for once daily dosing, which is considered a significant advantage for patient adherence compared to twice daily dosing regimens. Both drugs are available in paediatric drug formulations which will guarantee the adequate implementation of this multi-drug antimalarial combination for young children.

11.2.2 Artesunate-fosmidomycin-clindamycin (AFC)

The second ACT drug combination we propose here is artesunate-fosmidomycin-clindamycin. This combination of two registered drugs, which are used routinely in antimalarial therapy (artesunate, clindamycin) with fosmidomycin, constitutes a unique antimalarial class in late stage clinical development. The main feature of this innovative multidrug combination regimen is the matched short half-life pharmacokinetics and its collateral proven activity on a broad range of Gram-positive and Gram-negative bacterial pathogens.

The development of antimalarials with antibiotic properties has been repeatedly proposed but has not yet led to a registered product (Noedl, 2009, Njim, Dondorp, Mukaka, & Ohuma, 2018). The rationale for this ABC (antibiotic based combination therapy for malaria) termed treatment concept relies on

the fact that malaria cannot be clinically distinguished from bacterial sepsis and laboratory-based confirmation is often lacking in those regions most affected by malaria. Importantly, patients with bacterial sepsis often also harbour asymptomatic *Plasmodium* parasites in regions of high malaria transmission, which are readily detected by rapid diagnostic tests and therefore leading the clinical management into the wrong direction. Finally, malaria is known to increase the risk for certain bacterial blood stream infections including non-typhoidal *Salmonella spp*.. The rationale of developing an antimalarial regimen which concurrently covers the most important bacterial pathogens is therefore well justified from a clinical perspective. The lack of an available regimen is mostly due to the fact that previously used antibiotics with antimalarial activity have covered only a limited spectrum of clinically relevant bacterial pathogens therefore limiting their clinical usefulness.

Fosmidomycin is an antibiotic closely related to the widely used antibiotic fosfomycin and is highly active against the Gram-negative rods *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Enterobacter cloacae, E. aerogenes, Citrobacter, Salmonella spp*. and the Gram-positive rod *Bacillus anthracis* (Neu & Kamimura, 1981). It has originally been developed as an antibiotic for the treatment of urinary tract infections due to Gram-negative bacteria. Despite excellent results, its development as an antibiotic was stopped because of the concurrent and faster development of other antibiotics about thirty years ago. Due to its unique action on the isoprenoid synthesis pathway in *P. falciparum*, it was evaluated in a series of clinical trials for the treatment of malaria. It has been shown to be highly efficacious in combination therapy with either clindamycin or artesunate, was well tolerated and safe and it has a rapid antiparasitic action with complete parasite clearance between 44 and 54 hours (Lell et al., 2003; Na-Bangchang, Ruengweerayut, Karbwang, Chauemung, & Hutchinson, 2007).

Clindamycin on the other hand, is an antibiotic widely used in antibacterial therapy for infections due to Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and anaerobic pathogens including *Bacteroides fragilis* (Sanders et al., 2018). Its activity against *P. falciparum* malaria has been shown in clinical trials with several combination partners, mostly quinine or artesunate (Lell et al., 2003; Ramharter et al., 2005). Due to its favourable safety and tolerability profile it is recommended for the treatment of malaria in first trimester pregnancies. It has a unique mode of action on the apicoplast of *P. falciparum*. Due to its slow onset of action clindamycin requires combination with a rapidly acting antimalarial.

The combination of artesunate-fosmidomycin-clindamycin is anticipated to exert high efficacy based on the known antimalarial properties of these drugs in combination therapy. Whereas fosmidomycin selectively inhibits the non-mevalonate pathway of isoprenoid synthesis, clindamycin is known to act directly on the apicoplast of Plasmodium parasites and artemisinins exert again a different mode of action. The combination of three independently acting antimalarials therefore is anticipated to increase the barrier for the development of de novo drug resistance. All drugs have a favourable tolerability profile. The often-cited association of lincosamid antibiotics with Clostridium difficile colitis is of negligible clinical relevance in the setting of antimalarial therapy of otherwise healthy African children. To our knowledge there is no report of a Clostridium difficile associated colitis published which was associated with the antimalarial use of clindamycin.
The uniquely matched short half-lives of artesunate, clindamycin and fosmidomycin (all <5 hours) precludes the selection of drug resistance during sub-therapeutic drug levels. This feature is thought crucial in high transmission settings to improve the lifespan of antimalarials. At the same time this feature requires two daily dosing to provide adequate drug exposure for the therapeutic effect. Based

on the current knowledge of drug metabolism and the short half-life no clinically relevant drug-drug interactions are anticipated and there is no risk for accumulation of any of the partner drugs.

By combining artesunate-fosmidomycin-clindamycin in this study, we seek to address the typical issues concerned with risking drug resistance as described above, with the additional aspect of dealing with concomitant bacterial blood stream infection frequently encountered in malaria endemic settings (Noedl, 2009).

Table 2 summary of unique features of proposed drug combinations

APAP	**AFC**
high efficacy of individual drugs	independent modes of action
favourable tolerability and safety of individual drugs	proven efficacy of individual drugs in combination therapy
no drug interaction anticipated based on known drug metabolism	favourable tolerability and safety with potential use in pregnancy
once-daily dosing for three days with food intake	matched short half-life drugs mutually protecting each other
paediatric drug formulations available	no drug interaction anticipated based on known drug metabolism
no pre-existing drug resistance in sub-Saharan Africa	twice-daily dosing for three days
Matched pharmacokinetic profile of pyronaridine and atovaquone-proguanil with intermediate half-lives leading to limited duration of mutually protected elimination	no pre-existing drug resistance in sub-Saharan Africa
potentially synergistic impact on interruption of transmission	broad spectrum antibacterial activity against relevant Gram positive and Gram-negative bacteria besides the antimalarial activity
low toxicity	

11.3 Hypothesis

We hypothesize that pharmacokinetic characteristics and safety of multidrug combination of the proposed antimalarials are similar to those of a standard ACT and published data. For this purpose, a descriptive analysis is performed for each drug combination in this proof of concept trial.

11.4 Affected Area and Study site

After WHO Malaria report November 2018:
In 2017, an estimated 219 million cases of malaria occurred worldwide (95% confidence interval [CI]: 203–262 million), compared with 239 million cases in 2010 (95% CI: 219–285 million) and 217 million cases in 2016 (95% CI: 200–259 million).

Although there were an estimated 20 million fewer malaria cases in 2017 than in 2010, data for the period 2015–2017 highlight that no significant progress in reducing global malaria cases was made in this timeframe. Most malaria cases in 2017 were in the WHO African Region (200 million or 92%), followed by the WHO South-East Asia Region with 5% of the cases and the WHO Eastern Mediterranean Region with 2%. Fifteen countries in sub-Saharan Africa and India carried almost 80%

of the global malaria burden. Five countries accounted for nearly half of all malaria cases worldwide: Nigeria (25%), Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%). The 10 highest burden countries in Africa reported increases in cases of malaria in 2017 compared with 2016. Of these, Nigeria, Madagascar and the Democratic Republic of the Congo had the highest estimated increases, all greater than half a million cases. In contrast, India reported 3 million fewer cases in the same period, a 24% decrease compared with 2016.

For the purpose of this study, two study sites had been identified:
Site 1: Lambaréné, Gabon (to be performed at CERMEL)
Site 2: KCCR, Kumasi, Ghana (St. Francis Xavier Hospital, Assin Fosu)

12 Study objectives / purpose

12.1 Aim of the Study/research questions

The main objective of the project is to investigate two combinations of drugs already used in the market or in late stage clinical development but not yet tested in the presently proposed combination. These are Artesunate-Pyronaridin-Atovaquone/Proguanil (APAP) and Artesunate-Fosmidomycin-Clindamycin (AFC).

The two drug combinations will be investigated in a randomized controlled three group clinical phase II study. The aim of this study will be to describe:

- The pharmacokinetics of the investigated drugs when administered in combination therapy
- PCR corrected antimalarial efficacy over a 42 day follow up period
- Safety and tolerability

12.2 Primary objective

To evaluate the pharmacokinetics of APAP and AFC in comparison to the pharmacokinetics of the current standard treatment of artesunate-pyronaridine.

12.3 Secondary objectives

To evaluate safety, tolerability and efficacy in a randomized controlled clinical phase II trial antimalarial combination of:

1. artesunate-pyronaridine-atovaquone/proguanil
2. artesunate-fosmidomycin-clindamycin

12.4 Exploratory objective

None

13 Study endpoints

13.1 Primary endpoint

E1: Descriptive analysis of pharmacokinetics of each partner drug and their principal active metabolites

13.2 Secondary endpoint

E2.1: PCR corrected adequate clinical and parasitological response on Day 42 in per protocol population
E2.2: PCR corrected cure rate on day 28 in per protocol population
E2.3: PCR uncorrected cure rates on days 28 and 42 in intention to treat population
E2.4: Safety and tolerability of combination therapies in intention to treat population
E2.5: Parasite clearance dynamics of combination therapies
E2.4: Proportion of patients with sexual stage parasitaemia during follow up

14 Methodology

14.1 Study design

14.1.1 Randomization and group allocation

A 1:2:2 randomization will be performed for each age group using computer generated random permuted blocks. Allocation will be concealed until the randomization is performed by the investigator. No blinding/masking will be performed for clinical investigations in this open label clinical trial. Genotyping of reappearing parasitaemia will be performed in a single-blinded way by concealing treatment groups to the molecular biologist.

14.1.2 Study groups

At each study site, the following three groups will be investigated:
Group A: oral artesunate-pyronaridine standard treatment
Group B: oral artesunate-pyronaridine-atovaquone-proguanil therapy
Group C: oral artesunate-fosmidomycin-clindamycin therapy

14.2 Study duration

24 Months

14.3 Investigational Medicinal Product

14.3.1 APAP (artesunate/ pyronaridine & atovaquone/proguanil)

For a detailed background see 11.2.1 above. APAP combination tetra-therapy is to be administered as a fixed dose artesunate/pyrinaridine (Pyramax, Shin Poong Pharmaceutical Co., Ltd) and Atovaquone/Proguanil (Malarone, GlaxoSmithKline or generic). The combination of rapidly eliminated drug artesunate and proguanil (half-life < 1 day) with the intermediate half-life of pyronaridine (~7-10 days) and atovaquone (~3 days) lead to a faster drug elimination than the conventional partner drugs mefloquine or piperaquine and therefore shorter exposure of sub-therapeutic drug levels in regions of high malaria transmission. Both drugs are available in paediatric drug formulations which will guarantee the adequate implementation of this multi-drug antimalarial combination for young children.

14.3.2 AFC (artesunate-fosmidomycin-clindamycin)

See 11.2.2 for detailed background. AFC combination triple-therapy constitutes the second antimalarial multi-therapy to be investigated in this study (Group C).

