CLINICAL STUDY PROTOCOL

Sponsor's Reference Number: APM/18/1702001

Richmond Pharmacology Study Number: C18034

EudraCT Number: 2018-004968-60

TITLE:	A randomized, double-blind, placebo controlled parallel group study in heathy adult subjects to determine the tolerability and safety of atovaquone-proguanil (ATV-PG) co-administered with amodiaquine (AQ)
PHASE:	Phase 1
DRUG:	Atovaquone-proguanil (ATV-PG) and Amodiaquine (AQ)
SPONSOR:	Medicines for Malaria Venture
PRINCIPAL INVESTIGATOR:	Dr Ulrike Lorch, MD FRCA FFPM
STUDY CENTRE:	Richmond Pharmacology Ltd. St George's University of London Cranmer Terrace, Tooting London SW17 0RE, UK Telephone: +44 (0)20 7042 5800 Fax: +44 (0)20 7378 9135 Richmond Pharmacology Ltd. 1a Newcomen Street, London Bridge, London SE1 1YR, UK
Protocol Version and Date:	Version 6.0 29 July 2019

TABLE OF CONTENTS

List of Tables

List of Figures

PROTOCOL APPROVAL SIGNATURES

Version 6.0, dated 29 Jul 2019

Sponsor's Approval

This protocol has been approved by MMV.

Sponsor's Signatory:

Name: **Fiona Macintyre, PhD**

Senior Director, Clinical Sciences, Product Development

Signature: _____ Date: 2.q Io -;- /?-01q,

Name: **Stephan [illegible], MD, PhD**

VP, Experimental Medicine & Clinical

Signature: _____ **Date:** 2,q Vl vt 1AJC,

Name: **Andre-Marie Tchouatieu, MD**

Associate Director, Access & Product Management

Signature: -BS _____ **Date:** 21" /'\f-/ 2.01:[·

INVESTIGATOR'S AGREEMENT

I have read this MMV Protocol No. APM/18/1702001:

A randomized, double-blind, placebo controlled parallel group study in heathy adult subjects to determine the tolerability and safety of atovaquone-proguanil (ATV-PG) co-administered with amodiaquine (AQ)

I have fully discussed the objectives of this trial and the contents of this protocol with MMV representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical/regulatory review of the trial, without written authorisation from **MMV.** It is, however, permissible to provide information to a subject to obtain consent.

I agree to conduct this trial according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with ICH guidelines on GCP and with the applicable regulatory requirements .

I understand that MMV may decide to suspend or prematurely terminate the trial at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial I will communicate my intention immediately in writing to MMV.

Principal Investigator:

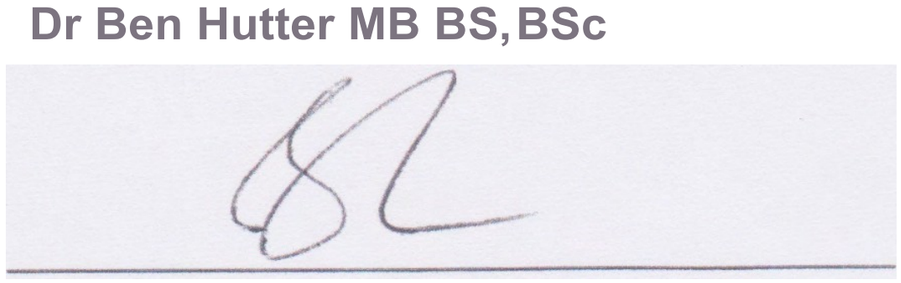
Dr Ulrike Lorch, MD FRCA FFPM

Signature: _____ **Date:** 29 Jul 2019

MEDICAL WRITERS

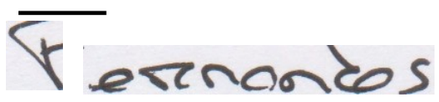
This protocol was written by RPL writers:

Name: **Dr Ben Hutter MB BS, BSc**

Signature: 

Date: 29 JUL 2019

Name: **Sara Fernandes, PhD**

Signature: ..... ::t.c 

Date: 29 JUL 2019

STUDY PERSONNEL

Sponsor's Representative	Dr Jörg Täubel MD FFPM Richmond Pharmacology Ltd.
Sponsor's Main contact	Heike Huegel- Koerpert Phone: +41 789 48 18 58, +43 699 12 77 1008 e-mail: huegelh-consultants@mmv.org
Sponsor's Medical Director	Stephan Chalon, MD, PhD Phone: +41 22 555 0379 e-mail: chalons@mmv.org
Sponsor's Chief Medical Officer	Stephan Duparc, MD Phone: +41 22 555 0351 e-mail: duparcs@mmv.org
Sponsor's Study Director	Fiona Macintyre, PhD Phone: +41 22 555 0319 e-mail: macintyref@mmv.org
Sponsor's Project Director	André-Marie Tchouatieu, MD Phone: +41 22 555 0392 e-mail : tchouatieua@mmv.org
Sponsor site	Medicines for Malaria Venture (MMV) ICC – Block G, 3rd floor 20, Route de Pré-Bois PO Box 1826 1215 Geneva 15 Switzerland
Principal Investigator	Dr Ulrike Lorch MD FRCA FFPM Phone: +44 (0)20 70425800 e-mail: u.lorch@richmondpharmacology.com
Co-investigators	Dr Jörg Täubel MD FFPM Dr Ben Hutter MB BS, BSc
Study Site	Richmond Pharmacology Ltd. 1a Newcomen Street, London Bridge, London SE1 1YR, UK
Clinical Laboratory	The Doctor's Laboratory 60 Whitfield Street London W1T 4EU, UK
Bioanalytical Laboratory (PK analysis)	Adnan Mohamed Abdi Swiss BioQuant AG Kägenstrasse 18 4153 Reinach

	Switzerland Phone: +41 61 716 9823 e-mail: adnan.abdi@swissbioquant.com
Data Management	Richmond Pharmacology Ltd.
Statistics	Richmond Pharmacology Ltd.
Qualified Person	Kastur Patel
Pharmacovigilance	Andreja Baricevic PrimeVigilance The Surrey Research Park, 1 Occam Court, Occam Road, Guildford, Surrey, GU2 7HJ, United Kingdom e-mail: andreja.baricevic@primevigilance.com Phone: +385 (1) 20 78 810
Medical Monitoring	Michael Marx ICON Clinical Research Heinrich-Hertz Str. 26 63225 Langen Germany Phone: +49 6103 904 1950 Michael.Marx@iconplc.com
Medical Writing	Dr Ben Hutter MB BS, BSc Sara Fernandes, PhD

LIST OF ABBREVIATIONS

Abbreviation	Explanation
ABPI	Association of British Pharmaceutical Industry
ACT	Artemisinin-based combination therapy
ADL	Activities of Daily Living
AE	Adverse event(s)
AESI	Adverse events of special interest
AR	Adverse Reaction
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AQ	Amodiaquine
AST	Aspartate transaminase
ASAQ	Artesunate-amodiaquine
ATV	Atovaquone
ATV-PG	Atovaquone-proguanil
AUC	Area under the curve
BMI	Body Mass Index
CG	Cycloguanil
C_{max}	Maximum plasma concentration
CRF	Case Report Form
CV	Coefficient of variation
DB	Database
DCF	Data Clarification Form
DEAQ	Desethyl-amodiaquine
DHA-PQP	Dihydroartemisinin-piperaquine
DHP	Data Handling Protocol
DSUR	Development Safety Update Reports
EPS	Extrapyramidal symptoms

GCP	Good Clinical Practice
GI	Gastrointestinal
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product(s)
LFT	Liver Function Tests
LLOQ	Lower Limit of Quantification
LOQ	Limit of Quantification
QC	Quality Control
MDA	Mass Drug Administration
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
MMV	Medicines for Malaria Venture
PE	Protective efficacy
PG	Proguanil
PK	Pharmacokinetic(s)
POMS	Profile of Mood scale
PT	Preferred term
RPL	Richmond Pharmacology Ltd
SAE	Serious Adverse Event(s)
SAP	Statistical Analysis Plan
SD	Standard Deviation
SMC	Seasonal Malaria Chemoprevention
SOC	System Organ Class
SOM	Standard Operations Manual
SOP	Standard Operating Procedure
SP	Sulfadoxine-pyrimethamine
SmPC	Summary of Product Characteristics
SRC	Safety Review Committee