14.3.3 Storage and transport conditions

14.3.3.1 Pyramax (Shin Poong Pharmaceutical Co., Ltd):
Do not store above 30°C. Store in the original package.

14.3.3.2 Malarone (GSK):
Store at 25°C; excursions permitted to 15° to 30°C

14.3.3.3 Artesunate (Generic; either GMP compliant, or on the WHO prequalification list)
Store at 25°C; excursions permitted to 15° to 30°C

14.3.3.4 Fosmidomycin (Deutsche Malaria Gmbh):
Store at 25°C; excursions permitted to 15° to 30°C

14.3.3.5 Cleocin HCl® (Pharmacia and Upjohn Company)
Store at controlled room temperature 20° to 25° C

14.4 Drug prescription and dosing

Artesunate-pyronaridine (Pyramax, Shin Poong Pharmaceutical Co., Ltd):
Three days once daily oral dosing of:
Paediatric dosing regimen:
5 <-8 kg: 1 sachet daily
8 -<15 kg: 2 sachets daily
15-20 kg: 3 sachets daily
1 sachet contains 20 mg artesunate and 60 mg pyronaridine

Adult dosing regimen:
20-<24 kg: 1 tablet daily
24-45 kg: 2tablets daily
45-<65 kg: 3 tablets daily
>65 kg: 4 tablets daily
1 tablet contains 60 mg artesunate and 180 mg pyronaridine

Atovaquone-proguanil (Malarone®, GSK or GMP compliant generic):
Three days once daily oral dosing with food of:
5-8 kg: Atovaquone/proguanil 125 mg/50 mg
9-10 kg: Atovaquone/proguanil 187.5 mg/75 mg
11-20 kg: Atovaquone/proguanil 250 mg/100 mg
21-30 kg: Atovaquone/proguanil 500 mg/200 mg
31-40 kg: Atovaquone/proguanil 750 mg/300 mg
>40 kg: Atovaquone/proguanil 1000 mg/400 mg

Artesunate (Generic; either GMP compliant, or on the WHO prequalification list):
Three days, twice daily dosing independent of food 2 mg/kg as calculated closest to the capsule strength.

Fosmidomycin (Deutsche Malaria Gmbh):
Three days, twice daily dosing independent of food 30 mg/kg as calculated closest to the capsule strength.

Clindamycin hydrochloride (Cleocin HCl®, Pharmacia and Upjohn Company):
Three days, twice daily dosing independent of food 10 mg/kg as calculated closest to the capsule strength (150 mg, 300 mg, 600 mg)

14.5 Pharmacokinetics

Whole blood sampling will be performed at predefined time points to assess the pharmacokinetics of all partner drugs and their principal active metabolites (artesunate: dihydroartemisinin, proguanil: cycloguanil, clindamycin: clindamycin sulfoxide). Blood samples will be taken and stored at -80° C until shipped on dried ice for further analysis by HLPC and LC-MS analysis. The bioanalysis will be performed at the Dept. of Clinical Pharmacy, Institute of Pharmacy, University of Hamburg, Germany. AFC regimen analyses require 2ml plasma from a heparinized blood collection tube per sampling time point and APAP regime analyses require both 2ml plasma each from a heparinized blood collection tube and from an EDTA-coated blood tube per sampling time point.

Pharmacokinetic modelling will be performed for all individual drugs using non-linear mixed effects modelling in NONMEM®. Patient characteristics (e.g. body composition, organ function, maturation) will be assessed as covariates of the pharmacokinetics of each drug. Potential drug-drug interactions of atovaquone-proguanil on artesunate-pyronaridine pharmacokinetics will be evaluated. The time-courses of the pharmacodynamic markers (PCR corrected antimalarial efficacy) will be modelled by multi-state pharmacometric modelling. The pharmacokinetics will be linked to pharmacodynamic models to derive the exposure-response relationships of each regimen. Patient covariates correlating with treatment success and/or failure will be evaluated. Time-to-event modelling will be employed on the recrudescence data from the follow-up period together with the linked pharmacokinetic-pharmacodynamic model to potentially identify predictive factors for treatment failure.

15 Study population and patient selection

15.1 Eligibility criteria

15.1.1 Inclusion criteria

1. Male or female patient age >6 months <66 years.
2. Body weight >5 kg <90 kg
3. Presence of mono-infection of *P. falciparum* with:
   a. Fever, as defined by axillary temperature ≥ 37.5°C or oral/rectal/tympanic temperature ≥ 38°C, or history of fever in the previous 24 hours
   b. Microscopically confirmed parasite infection, in range 1,000 to 100,000 asexual parasites /µL of blood.
4. Written informed consent provided by the adult patient, or parent or legally acceptable representative (LAR) of the minor patient or by an impartial witness (if the patient or patient's LAR is illiterate), stating that the information has been read and/or is understood, and by the medically qualified Investigator. Children will be asked to provide assent where appropriate. The age from which this will be sought will be defined by local legislation.

15.1.2 Exclusion criteria

1. Presence of severe malaria (according to WHO definition – WHO 2013)
2. Anti-malarial treatment in the last 6 weeks.
3. Known history or evidence of clinically significant disorders such as, cardiovascular, respiratory (including active tuberculosis), hepatic, renal, gastrointestinal, immunological (including active HIV-AIDS), neurological (including auditory), endocrine, infectious, malignancy, psychiatric, history of convulsions or other abnormality (including head trauma).
4. Mixed Plasmodium infection
5. Severe vomiting, defined as more than three times in the 24 hours prior to enrolment in the study or inability to tolerate oral treatment, or severe diarrhoea defined as 3 or more watery stools per day
6. Severe malnutrition (defined for subjects aged ten years or less as the weight-for- height being below -3 standard deviation or less than 70% of median of the NCHS/WHO normalised reference values, and for subjects aged greater than ten years, a body mass index (BMI) of less than 16 (WFP Manual, Chapter 1)).
7. Known history of hypersensitivity, allergic or adverse reactions to any of the study drugs
8. Known active Hepatitis A IgM (HAV-IgM), Hepatitis B surface antigen (HBsAg) or Hepatitis C antibody (HCV Ab).
9. Haemoglobin level below 8 g/dL.
10. Serum creatinine levels ≥2 x ULN
11. Female patients of child bearing potential must be neither pregnant (as demonstrated by a negative pregnancy test) nor lactating, and must be willing to take measures not to become pregnant during the study period and safety follow-up period.
12. Have received an investigational drug within the past 4 weeks.
13. Previous participation in any malaria vaccine study or received malaria vaccine in any other circumstance.
14. Refusal to participate and to provide written or witnessed informed consent or assent.

15.2 Sample size

Each group will consist of 20 evaluable patients (with 10 patients in the semi-immune sub-group allocated to the age groups 18-65 years and 11-17 years, respectively) and a consecutive group of 20 patients aged 6 months to 10 years, to allow for adequate pharmacokinetic characterization of study drugs. The total number of evaluable participants will be 100. The sample size was corroborated by a clinical trial simulation using NONMEM® (version 7.4, ICON development solutions, Hanover, USA). Simulation of 'virtual' clinical trials using the present trial design and literature information on the PK of the drugs indicated that the design supports estimation of structural pharmacokinetic parameters with high precision and accuracy (mean absolute bias: 5.9%, mean rRMSE: 12.1%). The design is suitable to detect differences in drug clearance of 20% between the groups B or C vs. A with adequate statistical power (> 81.8%).

Group A:	AP	(20 patients)
Group B:	APAP	(40 patients)
Group C:	AFC	(40 patients)

There are 3 age groups in the step-down procedure: 18-65y --> 11-17y --> 6 months - 10 y

For **Group A**:
It will be **0 patients** for 18-65y, **10 patients** for 11-17y and **10 patients** for 6 months - 10y

For **Group B**:
It will be **10 patients** for 18-65y, **10 patients** for 11-17y and **20 patients** for 6 months - 10y

For **Group C:**
It will be **10 patients** for 18-65y, **10 patients** for 11-17y and **20 patients** for 6 months - 10y

16 Study procedure and data collection

16.1 Visit planning

Treatment will be administered under direct supervision over a three-day period at the study centre. Patients will be followed up on days 0, 1, 2, 3, 7, 14, 21, 28, 35 and 42. Pharmacokinetic sampling will be performed following a predefined sampling schedule (0, 0.25, 0.5, 0.75, 1.5, 3, 5, 8, 48, 168, 336, 672 and 1008 h). Sampling times for the paediatric part of the study will be re-evaluated and sampling timepoint will potentially be reduced during the age-step-down procedure. Blood samples for parasitological assessment will be obtained using standardized finger prick filter paper collection and haematology, biochemistry and pharmacokinetics will be performed using peripheral blood samples.

16.2 Study flow chart

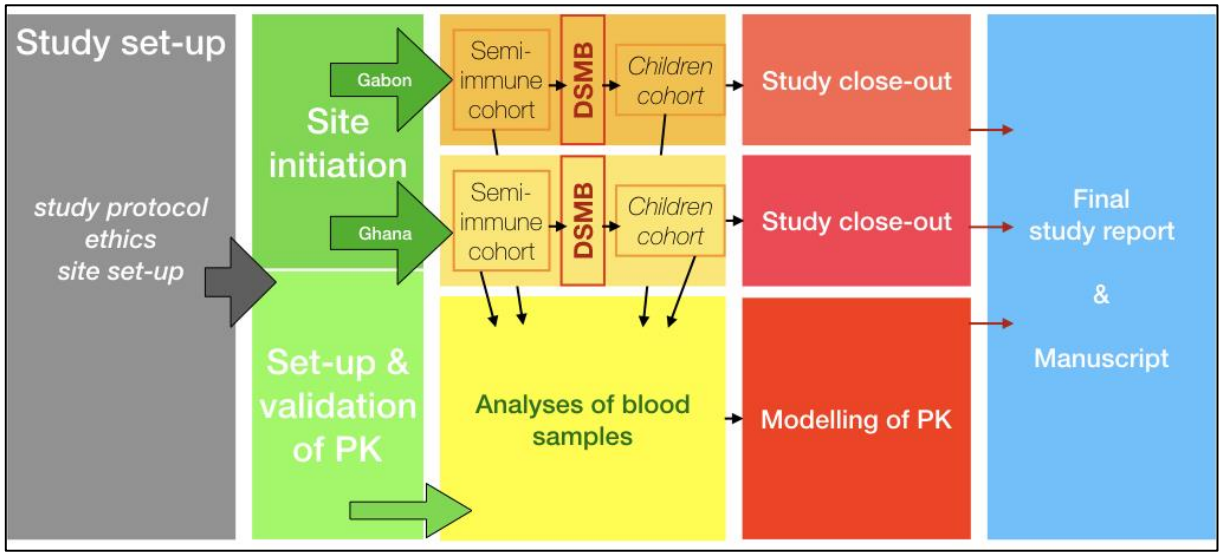

Figure 2 Study flow chart

16.3 Recruitment of participants and follow up visits

16.3.1 Study setting/location sites

Two study sites had been identified. These include
Site 1: Lambaréné, Gabon (to be performed at CERMEL) and
Site 2: KCCR, Kumasi, Ghana (St. Francis Xavier Hospital, Assin Fosu).

16.3.2 Patient numbering

Each participant included into the study receives a unique identifier. The format of the Study ID will be the following: **MM19-CC-nnn (-st)**

MM19: study abbreviation (MultiMal 2019)
CC: country code (see **Fehler! Verweisquelle konnte nicht gefunden werden.**)
nnn: sequential number of enrolled participants
st: sample type (see Table 4 Coding for sample types)

Table 3 Country coding for patient numbering

Country	Code
Gabon	GA
Ghana	GH

Table 4 Coding for sample types

Sample type	Code
Whole blood	WB
Urine	UR
Blood in heparinized tube	HEP
Blood in EDTA-coated tube	EDTA
Plasma from heparinized blood collection tube	PL-HEP
Plasma from EDTA-coated tube	PL-EDTA
Thick blood smear	TCBS
Thin blood smear	THBS
Dried blood spot	DBS

Data is recorded on paper-based source documents and then digitalized using the REDCap software system at monthly intervals.

16.3.3 Registration at the clinical study centre

Patients present to diagnostic facilities of the respective clinical research centres (passive participant recruitment). In case that during the diagnostic routine *Plasmodium* parasites will be detected in the peripheral blood of the respective patient they will be asked to participate in the study and in case of positive consent the study activities can begin (starting with “Screen visit”).