SUSAR	Suspected Unexpected Serious Adverse Reaction
T_{max}	Time at which the plasma concentration maximum occurs
TEAE	Treatment-emergent adverse event
UNL	Upper limit of normal
UK	United Kingdom
WHO	World Health Organization
WHOPAR	WHO Public Assessment Report

[For the purposes of this protocol, 'investigator' refers to the Principal Investigator or their delegate].

STUDY SYNOPSIS

Protocol Ref. APM/18/1702001 **Study drug:** Atovaquone-proguanil (ATV-PG), Amodiaquine (AQ)

Title of the study: A randomized, double-blind, placebo controlled parallel group study in heathy adult subjects to determine the tolerability and safety of atovaquone-proguanil (ATV-PG) co-administered with amodiaquine (AQ)

Principal Investigator: Dr Ulrike Lorch, MD FRCA FFPM

Study centre:

Richmond Pharmacology Ltd.

Registered address: St George's University of London, Cranmer Terrace, Tooting, London, SW17 0RE, UK

Clinical site: London Bridge, 1A Newcomen Street, SE1 1YR, UK

Study Parts: N/A **Clinical phase:** 1 (The study does not have therapeutic or prophylactic intent and does not plan to assess efficacy)

Objectives:

Primary

- To assess the safety and tolerability of the approved curative dose of ATV-PG (once daily for 3 days) and the adult equivalent of the approved Seasonal Malaria Chemoprevention (SMC) dose of AQ (once daily for 3 days) when administered alone and in combination, in comparison with placebo.

Secondary

- To determine the pharmacokinetics (PK) of atovaquone (ATV), proguanil (PG) and cycloguanil (CG), and amodiaquine (AQ) and desethyl-amodiaquine (DEAQ) following administration of ATV-PG and AQ alone and in combination.
- To determine the relationship between AQ and DEAQ and ECG parameters and to evaluate any impact of the combination with ATV-PG on this relationship.

Endpoints:

Primary

- Safety and tolerability as measured by the incidence of treatment-emergent adverse events (TEAEs) including the clinical signs, nausea, vomiting and diarrhoea, proportion of subjects with clinically relevant changes in laboratory safety tests (haematology, chemistry (in particular ALT, AST and bilirubin increases) and urinalysis), proportion of subjects with morphological and/or rhythm abnormalities on electrocardiogram (ECG), proportion of subjects with clinically significant changes in ECG time intervals (PR, QRS, QT and QTc intervals) and proportion of subjects with clinically significant changes in vital signs (systolic blood pressure, diastolic blood pressure and pulse rate).

Secondary

- PK parameters derived by non-compartmental methods including maximum observed plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from time zero to last detectable plasma concentration (AUC_{0-t}), area under the plasma concentration-time curve from time zero extrapolated to infinite AUC_{0-inf}, terminal elimination rate constant (λz), terminal elimination half-life ($t_{1/2}$).
- Baseline corrected QTc (ΔQTc) as a function of the concentrations of AQ and DEAQ when administered alone, and when administered in combination with ATV-PG.

Study Design:

The study is a randomized, double-blind, placebo controlled, parallel group study to determine the tolerability and safety of ATV-PG + AQ, ATV-PG + AQ placebo, ATV-PG placebo + AQ, and ATV-PG placebo + AQ placebo administered once daily for 3 days to healthy adult male and female subjects.

Fifty-two subjects will be enrolled and randomized to one of the four treatments in a ratio of 4:3:3:3 as described below:

- Treatment 1 (n=16) - ATV-PG 1000-400 mg + AQ 612 mg;
- Treatment 2 (n=12) - ATV-PG 1000-400 mg + AQ unmatched placebo;
- Treatment 3 (n=12) - ATV-PG unmatched placebo + AQ 612 mg;
- Treatment 4 (n=12) - ATV-PG unmatched placebo + AQ unmatched placebo.

Following the completion of dosing of the first 20 randomised subjects, Treatment 1 has been discontinued from further dosing in accordance with the Adverse Reaction (AR) rules in Section 4.4. Up to 24 further subjects will be included in the study, split in three cohorts of up to eight subjects.

The remaining three treatments will be re-randomised in such a way that across the whole study there will be still 12 subjects dosed with Treatment 2, 12 subjects with Treatment 3 and 12 Subjects with treatment 4. This is taking into account both the already dosed subjects with their originally randomised treatments and the newly randomised subjects yet to be included. Furthermore, within each cohort of up to eight subjects, each of the three treatments (2, 3, and 4) needs to be represented with a maximum of four and a minimum of one subject in the same cohort.

Subjects will be screened within 20 days prior to entering the study on Day -1. Each subject will receive verbal and written information followed by signing of the Informed Consent Form (ICF) prior to any screening procedures taking place. Subjects will be admitted to the study unit on Day -1 and will be discharged on Day 4.

All subjects will attend the unit for an outpatient visit on Days 8, 15, 22, 29 and a follow-up visit on Day 36 (+/- 1 day). All the assessments performed during the study are detailed in the study schedule of assessments (Table 1 and Table 2). Study design features as well as number of subjects may be adapted according to the Adaptive Features (Table 3). This study will use a sentinel dosing strategy, for full details see Section 3.3.4.

Number of subjects:

The planned total number of subjects for this study will be 52.

Main criteria for admission:

Black males or females, of sub-Saharan African origin (both parents are black and are of sub-Saharan African origin) will be included if they are aged between 18 and 45 years with a body mass index (BMI) between 18.0-25.0 kg/m^2. Subjects must agree to use effective methods of contraception.

Main exclusion criteria are: Current or recurrent disease (or condition) that could affect the action, absorption or disposition of ATV-PG or AQ, and any other significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, may influence the result of the study, or the subject's ability to participate in the study.

Anticipated test treatment(s) and mode of administration:

Oral doses of ATV-PG (1000-400 mg) and AQ (612 mg) administered once daily on Days 1 to 3 in the fed state (see Section 6.1.2 for details).

Reference treatment(s) and mode of administration:

Oral doses of unmatched placebo for ATV-PG and AQ.

Criteria for evaluation:

Safety analysis

Safety assessments will include standard laboratory safety tests (haematology, coagulation, biochemistry and urinalysis), vital signs (systolic blood pressure [SBP], diastolic blood pressure [DBP], Respiration rate, pulse rate and tympanic temperature), physical examinations, 12-lead ECG (RR, PR, QRS, QT, QTcF intervals and heart rate [HR]), continuous telemetry, and adverse event monitoring.

Pharmacokinetic analysis

The following pharmacokinetic parameters will be calculated from measured plasma concentrations of atovaquone (ATV), proguanil (PG), cyloguanil (CG), amodiaquine (AQ) and desethyl-amodiaquine (DEAQ): maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from time zero to last detectable plasma concentration (AUC_{0-t}), area under the plasma concentration-time curve from time zero extrapolated to infinity (AUC_{0-inf}), terminal elimination rate constant (λz), terminal elimination half-life ($t_{1/2}$).

ECG analysis

Intensive cardiac assessments will be performed using food effects on the ECG to establish assay sensitivity. Analysis of drug related QT/QTc interval changes relative to plasma PK concentrations will be conducted. The ECG utilised for this analysis require adjudication by qualified cardiologists in accordance with principles set out in the ICH E14 guideline and subsequent Q&A documents. All ECG recordings are in triplicate and will be compliant with the correct recording and manual adjudication of ECG in thorough QT/QTc studies.