16.3.4 Examination and data collection at inclusion and follow up

Once participants are included into the study, screening activities will be undertaken to ensure all inclusion criteria are present and all exclusion criteria are absent (see section 15.1 Eligibility criteria). Physical exams will be performed, demographics, medical history clinical signs and symptoms, as well as, prior and concomitant medication will be ascertained. Further, vital signs temperature (single), 12-lead ECG (single) will be evaluated. Further, peripheral blood samples will be collected to determine clinical laboratory safety, asexual and sexual *Plasmodium falciparum* parasitaemia and a pregnancy test will be conducted. Then, in case of favourable results the baseline visit will start immediately after

the screening visit: temperature (single) is measured, PK sampling performed and the participants are to be given his/her respective dose of therapy.

Beginning from inclusion into the study the participant is to be monitored for AEs.

During subsequent follow-up visits, details of parameters are to be collected as indicated in Annex 4 – Visit and Sampling Schedule.

16.3.5 Vital signs and Physical examination

The standardized study-specific clinical examination includes assessment of the general appearance, exams of head/neck, thorax and lungs and heart, the abdomen, the extremities and also includes a basic neurological assessment. The basic non-pathological findings are detailed below, which shall serve as an orientation for clinical investigators.

Table 3 Vital signs and physical examinations

General appearance	Patient awake, oriented to all qualities. Speech normal. General appearance age-appropriate, nutritional status normal. Skin colour normal, no icterus. Axillary temperature in °C.
Head and neck	No percussion pain over the sinuses, mucous membranes normal, throat not inflamed, no murmur over the carotid arteries. Thyroid gland not enlarged, no enlarged cervical lymph nodes.
Thorax	No deformities, no compression pain.
Lungs	Upon auscultation normal breathing sounds. No crackles, wheeze or rhonchi. Regular percussion. Pulmonary margins mobile during respiration. Respiratory frequency breaths / min, peripheral O2-saturation in % at room air as assess by pulse oximetry.
Cardiovascular	Heart sounds normal, no murmur auscultated. Blood pressure and heart rate within normal range.
Abdomen	Soft upon palpation, no pain, no local resistances, no rebound tenderness, no mass palpable. Liver and spleen not enlarged palpable. Auscultation of bowel sounds regular in all four quadrants. No percussion pain over the kidneys. No enlarged inguinal lymph nodes palpable.
Extremities	Free range of motion. Peripheral vascularization, motor function and sensibility normal. No peripheral oedema. No clinical sings of deep venous thrombosis.
Neurological status (basic)	Basic exam of the cranial nerves regular, no meningism. Pupils reacting to light, isocoria. No apparent general coordination or movement deficits. No apparent focal deficit.

16.3.6 Laboratory examinations

During the various visits, samples will be taken to assess asexual & gametocyte parasite count (thick and thin blood films), as well as blood spots for genotyping (PCR correction). Further, laboratory safety parameters will be ascertained as listed in section 17.1.3, Haematology and Biochemistry.

16.4 Participant withdrawal or termination

16.4.1 Patient lost to follow-up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

16.4.2 Withdrawal of consent

A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance, or administrative reasons.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

All study withdrawals should be recorded by the Investigator in the appropriate screens of the CRF and in the participant's medical record. In the medical record, at least the date of the withdrawal and the reason should be documented.

Withdrawal of consent for follow-up visits should be distinguished from withdrawal of consent for non-patient contact follow-up, e.g. medical record checks. The site should document any case of withdrawal of consent.

Participants who have withdrawn from the study cannot be re-randomized (treated) in the study. Their inclusion and treatment numbers must not be used.

16.4.3 Reasons for withdrawal or termination

16.4.4 Premature termination or suspension of the study

16.4.4.1 Temporary treatment discontinuation with investigational medicinal products

Temporary treatment discontinuation after incomplete treatment may be considered by the Investigator because of suspected AEs. Re-initiation of treatment with the IMP will be done under close and appropriate clinical/and or laboratory monitoring once the Investigator will have considered

according to his/her best medical judgment that the responsibility of the IMP(s) in the occurrence of the concerned event was unlikely.

16.4.4.2 Permanent treatment discontinuation with investigational medicinal products

Permanent treatment discontinuation is any treatment discontinuation associated with the definitive decision from the Investigator or the patient not to re-expose the patient to the IMP at any time after incomplete treatment.

16.4.4.3 Handling of patients after permanent treatment discontinuation

Patients will be followed-up according to the study procedures as specified in this protocol up to the scheduled date of study completion, or up to recovery or stabilization of any AE to be followed-up as specified in this protocol, whichever comes last.

16.4.4.4 Procedure and consequence for patient withdrawal from study

The patients may withdraw from the study before study completion if they decide to do so, at any time and irrespective of the reason:
If possible, the patients are assessed using the procedure normally planned for the end-of-study visit including a pharmacokinetics sample.
For patients who fail to return to the site, the Investigator should make the best effort to recontact the patient (eg, contacting patient's family or private physician, reviewing available registries or health care databases), and to determine his/her health status, including at least his/her vital status at the planned D42 date. Attempts to contact such patients must be documented in the patient's records (eg, times and dates of attempted telephone contact, receipt for sending a registered letter).
Patients who have withdrawn from the study cannot be re-randomized (treated) in the study. Their inclusion and treatment numbers must not be reused.

The Sponsor, Principal Investigator, Ethics Committee (EC) and Regulatory Authorities independently reserve the right to discontinue the study at any time for safety or other reasons. This will be done in consultation with the Sponsor where practical. In the occurrence of premature trial termination or suspension, the above-mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension (with the exception of the sponsor's responsibility for notifying the Regulatory Authorities). After such a decision, the Sponsor and the Investigator will ensure that adequate consideration is given to the protection of the participants' interest. The investigator must review all participants as soon as practical and complete all required records.

16.4.5 Strategies to assure compliance with the study are:

a. detailed explanation and discussion with participants at inclusion of the study ensuring complete understanding of the study and all study procedures,
b. participants are accompanied to their homes and their mobile phone numbers are recorded with their agreement for being able to actively look for participants who have not presented for a follow up visit,
c. participant transport is organized and costs are covered by the study team for all follow up visits,
d. the longstanding and positive interaction with the local communities is a further reason for high retention rates in clinical trials. One reason of participants not following the entire study protocol is withdrawing of informed consent. In case of withdrawal of consent, the participant is contacted to ask whether he is willing to discuss the reasons for withdrawal with the investigator. However, the participant must not be unduly influenced or coerced to remain in the study as withdrawal of consent is the right of the participant and has to be accepted by the study team and this decision is therefore fully respected. In case of withdrawal of consent, the investigator will provide

recommendations for further clinical care in routine management so that the participant has not disadvantage from withdrawing from the study.

16.5 Investigational product/ study agent

16.5.1 IMP management (storage, stability and accountability (including Placebo where applicable))

Investigators or other authorized persons (e.g., study pharmacists) are responsible for storing the IMP in a secure and safe place in accordance with labelling specifications.
Drug accountability will be executed to ensure that research participants receive the correct study drug. This also includes documentation of study drug reception, storage, handling, dispensing, as well as documentation of study drug administration, return and/or destruction of the drug to ensure that IMP has been used according to the protocol. Control of IMP storage conditions, especially temperature control and information on in-use stability and instructions for handling should be managed according to the rules provided by the sponsor.

16.5.2 Expected risks

Table 4 Expected risks

Group	Agent	Adverse events
A	Artesunate	hypersensivity, anaemia, gastrointestinal disorders (nausea, vomiting)
	Pyronaridin	headache, eosinophilia, neutropenia, anaemia, increased platelet count, bradycardia, hypoglycaemia, fatigue, transient liver transaminase elevations, gastrointestinal disorders (abdominal pain, vomiting, nausea)
B	Artesunate	*see Group A*
	Pyronaridin	*see Group A*
	Atovaquone/ Proguanil	headache, dizziness, cardiac arrhythmias symptoms, insomnia, intolerance due to lactose ingredient, gastrointestinal disorders (abdominal pain, diarrhea, vomiting, nausea)
C	Artesunate	*see Group A*
	Fosmidomycin	headache, allergic cutaneous reaction, intolerance due to fructose ingredient, gastrointestinal disorders (abdominal pain, diarrhea, vomiting, nausea, loose stools, flatulence)
	Clindamycin	maculopapular rash, urticaria, intolerance due to lactose ingredient, gastrointestinal disorders (abdominal pain, diarrhea, colitis, vomiting, nausea)

16.6 Randomization and group allocation

A 1:2:2 (Group A: Group B: Group C) randomization will be performed for each age group using computer generated random permuted blocks. Allocation will be concealed in opaque, sealed envelopes until the randomization is performed by the investigator. No blinding/masking will be performed for clinical investigations in this open label clinical trial. Genotyping of reappearing parasitaemia will be performed in a single-blinded way by concealing treatment groups to the molecular biologist.

17 Subject safety

17.1 Assessment of safety

Before inclusion, a standardized study-specific clinical examination will be performed on each patient to determine the current health status. Special attention will be paid to whether the patient is eligible for the study or not. The examination needs to strictly adhere to the standard examination given in the Source Document. Throughout the study, further physical examinations, including weighing, temperature measurements, measurements of vital signs, haematological and biochemical safety analyses and urinalysis need to be performed.

17.1.1 Physical examination

The standardized study-specific clinical examination includes assessment of the general appearance, exams of head/neck, thorax and lungs and heart, the abdomen, the extremities and also includes a

basic neurological assessment. The basic non-pathological findings are detailed in Table 3 Vital signs and physical examinations , which shall serve as an orientation for the clinical investigator.

17.1.2 Clinical parameters

Clinical parameters will include malaria signs and symptoms as well as vital parameters.
Vital parameters include:

- body temperature,
- pulse rate and
- blood pressure.

Blood pressure and pulse rate will be measured after the participant has rested for 10 minutes.

17.1.3 Laboratory safety variables

Local laboratory ranges will be used to define an abnormal laboratory finding. Investigators will document whether an abnormal laboratory finding is clinically significant.

17.1.3.1 Haematology:

The following parameters will be analysed throughout the study. Further parameters may be analysed if medically required.

- RBC
- Haemoglobin
- Haematocrit
- Platelets
- WBC
- Neutrophils
- Eosinophils
- Basophiles
- Lymphocytes
- Monocytes
- reticulocyte count if possible at local lab

17.1.3.2 Biochemistry:

The following parameters will be analysed throughout the study. Further parameters may be analysed if medically required.

- Total bilirubin
- Direct bilirubin (if total bilirubin > ULN)
- Albumin
- ALT
- AST
- Alkaline phosphatase
- LDH
- Creatine kinase
- Urea
- Creatinine
- Haptoglobin
- Sodium
- Potassium
- Glucose

17.2 Training

Staff training at each site will entail an initial training session to ensure all staff members involved with this study are aware of the details, expected timelines, and how this study will fit into their other activities on site. Subsequently, site training sessions for staff members will be taking place as the PI recognizes the need therefore. Regular group meetings (weekly unless stated different) will update the staff at the site on the current state of the project in addition to their other projects and activities. Consortium training will take place in combination with the kick-off meeting and will involve all aspects of the study, including Project management, Financial management, Study procedures, subject safety, Data security, etc.)
All study members will be in possession of a valid (not older than 2 years) GCP certificate, and where applicable an GLP certificate too.

17.3 Adverse Events and Serious Adverse Events

17.3.1 Adverse Event (AE)

An AE is a sign, symptom, syndrome, disease or biological anomaly suffered by a participant or a subject participating in a clinical study and receiving a medicinal product. This term does not imply a causal relationship with the concerned treatment. Clinical signs typical of an acute malaria episode will not be considered AEs unless the healthcare personnel consider these events as exceptional due to their evolution, their seriousness, or another factor related to these events (See Annex 3: Grades of Adverse Events).

17.3.2 Serious Adverse Event (SAE)

A SAE is an adverse event which:
- causes death or
- is life-threatening or
- necessitates or prolongs hospitalisation or
- results in persistent or significant disability/incapacity or
- is a congenital defect or malformation
- is another medically important event

A decision on medical and scientific grounds is required to assess whether an immediate notification of an event is warranted in other situations, such as medically important events which are not life-threatening, fatal or cause hospitalization, but could endanger the patient or required an intervention to prevent one of the above conditions developing.

Remark: Examples of such events are intensive care in the emergency room or at home to treat a bronchospasm; convulsions not causing hospitalisation, or the development of drug addiction or drug abuse.

17.3.3 Adverse Event of special interest

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

AESIs in this study are defined as events fulfilling the criteria of Hy's law. In case of an AESI confirmed by the study physician, the medical monitor, sponsor, clinical monitor and the international study coordinator shall be informed within 24 hours. Notification will occur through the use of an AESI form.

Hy's law

Possible Hy's law case is defined as a subject with any value of ALT or AST >3x the upper limit of normal (ULN) together WITH an increase in bilirubin to a value > 2xULN (>35% direct) and NOT associated to an ALP value > 2xULN. Collect if not scheduled urine, blood, or relevant biological fluids for additional diagnostic tests. Appropriate medical attention and appropriate follow up shall be ensured.

17.3.4 Monitoring of AEs

The Site Principal Investigator (PI) will make a causality assessment. He should be informed about any new data on a participant for whom a causality link had already been established to reconsider its analysis and if necessary reassess the causality. Data shall only be modified following an official query procedure. Source Document review meetings shall be organised regularly, during which the site PI and the local study team shall review AE data (intensity, causality, and date of event manifestation in relation to the start of treatment). The site PI and his team will also make a first analysis of the safety reports received in order to assess completeness. Safety data should be entered immediately in the eCRF.
The SAE reporting must follow notification requirements in Section 17.3.6. Safety reports are reviewed by the sponsor for quality control.

One Medical Monitor will be available for the trial site to resolve arising safety related questions and uncertainties. He will oversee the medical aspects of the clinical trial and will actively collaborate with the sites. The medical monitor will have access to the eCRF and can address a request to the site PI concerned to add any further information needed for the safety analysis. The medical monitor will conduct the final review of the eCRF for any participants who have had an adverse event.

An independent study-specific DSMB will conduct periodic safety reviews. If necessitated by special events, unscheduled safety reviews can be convened.

17.3.5 Assessment of AEs

17.3.5.1 Severity

The investigator will assess the severity/intensity of the adverse reactions and clinical laboratory changes using the following guidelines:

- **Mild:** awareness of sign or symptom, but easily tolerated
- **Moderate:** enough discomfort to cause interference with usual activity
- **Severe:** incapacitating with inability to work or do usual activity
- **Life-threatening** (Note: this must also be reported as Serious Adverse Event)

Clarification of the difference in meaning between "severe" and "serious":
The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on the outcome or criteria defined under the serious adverse event definition. An event can be considered serious without being severe if it conforms to the seriousness criteria; similarly, severe events that do

not conform to the criteria are not necessarily serious. Seriousness (not severity) serves as a guide for defining reporting obligations.

Relationship or association with the use of study drug or study procedure
The investigator will assess the relationship of the event with the study drug using the following guidelines and terminology:

Table 5 Relationship definitions of drug and procedure

Definite	Clear-cut temporal association, with a positive re-challenge test or laboratory confirmation; Event or laboratory test abnormality, with plausible time relationship to drug intake; Cannot be explained by disease or other drugs; Response to withdrawal plausible (pharmacologically, pathologically) event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon)
Probable	Clear-cut temporal association, with improvement upon drug withdrawal and not reasonably explained by the participant's known clinical state. Event or laboratory test abnormality, with reasonable time relationship to drug intake. Unlikely to be attributed to disease or other drugs response to withdrawal clinically reasonable
Possible	Event or laboratory test abnormality, with reasonable time relationship to drug intake. Less clear temporal association, other aetiologies are possible. Could also be explained by disease or other drugs, other possible aetiologies should be recorded on the Source Document Information on drug withdrawal may be lacking or unclear
None	No temporal association with the study drug; related to other aetiologies such as concomitant medications or conditions or subject's known clinical state. Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) Disease or other drugs provide plausible explanations

The investigator should use clinical judgement in assessing whether the event is more likely due to other causes or to concomitant medication rather than the IMP; i.e. that it is unlikely due to the IMP. If it is considered 'unlikely' then it should be marked as "not related/none". If the investigator cannot exclude the possibility of a relationship to the drug then a "possible relationship" should be recorded.

17.3.5.2 Action Taken

The investigator will document the taken action(s) following identification of the adverse event:

- Study drug discontinued
- Participant withdrawn from study
- Concomitant medication required
- Hospitalization required or prolonged (this should also be reported as a SAE)
- Other

17.3.5.3 Outcome

The investigator will follow-up the adverse event until resolution or until no further medically relevant information can be expected. Adverse event outcome will be classified as follows:

- Resolved
- Resolved with sequelae
- Continuing
- Death

17.3.6 Obligation of AE and SAE notification

17.3.6.1 Adverse Events

The AEs, regardless of their seriousness and causal relationship to the study drug, arising between the first administration of study medication and the last study visit (as per the protocol), must all be recorded on the participant's Source Document (AE recording section). When possible, the symptoms must be regrouped within a single syndrome or diagnosis. The healthcare personnel shall have to specify the date of manifestation of the event, its intensity, final evolution, the measures taken and the treatment undertaken (if any).

17.3.6.2 Serious Adverse Events

In case of SAEs, the healthcare personnel must immediately contact the Investigator for validation of the seriousness and determination of the causality. Subsequently, the procedure described below must be followed, independent of causality:

- Send (within 24 hours of knowledge) the signed and dated copy of the "Adverse Event form" and the "SAE form" electronically to the medical monitor, sponsor, clinical monitor and the international study coordinator
- Contact immediately (the same day) the medical monitor, responsible for safety in case of death or life-threatening events.
- Inform the Ethics Committees of the occurrence of any SAE or if applicable as per local regulation.
- The follow-up of each fatal or life-threatening AE must be provided to the medical monitor, sponsor-coordinating PI, clinical monitor and the international study coordinator within the same timeline as the initial report (within 24 hours of knowledge and preferably by email).
- Attach to the Source Document the photocopy of all available results and examinations which were undertaken (and their date). Analysis results must be accompanied by the laboratory normal ranges. Special consideration shall be taken to ensure participant anonymity, and to the correct completion of the participant's study specific identifier in the copies of the source documents provided to the sponsor.

17.3.6.3 Adverse Events of Special Interest

In case of AESIs, the healthcare personnel must immediately contact the Investigator for validation of the seriousness and determination of the causality. Subsequently, the procedure described below must be followed, independent of causality:

- Send (within 24 hours of knowledge) the signed and dated copy of the "Adverse Event form" and the "AESI form" electronically to the medical monitor, sponsor-coordinating PI, clinical monitor and the international study coordinator
- Attach to the Source Document the photocopy of all available results and examinations which were undertaken (and their date). Analysis results must be accompanied by the laboratory normal ranges. Special consideration shall be taken to ensure participant anonymity, and to the correct completion of the participant's study specific identifier in the copies of the source documents provided to the sponsor.

17.3.7 Emergency procedures

The site PI is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study.

17.3.8 Follow-up of Adverse Events

The healthcare personnel must take all appropriate measures to protect the safety of the participants. Personnel must ensure to document follow-up of the evolution of each AE (clinical, biological or other) until resolution or until the stabilization of the participant's status.
In case of a SAE the participant must be followed until complete resolution and normalization of all analysis results, or until chronicity of the participant's status. This can imply that the follow-up of the participant must continue beyond the period of follow-up per protocol, and that additional investigations could be requested by the sponsor.
All new relevant information concerning the initial SAE shall be recorded on the "SAE form" by the local study team, and shall be validated by the site PI/co-PI who shall transfer the form to the medical monitor, sponsor-coordinating PI, clinical monitor and the international study coordinator.

17.4 Prior, Concomitant Medication, Prohibited Medications, Treatments and Procedures

17.4.1 Prior medication

Record prior medication including anti-malarial treatment and antibiotics taken within the timeframes specified in the Exclusion Criteria section. Ensure that no excluded medications have been taken in line with the restrictions in Exclusion Criteria and Section 15.4.5 below.

17.4.2 Concomitant Therapy

Concomitant medication is defined as any medication, other than the Investigational Medicinal Product (IMP), which is taken during the study, including prescription and over- the-counter medicines, and any traditional or herbal remedies.

The only additional medication likely to be given will be paracetamol (15 mg/kg 4 hourly as required) as an antipyretic, and metopimazine for repeated vomiting (or if not available, any other antiemetic which is not known to prolong QT and/or cause torsade de pointes).

Metoclopramide is contraindicated from the period prior to screening to Day 5 post-dose (120 hours).

Beta-lactam antibiotics can be given in case of a bacterial infection appearing after enrolment. All the other antibiotics, new-quinolones included, should be avoided where possible. All concomitant medication taken while the patient is participating in the study will be recorded.

17.4.3 Post- Treatment Therapy

17.4.4 Rescue Therapy

Rescue treatment will be administered according to local regulations. In case of uncomplicated malaria, artemether-lumefantrine is suggested as treatment of choice but the ultimate decision lies with the treating physician and must be taken in the interest of the patient. Rescue treatment will be administered in case of

- **<u>early treatment failure</u>**
    - danger signs or severe malaria on day 1, 2, or 3 in the presence of parasitaemia

- parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature
- parasitaemia on day 3 with axillary temperature 37,5°C
- parasitaemia on day 3, 25% of count on day 0

• **late clinical failure**
- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in participants who did not previously meet any of the criteria of early treatment failure
- presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature 37.5°C in participants who did not previously meet any of the criteria of early treatment failure

• **late parasitological failure**
- presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature <37.5°C in participants who did not previously meet any of the criteria of early treatment failure or late clinical failure

17.4.5 Prohibited Medications

Study participants should not take medicines as listed under section 15.1.2 “exclusion criteria” and below:

Excluded medication unless explicitly given as study medication or rescue medication (both in the period prior to Screening and during the patient’s participation):

- 4-Aminoquinolines chloroquine and piperaquine Arylaminoalcohols quinidine
- Quinine
- Mefloquine
- 9-phenanthrene methanol halofantrine
- Dibutyl-amino ethanol lumefantrine
- Artemisinin derivatives such as artemether, arteether, artesunate and dihydroartemisinin
- Antimetabolites proguanil
- Chlorproguanil
- Methotrexate and other folate antagonists
- Sulfalene
- Pyrimethamine
- Sulfonamides
- Sulfadoxine
- Sulfisoxazole
- Sulfadiazine
- Sulfasalazine
- 8-Aminoquinolines primaquine, tafenoquine
- Hydroxynaphtolquinone atovaquone
- Antibiotics, various classes doxycycline
- Antibiotics, various classes new quinolones
- Azythromycin
- Erythromycin
- Pentamidine
- Clindamycin

- Rifampin
- Dapsone
- Combinations of sulfadoxine pyrimethamine
- Trimethoprim sulfamethoxazole
- Atovaquone
- Pyronaridine
- Metoclopramide (period prior to screening to Day 5 post-dose (120 hours)).

Traditional and herbal remedies are not permitted from 7 days prior to dosing and during the study.

17.5 Pregnancy

A urine pregnancy test will be performed for all women of child-bearing potential at initial presentation. Women of child-bearing potential are defined as women who have experienced menarche and who are not permanently sterile or postmenopausal, defined as 12 consecutive months with no menses without an alternative medical cause. The investigator shall decide whether this is applicable or not based on the medical history of the patient.

In case of pregnancy on initial presentation, the anti-malarial treatment shall be the one recommended by the national malaria control program (NMCP). The patient will not be included in this study.

Female patients will be encouraged to communicate to their village CHW if they become pregnant within a period of two months after the start of the (drug) treatment.

The evolution of the pregnancy will be monitored with visits at 3, 6 and 9 months and at 7 days after the delivery. Information on the drugs taken during the pregnancy as well as AEs/AESIs/SAEs and the health status of the newborn/s will be collected.

Pregnancy is not an adverse event unless the outcome of the pregnancy fulfils one of the serious criteria as defined in Section 15.1. Pregnancy should be reported by using the appropriate pregnancy report form within 24 hours of knowledge to safety-multimal@bnitm.de.

17.6 Sponsor obligations

17.6.1 Safety

Throughout the study investigator should report expedited all SAEs including SUSARs to the sponsor. The sponsor reports SUSARs within 7 days for fatal SUSARs and 15 days for non-fatal SUSARs to the regulatory authorities. For this (multicentre) trial the sponsor will share /circulate the SUSARs information to other investigators immediately after reporting them to the regulatory authorities.

17.6.2 Quality Assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigators and associated personnel before the study, periodic monitoring visits by the assigned study monitors on behalf of the Sponsor, and direct transmission of clinical laboratory data from central laboratories into the study database.
Written instructions will be provided for collection, preparation, and shipment of blood samples and for thick blood smear procedures.

Guidelines for eCRF completion will be provided and reviewed with study personnel before the start of the study.

CRFs will be reviewed for accuracy and completeness during on-site monitoring visits by the study monitor, any discrepancies will be resolved with the investigators or designee, as appropriate. Following ongoing transfers of the data into the clinical study database further validation and checks will be performed by Data Management and any queries will be communicated to the investigator or designee for subsequent resolution.

Steps to be taken to ensure the accuracy and reliability of data include:

- the selection of qualified investigators and appropriate study sites,
- review of protocol procedures with the investigators and associated personnel before the study,
- periodic monitoring visits by the assigned study monitors on behalf of the sponsor,

Sites will be assisted in the development of SOPs and the implementation of local quality management systems.

Besides remote monitoring activities, on site monitoring will be performed to verify that the quality requirements of the study activities have been fulfilled. Source Data Verification will be performed by the study monitors to ensure that the trial is performed and the data are generated, recorded and reported in compliance with GCP guidelines, the study protocol and applicable regulatory requirements.

Quality Control (QC) measures will include validation of clinical laboratory equipment and external QC of malaria microscopy.

Written instructions will be provided for clinical examinations, for the collection and analysis of blood parameters, for preparation and shipment of blood spots, and for blood smear procedures (study manual).

CRFs will be reviewed for accuracy and completeness during on-site monitoring visits by the study monitor, any discrepancies will be resolved with the investigators or designee, as appropriate. Following ongoing transfers of the data into the clinical study database further validation and plausibility checks will be performed by Data Management and any queries will be communicated to the investigator or designee for subsequent resolution. Guidelines for eCRF completion will be provided and reviewed with study personnel before the initiation of the study. All data captured in an electronic device (eCRF) will be uploaded on a daily basis into the main database. After that, data can be checked for plausibility as well as for completeness.
Audits may be commissioned by the sponsor before during and after the conduct of this clinical trial focussing on any aspect of the project.

18 Statistical considerations

18.1 Analytical Plan

The statistical analysis plan will be prepared in parallel to the study set up ideally before recruitment of the first patient.

18.2 Data analysis

The Safety Population includes all subjects who receive at least one dose of the study drugs after the baseline visit. All analyses will be based on the Safety Population. Study data will be summarised as described below. These results will be presented to the DSMB, who will evaluate the nature and significance of potentially reported adverse events and treatment failures.

Data processing, tabulation of descriptive statistics, and graphical representations will be performed using Stata version 15.

18.2.1 Interim Analysis

No formal interim analysis is planned for this proof-of-concept study. However, the study will be monitored by a DSMB, which will advise the sponsor regarding the continuing safety of already recruited and potential study subjects, as well as the validity and scientific merit of the trial.

18.2.2 Analysis of Adverse Events and Severe Adverse Events

The following summary statistics on adverse events will be presented by treatment group:

- Number and percentage of participants with any AE during the study period
- Number and percentage of participants with any SAE during the study period
- Number and percentage of participants with any AEs of special interest (AESIs) during the study period
- Number and percentage of AEs which caused early discontinuation of IMP
- Description of all deaths during the study period
- Number and percentage of reported pregnancies

All participants in the treatment group are considered in the denominator, unless otherwise specified. Participants with multiple occurrences of events will only be counted once considering the most severe event.

18.2.3 Analyses of safety data

The summary statistics (including number, mean, median, interquartile range, standard deviation, minimum and maximum) of selected clinical parameters and laboratory safety variables (values and changes from baseline) will be calculated for each visit by treatment group. The summary statistics of demographic variables at baseline will be calculated by treatment group.

The proportion of participants who completed the day 28 treatment will be presented as an indicator for tolerability of the treatment. This includes the

- Number and percentage of participants completing 28 days of treatment by treatment arm
- Number and percentage of participants with treatment discontinuation (withdrawn from the study and AEs that led to study drug discontinuation)
- Proportion of participants vomiting their medication within 30 minutes after administration, each day a drug is administered

18.3 Assessment of efficacy

18.3.1 Measurement of parasitaemia

Blood samples for parasitological assessment will be obtained using capillary or venous blood by applying finger pricks and venipunctures, respectively. Whenever blood is available from venous blood draws that are applied during a respective visit (e.g. PK sampling), venous blood shall be used for parasitological sample preparation to increase patience satisfaction. Standardized light microscopy samples and filter paper samples will be prepared twice daily until parasite clearance and subsequently once per visit. Parasitological assessment will be conducted at day 0 (dosing), at days 1, 2, 3, 7, 14, 21, 28, 35, 42 post-dose and in case of an unforeseen visit (See Appendix 4).
Thick and thin blood smears will be used to determine parasite count and species. Only *P. falciparum* mono-infections will be included in the study.
Blood films will be read by two independent expert microscopists according to WHO standard. In case of deviating results of more than 50% of parasitaemia or one positive and one negative result, the concerning slide will be read by a third independent microscopist and the mean of the two nearest results will be documented. A blood film will be considered negative when the examination of 1000 white blood cells reveals no asexual parasites.

18.3.2 Parasite genotyping

Dried blood spots (DBS) will be collected at all time points of slide preparation. In case of reappearing microscopic parasitaemia, the pre-treatment DBS (day-0) and the DBS of the time-point at which reappearance of parasites occurred (day-failure) will be analysed by PCR-based genotyping of polymorphic parasite loci (glurp/msp2/msp1 and/or microsatellites). The presence of at least one identical allele shared by day-0 and day-failure parasite samples will be interpreted as parasite recrudescence. Other results will be interpreted as a new infection.

18.4 Safety analysis and review

18.5 Determination of sample size

Each group will consist of 20 evaluable patients (with 10 patients in the semi-immune sub-group allocated to the age groups 18-65 years and 11-17 years, respectively) and a consecutive group of 20 patients aged 6 months to 10 years, to allow for adequate pharmacokinetic characterization of study drugs. The total number of evaluable participants will be 100. The sample size was corroborated by a clinical trial simulation using NONMEM® (version 7.4, ICON development solutions, Hanover, USA). Simulation of 'virtual' clinical trials using the present trial design and literature information on the PK of the drugs indicated that the design supports estimation of structural pharmacokinetic parameters with high precision and accuracy (mean absolute bias: 5.9%, mean rRMSE: 12.1%). The design is suitable to detect differences in drug clearance of 20% between the groups B or C vs. A with adequate statistical power (> 81.8%).

19 Data management

19.1 Collection and validation of data

Patient data should be captured in the patient record at each site and documented in the study specific CRF.

Frequency of visits and responsibilities of the study monitor includes an interim site monitoring visit every 3 months, during which the following activities will be performed: Source data verification, inspection of the investigational product and its storage, inspection of randomisation lists, inspection of CVs and qualifications of involved investigators and study personnel, inspection of laboratories and inspection of the investigator site file. Further, it is the responsibility of the monitor to prepare a site initiation visit and a close out visit at each recruiting site. The sponsor may include other study related activities to the list of monitoring responsibilities at a later time point. Further, additional for cause visits can be added if deemed necessary by the sponsor.

Source data verification (SDV) will be conducted on all informed consent forms, eligibility criteria, primary endpoint and AEs, SAEs and AESIs by the study monitor during monitoring visits.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the eCRF are consistent with the original source data.

The study coordinator shall organise regular capture of these data by each study centre together with the results of clinical examinations, parasitological and other study-related analyses.

Interim data will be assessed under the DSMB Charter by the independent DSMB.

The identification of any data to be recorded directly on the CRFs (i.e. no prior written or electronic record of data), and to be considered to be source data.

19.2 Data handling

Data management will adhere to good clinical data management practices. There will be one main server at the DTM which will collect the data of all study sites. Each study site will have a host computer on which the data from health facilities and the field will be merged. Data generated during field work will be recorded on paper-based case report forms and subsequently entered in an online data base. Data entry shall be executed in a timely fashion by the recruiting sites and supervised by the local PI to ensure data will be available in the online data base, so that the Sponsor is aware of the current state of recruitment and follow-up. Secure mechanisms to store and access data will be used, such as the open source platforms REDCap.
The database software will be able to manage multiple users and thus can control concurrent data access. It also will have a backup scheme and Log file/Audit trail functions that allow to supervise the data entry process and to restore data in case of unexpected errors or malfunctions with the threat of data loss. Furthermore, it will offer access control functions with allocation of different access authorizations. Plausibility checks and other data quality methods (e.g. defining ranges, mandatory fields) will be implemented to guide the data entry clerk through the eCRF to ensure high data quality while entering the data.
The data management strategy will be established with all the partners, in order, for each dataset produced, to share the same objectives in terms of data sharing and data exploitation, to check any ethical and legal/IPR aspects, and to determine the timeframe.
If necessary, a defined period for data embargo or restricted access will be applied to some datasets.

Data curation and repository costs are included in the proposed budget

19.3 Validation of data

A query and data validation system such as plausibility checks, ranges, coded lists or specific entry formats will be implemented according to the sponsors definitions within the database in order to guarantee the good quality of the data. Those tests will run during data entry and/or data upload to the main server at DTM. A regular check-up of the data will be performed at the DTM as well and possible discrepancies or inconsistencies will be discussed with the field team on a regular basis. Before real time data collection, test procedures and test data for the checks will be used to perform database validation.

When no more updates or changes to the data are expected the database is locked.

19.4 Recordkeeping

Appropriate medical and research records will be maintained for this trial, in compliance with ICH E6 GCP, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. Site monitoring will be conducted to ensure participant protection, as well as to ensure study procedures, laboratory processes, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH and, when appropriate, regulatory guidelines.
The data will be collected and recorded in the medical record or the source documents first by the PI or the designated clinical personnel and then entered onto the CRF.
The Investigator and the clinical personnel involved in the study will ensure that all recorded data are accurate, complete and consistent as in accordance with the ICH GCP requirements and the site-specific protocol for completion of documentation. The clinical staff involved in the study will also ensure that all records for receipt, shipment and other disposition of the study products are maintained, complete and accurate during the clinical study.

19.5 Documentation and utilization of study results

A scientific committee will be formed, comprising the coordinating PI, management team and others as agreed by these parties, with the responsibility for the presentations and/or publications of the results. Each subsequent presentation or publication should be approved by the scientific board.

The final decision on the publication of a manuscript/summary/presentation will be taken by the scientific committee in order to allow for an internal review and the possibility of providing comments.

19.6 Scientific/academic dissemination of results

Data from this study will be presented as follows:

- As articles in international peer-reviewed open access scientific journals with a high impact factor.
- As thesis publications from students which will typically include significant details on the methodology and findings which can be beneficial to other students and others not well-versed in clinical trials.

- As reports for filing at the institutional level and various stakeholder who might be in a position to fund further studies and which will typically contain detail which goes beyond the usual scientific publications in journals.
- In national and international conferences and symposia for the awareness of scientific community, stakeholders and policy makers of our findings.

No country-level dissemination is foreseen for the pilot study results. However, Ministry of Health and other applicable regulatory bodies will be informed of our findings to allow a smooth transition to the main trial.

19.7 Quality control of data on site

Audits may be carried out by DTM Quality assurance manager for clinical trials, representatives of the sponsor and other applicable national or international bodies. All documents pertinent to this study must be made available for such inspection after adequate notice of intention to audit.

19.8 Data entry

In short, relevant data will be recorded in a Clinical File and a Case Report Form (CRF, for protocol specific data. Each participant will have a Clinical File which is source data.
All recording will be done only in permanent ink.
Corrections will only be made by drawing a single line through the incorrect entry, writing the correction in the nearest practicable space and initialling and dating the correction. A log of names, signatures and initials of all staff entering data into a participant's Clinic File and CRF will be kept. Any corrections made after the review and signature of the Principal Investigator will be noted with the initials of the person making the change and countersigned by the Principal Investigator. Correction fluids are not allowed.
All deviations from this study protocol will be included in Trial Master File (TMF) and included in the final study report. An assessment of the significance of each protocol deviation will be given in the study report.
All CRFs will be reviewed internally by the CRU at the completion of each study visit for any omissions or apparent errors so that these can be corrected without delay.

19.9 Data cleaning and data base locking

At the end of the study the centrally aggregated data, will be verified according to the above-mentioned documented procedures. Once declared clean, the database will be locked before data analysis is performed.

20 Tasks and responsibilities

(after ICH GCP E6 R2, November 2016)

20.1 Sponsor

The study sponsor's responsibility is toward the study team at the study site and the health authorities and shall take all reasonable measures to ensure the good conduct of the study with regards to ethics, protocol compliance, integrity and validity of the information recorded in the participants' Source Documents and eCRFs, as well as, with regards to the availability of the adequate resources to ensure appropriate conduct of the study. In this respect, the principal function of the sponsor with the support

of the clinical trial coordination team (WP2) is to maintain high levels of ethical, scientific, technical and regulatory standards for all study-related aspects of ethics, regulations and administrative rules at the study site.

For this study the sponsor's representative acts as well as coordinating PI.

20.2 Study team on site

The site PI should be qualified by education, training and experience to assume responsibility for the proper conduct of the trial. The study team members shall be responsible for performing the clinical trial in accordance with this protocol and in accordance with the legislation and international guidelines under the direction of the site PI.

They are responsible for obtaining an informed consent prior to inclusion in the study, for completing the study documents (screening registry and participant Source Document) and for recording all relevant data in relation to the study. Each study team member shall ensure that the information reported in the document is precise and accurate.
They must inform the patient of all relevant aspects of the study, including the information in the patient information sheet. All this information shall be provided to the patient in layman's terms. Patient confidentiality is paramount.

20.3 Medical monitor

The role of the medical monitor is to oversee the medical aspects of the clinical trial and to collaborate actively with the investigators to protect the safety of the study participants. This work gives an additional layer of activity safeguarding participants' safety in addition to the more programmatic review by the DSMB. The medical monitor works in conjunction with the coordinating PI and actively collaborates with the study site.

20.4 Study specific DSMB

This study specific DSMB is an independent board governed by a specific charter which identifies the frequency of meetings and methodology of reporting. The minutes of the Open Sessions of the DSMB are to be made available to the sponsor.

This DSMB shall be in charge of the regular review (not more than every four months) of the listings of the adverse events collected during the study. The panel shall also receive the notification of serious adverse events, adverse events classified as severe and adverse events of special interest.

20.4.1 Responsibilities of the study specific DSMB

This study specific DSMB is composed of scientific experts in the field of malaria and/or tropical medicine and/or clinical medicine and of one statistician. If required, it shall be possible to consult ad hoc experts in other fields (haematology, cardiology, dermatology, hepatology etc.), who can join committee meetings.

The DSMB shall be involved in the approval of this protocol and its amendments, and be regularly updated on study progress, in particular on the number of inclusions. The DSMB shall receive data and reports issued, as required by the DSMB Charter, and prepared by Investigators, as well as notifications

of serious adverse events. If clear signals of safety concerns are present, the DSMB can recommend halting the inclusion of further patients. The decision to halt the study shall be made after discussion and agreement between the members of the DSMB and the Sponsor.

The DSMB will have meetings or telephone conferences, prior to the start of the study to review the DSMB Charter, and then in accordance with the schedule provided in the Charter over the course of the study. At meetings the DSMB will review adverse events, consider and determine any possible safety signals, and discussion recommendations on the progress of the study. Finally, the DSMB shall meet for the release of the final results.

In between, conference calls can be organized at the request of the Sponsor, of an investigator, or of a member of one of the local monitoring committees.

All the decisions taken by the study specific DSMB shall be documented in writing; they will be transmitted to the Sponsor with the PIs of all the sites on copy and attached to the final study report. Any further local reporting will be in line with local regulations.

20.5 Project manager/Project management team

The project manager is responsible for the day-to-day activities of the study. He/she/they will ensure timely fulfilment of the scheduled tasks for the consortium, periodic review/reporting, meetings, correct distribution and usage of funds, assist communication to the funder and arrange application for additional funds should it become necessary.

20.5.1 Project management plan

Annex 6

20.5.2 Financing

This study is being financed by Deutsches Zentrum für Infektionsforschung (DZIF) providing the core funding.

20.6 Responsibilities of the clinical staff of the health facilities

The staff shall be responsible to performing the study in accordance with this protocol and in accordance with the legislation and international guidelines under the direction of the local PI.

They are responsible for obtaining an informed consent prior to inclusion in the study, for completing the study documents (screening registry and patient CRF) and for recording all relevant data in relation to the study. Each staff member shall ensure that the information reported in the document is precise and accurate.

They must inform the patient of all relevant aspects of the study, including the information in the patient information sheet. All this information shall be provided to the patient in layman's terms. Patient confidentiality is paramount.

Prior to study inclusion, the informed consent form shall have to be personally completed (first name, surname), dated and signed by the patient, the patient's parent(s) or a guardian authorised representative. The person who has conveyed the information on the study to the patient shall also

sign and date the informed consent form approved by the Ethics Committee. The informed consent forms will be translated into local languages for the benefit of those who do not understand English or French.

In case of patients unable to read and sign the patient information sheet and informed consent form, these documents will be read and explained to the patient in local language in the presence of a witness. The patient or the parent(s)/guardian in case of children below 18 years old, shall put her/his fingerprint on the informed consent form and the witness shall also sign the consent form to confirm that the patient has consented willingly.

A copy of the information sheet and the signed consent form shall be handed over to the patient or the parent/guardian.

21 Ethical and regulatory aspects

21.1 Regulations

The study shall be conducted in compliance with the text of the Declaration of Helsinki adopted by the 18th World Medical Association Assembly in 1964, and with its amendments (Annex 1).
The study will seek approval from the local institutional review boards / ethics committees (IRBs/ECs). This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any), the informed consent form, applicable recruiting materials and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents and approved versions being approved. This study shall be conducted in accordance with the principles of the Good Clinical Practices.

The study shall be conducted in compliance with the international and national laws and regulations in effect, and in accordance with the applicable directives in Germany, Gabon and Ghana in particular concerning the submission to the Ethics Committee and the protection of personal data.

Study related documents (protocol, case report from, informed consent form) shall be submitted to the National Ethics Committees or to the Institutions from the participating countries. Upon signature of the protocol, the Investigator accepts to respect the instructions and procedures described in the protocol, as well as the Good Clinical Practices and Good Laboratory Practices, to which he/she conforms.
The Investigator shall obtain from the patient or his/her legal representative, a signed (fingerprint and signature from a witness for patients unable to read and write), written consent. Assent will be obtained for minors able to understand the study procedures, according to the regulations in each of the participating countries. If informed consent is not obtained, the patient will not be enrolled. Furthermore, patients enrolled shall be entirely taken charge of for the treatment of their malaria for the duration of the study.

21.2 Informed consent

The investigator is responsible for ensuring that informed consent is obtained from each patient or legal representative and for obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study medication.

The Investigator shall explain to each study participant or his/her legal representative the nature of the study, its objective, the procedures involved, its risks and potential benefits and any discomfort it may generate. The patient must be informed that his/her participation is entirely voluntary, that he/she can withdraw from the study at any time and that withdrawal will not affect his/her subsequent medical treatment nor his relationship with the treating physician. The patient or his/her legal representative will sign on the informed consent sheet after having read and voluntarily agreed to it. A translation in local language for subjects unable to read and understand it will be available. Where the patient or his legal tutor are unable to read, in this case, an impartial witness should be present during the entire informed consent discussion. After inclusion, the patient may elect to withdraw from the study when he/she so wishes. The same level of attention will be dispensed to the patient.

21.3 Data protection and confidentiality

The personal data of the patients which could be included in a Sponsor database or study database shall be treated in accordance with all local laws and regulations.
The investigator and the concerned personnel of the health centres and the study sites must keep all study documentation confidential and must take all necessary measures to prevent accidental or premature destruction of these documents.
The regulations or national laws in force on patient record keeping shall be applied.
At the time of archiving or management of the personal data pertaining to the nursing staff and/or patients, the Sponsor shall take all appropriate measures to secure and protect these data against access by a third non-authorised person.
A data entry station will be localized at the study site where data captured on electronic devices or paper Source Documents will be shared with the central database located at DTM via REDCap software. Subject identifying data will not be entered in the database. At DTM a protected server will guarantee the confidentiality of the data. All source documents for personal and clinical data will be kept in a secured environment on site.
Unless authorized by the sponsor, external removable data storage devices are not permitted to be used.

21.4 Insurance

The Sponsor certifies to have subscribed for this study under its sponsorship, a participant insurance, which is in agreement with the local laws and recommendations. The Sponsor's insurance shall not dismiss the investigator and his collaborators of their obligation to have their own civil liability insurance in line with the laws in force. Investigators' insurance is covered by the respective study sites.

A copy of the insurance certificate shall be available for provision to investigators and / or ethics committees who would request it.

21.5 Premature termination of the study

The Sponsor can decide at any time and for whatever reason to prematurely suspend or interrupt the study. The decision and the justification shall be communicated in writing to the study specific DSMB.

The local authorities, ethics committees and competent authorities shall have to be informed in line with local legislation.

21.6 Competent authority inspections

The study sites and delegated responsible parties as well as the principal investigators accept to grant direct access to study source dossiers to auditors/inspectors for review, with the understanding that these people are bound by professional secrecy and shall not disclose any identity or medical information of a personal nature.
They shall undertake any effort in support of the audits and inspections by facilitating the access to equipment, data and necessary documents.
The confidentiality of the verified data and the protection of the patients shall be respected during these inspections.

All results and all information resulting from these inspections by the regulatory authorities shall be immediately communicated to the Sponsor.

The study sites and delegated responsible parties as well as the principal investigators shall take the appropriate measures in order to lead the corrective actions to all problems identified during the audits or inspections.

22 Protocol amendments

Each change to the protocol will be reported in a written amendment which will be signed by both the Principal Investigators and the Sponsor. The signed amendment will be added to the protocol.

Following the national legislation, the protocol amendment may require a regulatory submission (for example to the Ethics Committee) before implementation. Sometimes, an amendment may result in changes to the informed consent form. The Sponsor/Principal Investigators must receive an approval/favourable opinion from the Ethics Committee on the revised informed consent form before use.

23 Documentation and utilization of study results

23.1 Properties and use of the study data and results

All results, data, documents and inventions obtained, directly or indirectly, from the trial, will be owned by the Sponsor unless a law or local regulation states otherwise. The Sponsor can use or exploit all results for their own use without any limitation of its industrial property (territory, area, duration) in consultation with the study centres. The full data base will be the property of the sponsor and will be utilized for producing the final study report. Data will be made available in electronic form for the site PI after production of the final study report.

23.2 Publications

A scientific committee will be formed, comprising the Sponsor, Funder and Coordinating Principal Investigator and others as agreed by these parties, with the responsibility for the presentations and/or publications of the results. The results of the study will be submitted to the Committee before each publication. Each subsequent presentation or publication should be approved by the scientific board.

The final decision on the publication of a manuscript/summary/presentation will be taken by the scientific committee after notification of the Sponsor in order to allow for an internal review and the possibility of providing comments. Each manuscript, summary, presentation will be submitted to the co-authors for internal review and possible comments at least 45 days before the submission to the journal and at least 20 days before the submission of the summary. The Sponsor may request that their name and/or the name of one of their employees is present or not present on the publication. The Sponsor may delay each publication or communication during a limited time frame in order to protect the confidentiality or the proprietary information present in the document.

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25 Annexes

Annex 1: Declaration of Helsinki
Annex 2: Definition of Severe Malaria
Annex 3: Guidance for gradation of clinical symptoms
Annex 4: Visit and Sampling schedule
Annex 5: Drug summary
Annex 6: Project management plan

26 Annex 1: Declaration of Helsinki

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations

of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote

the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

27 Annex 2: WHO definitions for severe malaria

After: WHO. Tropical Medicine and International Health is published by John Wiley & Sons., 2014. 19 (Suppl. 1), 7–131

Tropical Medicine and International Health VOLUME 19 SUPPL 1 PP 7–131 SEPTEMBER 2014

Table 3 Outline bedside clinical classification of severe malaria in adults

Group 1	Adults at increased risk of dying immediately who require parenteral antimalarials and appropriate supportive therapy Prostrated or obtunded adults (prostration is the inability to sit or to drink). Four subgroups of increasing severity should be distinguished: Prostrate but fully conscious Prostrate with impaired consciousness but not in deep coma (GCS > 11) Confusion and agitation (GCS > 11) Coma (the inability to localise a painful stimulus) (GCS < 11) Respiratory distress (acidotic breathing) Mild – sustained nasal flaring and/or mild intercostal indrawing (recession) Severe – the presence of either marked indrawing (recession) of the bony structure of the lower chest wall or deep (acidotic) breathing Shock (hypotension:systolic BP < 80 mmHg) Anuria Significant upper gastrointestinal haemorrhage
Group 2	Adults who, although able to be treated with oral ACTs, require supervised management because of the risk of clinical deterioration but who show none of the features of group 1 (above)*. This group includes adults with any of the following: Haemoglobin <7 g/dl or haematocrit <20% One or more convulsions within a 24-h period Haemoglobinuria (blackwater) Jaundice
Group 3	Adults who require parenteral treatment because of persistent vomiting but who lack any specific clinical or laboratory features of groups 1 or 2 (above)

*If parasite counts are immediately available a parasitaemia over 4% should be included in group 2.

28 Annex 3: Grades of Adverse Events

The CTCAE system is a product of the US National Cancer Institute (NCI). The current version 5.0 was released on November 27, 2017.
The NCI Common Terminology Criteria for Adverse Events is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

CTCAE Terms
An Adverse Event (AE) is any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each CTCAE v4.0 term is a MedDRA LLT (Lowest Level Term). For this study local laboratory ranges will be used to define an abnormal laboratory finding.

CTCAE: Common Terminology Criteria for Adverse Events

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1
Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2
Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL*.

Grade 3
Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated, disabling; limiting self-care ADL**.

Grade 4
Life-threatening consequences; urgent intervention indicated.

Grade 5
Death related to AE.

ADL = Activities of Daily Living

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

29 Annex 4: Visit and sampling schedule

Day[a]		0									1		2[b]		3	7	14	21	28	35	42	Unscheduled[c]
	Screen	0	15 m	30 m	45 m	90 m	3 h	5 h	8 h	12 h	24 h	36 h	48 h	60 h	72 h							
Informed consent	x																					
Inclusion/exclusion criteria	x																					
Physical exam, Malaria signs & symptoms	x									x	x	x	x	x	x	x	x	x	x	x	x	x
Demography, medical history	x																					
Prior and concomitant medication	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Pregnancy test[d]	x																					x
Clinical laboratory safety[e]	x												x		x	x	x		x			
Asexual & gametocyte parasite count (thick and thin blood films) ≥35 kg[f]	x[g]								x	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood spot for parasite genotyping (≥35 kg)[h]	x								x	x	x	x	x	x	x	x	x	x	x	x	x	x
12-Lead ECG (single)[i]		x								x[i]	x	x[i]	x	x[i]	x	x						x
Vital signs (single)	x	x								x	x	x[i]	x	x[i]	x	x	x	x	x		x	x

[a] At each patient contact post dose, circumstances for established anti-malarial treatment will be assessed

[b] Discharge at 48 or 72 hours (or up to Day 7) depending on age, parasite and fever clearance, clinical judgment and patient convenience. If discharged prior to Day 7, will return to Clinical Unit for further assessment on all scheduled times up to Day 7

[c] Unscheduled visits should be completed if a patient is readmitted at any time during the study with elevated temperature (> 37.5 ^{o}C)/feels unwell. Assessments to measure parasitaemia should be taken as scheduled and at the Investigator's judgement until parasite clearance or rescue medication is given (if required). Safety assessments should be performed at the Investigator's judgement.

[d] The patient's menstrual and contraceptive history will be taken, and a test for the presence of HCG will be performed at Screening, to exclude pregnancy. Result must be confirmed negative prior to dosing.

[e] Laboratory safety: haematology, including haemolysis, clinical chemistry, and urinalysis. **For children ≤5 years**: Screening (baseline) and Day 2, 7, 14 and 28. D3 should be done if results are abnormal at D2, and D5 should be done if results are abnormal on D3. Local laboratory ranges will be used to define an abnormal laboratory finding.

[f] Blood films (thick and thin) and temperature measurements need to be confirmed as follows: when 1st parasite clearance and 1st temperature <37.5 °C, measurements need to be confirmed with second reading 6 to 12 hours after the 1st measurement. The first measurement (if confirmed) will be considered the 'Clearance Time'. **Patients <35kg**: samples for blood films will be taken at **Screening/pre-dose, 8, 24, 36, 48 and 72 hours only**; **thereafter the same as adult schedule**. Additional temperature recordings and blood films may therefore be taken in order to confirm fever and parasite clearance and reported using the unscheduled visit form. In case of available venous blood from other (e.g. PK) samplings, venous blood shall be used for preparation of smears for higher patient convenience.

[g] Measurement required within 4 hours prior to dosing

[h] All blood spot samples will be taken at the same time points as the blood films for patients >35kg and patients <35kg.

[i] Patients should rest supine prior to measurement for a minimum of 10 minutes. Vital signs and single ECG to be taken before each dosing (**AP and APAP**: H0, H24, H48; **AFC**: H0, H12, H24, H36, H48, H60) and at other time points marked in the 'visit and sampling schedule'.

Temperature (single)[j]	x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PK sampling (≥35 kg)[k]		x	x	x	x	x	x	x	x		x		x			x	x[L]	x[L]	x[L]	x[L]	x[L]	x
Dosing (see table 2)		x								(x)	x	(x)	x	(x)								
AEs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Table 6 PK sampling for participants < 35 kg, number of samples to be taken per sampling time point

Patient weight	PK samples necessary for AP, APAP and AFC	Additional PK samples necessary for AP and APAP[L]
>5 to < 10 kg	0h, 1h, 8h, 24h, 48h, Day 7	Day 14, Day 42
>10 to <20 kg	0h, 1h, 8h, 24h, 48h, Day 7	Day 14, Day 28, Day 42
>20 to < 35 kg	0h, 1h, 5h, 8h, 24h, 48h, Day 7	Day 14, Day 28, Day 35, Day 42

AP... Artesunate/pyronaridin
APAP ... Artesunate/pyronaridin/atovaquone-proguanil
AFC ... Artesunate/fosmidomycin/clindamycin

Table 7 Dosing

Dosing	Food/Milk	Baseline	12h	24h	36h	48h	60h
AP	independent	x		x		x	
APAP	with	x		x		x	
AFC	independent	x	x	x	x	x	x

AP... Artesunate/pyronaridin
APAP ... Artesunate/pyronaridin/atovaquone-proguanil
AFC ... Artesunate/fosmidomycin/clindamycin

[j] Axillary temperature should be recorded. If the axillary method is not possible, an alternative route (oral, tympanic, rectal) may be used. Within an individual patient the same method of temperature measure should be used throughout the study

[k] PK samples should be taken by venepuncture. Where required, PK sampling should be performed after ECG, vital signs and temperature measurement to ensure that physiological measurements are not taken within 10 minutes of venepuncture or finger-prick. PK sample should be taken at the nominal time whenever possible, but actual time should be recorded on a minute basis. PK sampling schedule applies to patients > 35 kg who receive AP or APAP. **For patients <35kg or patients receiving AFC, see Table 1**. In addition, for all patients where possible, a PK sample should be obtained when recrudescence / re-infection occurs. AFC regimen analyses require 2ml plasma from a heparinized blood collection tube per sampling time point and AP & APAP regime analyses require both 2ml plasma each from a heparinized blood collection tube and from an EDTA-coated blood tube per sampling time point.

30 Annex 5: Drug summary

	Artesunate (AS)/Pyronaridin = Pyramax	Atovaquone/Proguanil	Clindamycin	Fosmidomycin	Artesunate (mono)
Efficacy, Africa	Excellent (> 95% in WHO therapeutic efficacy studies)		Study in Gabon: PCR-corrected cure rate over 90% (n=88) in children aged 3 to 14 years in response to the co-administration of **fosmidomycin and clindamycin** orally in doses of 30mg/kg and 10mg/kg respectively twice daily for three days	Studies of fosmidomycin in the treatment of acute uncomplicated P falciparum malaria have revealed unacceptably high rates of recrudescence precluding its use as monotherapy. The overall efficacy was considered to be too low to justify the continued development of fosmidomycin as monotherapy. Moreover to have done so would have contradicted the recommendations of WHO that only combination therapy should be used orally. Study in Gabon: PCR-corrected cure rate over 90% (n=88) in children aged 3 to 14 years in response to the co-administration of **fosmidomycin and clindamycin** orally in doses of 30mg/kg and 10mg/kg respectively twice daily for three days Fosmidomycin and piperaquine together fulfil the WHO criteria for combination	

				therapy by meeting the three key parameters of different modes of action, different biochemical targets and independent blood schizonticidal activity.	
Drug resistance, Africa	No resistance to artemisinin derivatives robustly documented yet	No resistance to artemisinin derivatives robustly documented yet .Is effective against drug sensitive and drug resistant .P. falciparum is especially recommended for prophylaxis and treatment of P. falciparum malaria	No resistance to artemisinin derivatives robustly documented yet .Resistance to clindamycin usually occurs via macrolide-lincosamide-streptogramin B (MLSB) type of resistance, which may be constitutive or inducible. .Clostridia spp. .Enterococci .Enterobacteriacea e	No resistance to artemisinin derivatives robustly documented yet	

Mode of action	Pleiotropic alkylation of parasite proteins		Clindamycin, a lincosamide, is a semi-synthetic derivative of lincomycin that was introduced as an antibacterial agent. It is still widely used for the treatment of infections due to Gram-positive and anaerobic bacteria. It inhibits protein synthesis by attaching to the 50S subunit of the bacterial ribosome. It has been demonstrated that clindamycin targets the prokaryote-like ribosomes of the apicoplast and by this means inhibits self-replication of the organelle. As a consequence of this mechanism, clindamycin displays a typical delayed kill kinetic effect, the growth of the parasites being unaffected until the second cycle of replication after drug exposure. Under such conditions, the in vitro growth of P. falciparum is inhibited with an IC50 and IC90 of approximately 25 and 50 nM respectively. Clindamycin is a semi-synthetic antibiotic prepared from lincomycin, which is produced by Streptomyces	Fosfomycin is indicated for the treatment of acute uncomplicated lower urinary tract infections in adults, caused by pathogens sensitive to fosfomycin. Fosfomycin trometamol is a broad spectrum antibiotic, derived from phosphonic acid. It inhibits the enzyme phosphoenolpyruvate transferase, which catalyses the formation of n-acetylmuramic acid from n-acetyl aminoglucose and phosphoenolpyruvate. N-acetylmuramic acid is required for the build-up of peptidoglycan, an essential component of the bacterial cell wall. Fosfomycin has a mainly bactericidal action. Fosfomycin is indicated for periprocedural prophylaxis in diagnostic and surgical transurethral procedures. .Fosmidomycin is an inhibitor of DOXP reductoisomerase, the second enzyme in the reaction cascade of the DOXP pathway for isoprenoid	

			lincolnensis var. lincolnensis.	biosynthesis. Recombinant DOXP reductoisomerase from P. falciparum is inhibited by fosmidomycin with an IC50 of 28 nM. The growth of wild-type and multi-resistant strains of P. falciparum are inhibited in vitro with IC50s ranging from 300 to 1200 nM. .It blocks the non-mevalonate pathway of isoprenoid biosynthesis on which mammalian metabolism is not dependent .It was originally isolated as a natural antibiotic from Streptomyces lavendulae. It is now chemically synthesised.	
Activity against	ABS + early gametocytes	Activity against hepatic schizonts of Plasmodium falciparum	Active against different apicoplexan parasites including P. falciparum.	Activity against several pathogenic gram-positive and gram-negative bacteria, including the genera Bacillus, Sarcina, Escherichia, Proteus, Salmonella, Pseudomonas, Shigella and Enterobacter. There is no significant activity against bacteria, such as Staphylococcus aureus, that depend on the mevalonate pathway for isoprenoid biosynthesis.	

				It is active against existing drug-resistant parasites, including artemisinin, piperaquine and multidrug resistant strains	
Duration of post-treatment prophylaxis	3-4 weeks				
Pediatric formulation	Yes (possibility to dissolve tablets in water)	Not sure, film-coated tablet	.No, hard capsules. Size "0" -> alternative formulation	Yes, intravenously and intramuscular injection possible. Otherwise capsules	
Administration	Should not be taken with a high fat meal	.Oral .Should be taken with food or a milky drink at the same time each day .If patients are unable to tolerate food, Atovaquone/Proguanil Hydrochloride should be administered, but systemic exposure of atovaquone will be reduced .In the event of vomiting within 1 hour of dosing a repeat dose should be taken	.Oral .Should always be swallowed whole with a full glass of water .Not appreciably modified by the presence of food. .Intramuscular .Intravenous .Vaginal	.Oral/e.x. during urinary tract infection: should be taken on an empty stomach, either 1 hour before or at least 2 hours after meals and preferably before bedtime after emptying the bladder. The contents of a sachet should be dissolved in a glass of water and taken immediately after its preparation. .Intravenous .Intramuscular	
Hepatic metabolisation	Artesunate is rapidly metabolized to DHA by plasma esterases 3. A small amount may undergo metabolism by CYP2A6. DHA is further metabolized by glucuronidation in the liver. α-dihydroartemisinin-β-glucuronide has been identified as a mjor metabolite in the urine	Some evidence suggests limited metabolism (although no metabolites have been identified) (URL:https://www.drugbank.ca/drugs/DB01117)	Yes	No?! Piperaquine is mainly excreted in the feces with a negligible amount in the urine	

	(URL: https://www.drugbank.ca/drugs/DB09274)				
Enzymes	Esterase, CYP2A6	CYP2C19	Lincosamides		
Active metabolite	DHA				
Pharmacokinetics, T_{max}	0.25h (AS), 0.75h (DHA)			2 (1-4) hr monotherapy 2 (1-4) hr + Clindamycin	
Elimination half-life	AS, DHA: 0.5h, 1.5h	The elimination half life of atovaquone is about 2-3 days in adults and 1-2 days in children. The elimination half lives of proguanil and cycloguanil are about 12-15 hours in both adults and children.		Is fast acting with a 0-48 hr Log10 parasite reduction ratio >3 and parasite clearance times <50hours	
Food effect	If taken with fat meal compared with fasting: C_{max} (DHA): 1.9-fold decrease AUC (DHA): 1.06-fold decrease	.4.018 mg lactose monohydrate	.67.82 mg anhydrous lactose .Absorption of Clindamycin is not appreciably modified by the presence of food.		

Drug-drug interactions	.HIV & TB drugs ; CYP2A6 & CYP2C8 inhibitors .The metabolism of Artesunate can be decreased when combined with Atovaquone. .The metabolism of Clindamycin can be decreased when combined with Artesunate. .The metabolism of Piperaquine can be decreased when combined with Artesunate.	.Oral anticoagulants .Concomitant administration of rifampicin or rifabutin (reduces plasma concentrations of atovaquone levels by approximately 50% and 34%). .Concomitant treatment with metoclopramide has been associated with a significant decrease (about 50%) in plasma concentrations .Efavirenz or boosted protease-inhibitors, atovaquone concentrations have been observed to decrease as much as 75% .Concomitant treatment with tetracycline .The metabolism of Artesunate can be decreased when combined with Atovaquone. .The metabolism of Clindamycin can be decreased when combined with Atovaquone. .The metabolism of Piperaquine can be decreased when combined with Atovaquone.	.Neuromuscular blocking properties: CAVE-> other neuromuscular agents .Erythromycin antagonism .Vitamin K antagonists .The metabolism of Clindamycin can be decreased when combined with Piperaquine. .The metabolism of Clindamycin can be decreased when combined with Atovaquone. .The metabolism of Clindamycin can be decreased when combined with Artesunate.	.Concomitant administration of metoclopramide has been shown to lower serum and urinary concentrations and should be avoided. .No cross-resistance with other antimalarials .The combination of fosmidomycin and piperaquine had an additive effect with FIC values for both the IC50 and the IC90 in the range of 1.1 to 1.5. There was no significant difference in the efficacy between the CQ sensitive and the CQ resistant strains.	

Contraindications	Retinopathy; liver injury & haemato. pb with AQ	.Lactose intolerance .Anorexia(<40 kg) .Severe hepatic impairmen .Severe renal impairment (creatine clearance <30 mL/min) .Hypersensitivity to the active substances or to any of the excipients .Acute lymphoblastic leukaemia .While nursing .Stomatitic .Cardiac disorders	.Lactose intolerance .Severe renal impairment or anuria .Severe hepatic impairment .Existing diarrhoea .History of gastro-intestinal disease (colitis) .Pregnancy/while nursing	.Severe renal insufficiency (CLcr<10ml/min) .Undergoing haemodialysis .This medicine contains 1,923 g of sucrose per sachet. Patients with rare hereditary problems of fructose intolerance, glucose - galactose malabsorption or sucrase-isomaltase insufficiency should not take this medicine .While nursing	
Dosage	4 mg / kg bw	250mg/100mg	300mg (325.78 mg clindamycin hydrochloride equivalent to 300 mg clindamycin)	.30mg/kg (one 3g sachet during urinary tract infection >12 years) Capsules in three sizes of 450mg, 225mg and 75mg containing 504mg, 252mg and 84mg of fosmidomycin monosodium salt respectively	
Intake timing	Once per day, x3 days	<u>Prophylaxis:</u> .Commence 24 or 48 hours prior to entering a malaria-endemic area, .Continue during the period of the stay, .Continue for 7 days after leaving the area. .Up to 12 weeks, average 27 days		.Twice a day for three days (?) .Study Fuji/81/2: orally 500mg three times a day for five days .Study FR 31564: 1g-2g intravenously every 8 hours for 6-14 days .Study CLR 840018: 2g by intramuscular injection 12 hourly for five days	

Formulations (pediatric 1, p1)	AS / AQ: 25mg / 67.5mg ³4.5kg to <9kg 1 tablet of p1/per day ³9kg to <18kg 1 tablet of p2/per day	11-20 kg 1* 250mg/100mg daily for three consecutive days	3 - 6 mg/kg every six hours depending on the severity of the infection.	Children aged six months to three years 30mg/kg 10mg/kg x2 daily for three days (?)	
(pediatric 2, p2)	50mg / 135mg ³18kg to <30kg 1 tablet per day ³30kg to <36kg 1 tablet per day	21-30 kg 2* 250mg/100mg as a single dose for three consecutive days 31-40 kg 3* 250mg/100mg as a single dose for three consecutive day	3 - 6 mg/kg every six hours depending on the severity of the infection.	Children aged 3-7 years 30mg/kg 5mg/kg x2 daily for 3 days (?) Children aged 7-12 years 30mg/kg 5mg/kg x2 daily for 5 days (?)	
(adult, a)	100mg / 270mg ≥ 36kg 2 tablets per day	> 40 kg 4* 250mg/100mg as a single dose for three consecutive day	<u>adult & elderly:</u> Moderately severe infection: 150 - 300 mg every six hours Severe infection: 1200 - 1800 mg daily in divided doses given every six to eight hours	1200mg x 3 daily for seven days (?)	

Literature:
Atovaquone/Proguanil: SmPC; https://www.medicines.org.uk/emc/product/638

Clindamycin: SmPC; https://www.medicines.org.uk/emc/product/7337

Artesunate/Pyronaridin: SmPC; https://www.ema.europa.eu/en/documents/medicine-outside-eu/pyramax-product-information_en.pdf

Fosmidomycin: Investigator brochure (IB)

URL: www.drugbank.ca 2019/09/16

31 Annex 6: Project management plan