Statistical Methods:

Statistical analysis of safety parameters

Adverse events (AE), adverse events of special interest (AESIs), serious adverse events (SAEs), vital signs, ECG parameters, Profile of Mood States (POMs) questionnaire and clinical laboratory data will be listed and summarised using descriptive statistics. Basic Neurological Examination data will be listed only.

All AEs will be summarised and listed by using system organ class (SOC) and preferred term (PT) assigned to the event using Medical Dictionary for Regulatory Activities (MedDRA). Furthermore, these events will be summarised by the maximum intensity. The number of subjects who had drug-related AEs will also be summarised. Any AESI, SAEs and/ or AEs that led to withdrawal will be summarised and listed.

Statistical analyses of pharmacokinetic parameters

Non-compartmental analysis will be used for estimation of pharmacokinetic parameters.

For individual plasma concentration data, the actual time of ATV-PG and AQ administration and actual blood sampling time will be used in the derivation of the PK parameters. If there is any doubt in the actual time a sample was taken, then the scheduled time will be used.

AUC values will be calculated using the linear/log trapezoidal method, applying the linear trapezoidal rule up to C_{max} and the log trapezoidal rule for the remainder of the curve. Samples below limit of quantification (LOQ) prior to the first quantifiable concentration will be set to zero. Samples with concentrations below LOQ after the first quantifiable concentration will be set to 'missing' and omitted from the analysis. Other pharmacokinetic parameters will be calculated according to standard equations.

Statistical analyses of pharmacodynamic parameters

Two complementary sets of analyses will be performed: a per timepoint analysis and a concentration-QTc analysis.

For all quantitative ECG parameters, descriptive statistics will be given for the change from baseline. In addition, for QTc, a linear model will be fitted for each timepoint and the difference between each of the active treatments and placebo will be estimated based on this model and two-sided 90 % confidence intervals be given.

This analysis will be based on the change of QTc from baseline and will use baseline QTc and the concentrations of AQ, DEAQ and relevant analytes/metabolites (when AQ administered alone and in combination with ATV-PG) as covariates. Treatment and time will be used as discrete fixed effects. A series of linear models including one or more of the concentrations will be fitted and the most appropriate one will be selected based on the AIC (Akaike Information Criterion) and the size of the fixed treatment effect. A significant treatment effect (based on an F-test) will be considered an indication for model misfit. For the best fitting linear model, predictions of the effect on QTc will be made at the mean concentrations of the moieties involved at the t_{max} for each of these moieties.

1. INTRODUCTION

Seasonal Malaria Chemoprevention (SMC) and Mass Drug Administration (MDA)

In 2017, an estimated 219 million cases of malaria occurred worldwide (95% CI: 203–262 million) (approximately 92% occurring in WHO African Region), leading to 435,000 deaths [1], of which about two thirds were in children under five years of age [1, 2].

As recommended by WHO, Seasonal Malaria Chemoprevention (SMC) is monthly administration of amodiaquine plus sulfadoxine-pyrimethamine (AQ + SP) to children between 3 months and 5 years of age (10 years of age in Senegal) in areas of highly seasonal malaria transmission and is delivered monthly throughout the malaria season for 3 to 4 consecutive months [3]. The AQ-SP product is co-packaged; AQ is administered once-daily for 3 days, and a single dose of SP is administered on the first day of treatment only (along with the AQ) [3]. Deployment of SMC is limited to the sub-Sahel region [3], due to high prevalence of multiple SP resistance markers in southern and eastern Africa [4]. It is believed that areas of southern and eastern Africa have sufficiently high burden of malaria to benefit significantly from the deployment of SMC.

Where deployed, SMC prevents three quarters of all malaria cases, thus significantly reducing morbidity and mortality [5] and the intervention has achieved widespread community acceptance.

The total number of children eligible for SMC in the 12 countries (Burkina Faso, Cameroon, Chad, Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Nigeria, Senegal and Togo), as estimated by the national programmes, is 29.3 million and the number treated in 2017 was estimated to be 15.7 million, leaving a gap of 13.6 million children in eligible areas not yet served by current SMC programmes [1]. Surveys carried out in 2017 show that coverage of four treatments was 67% in Chad, 63% in Guinea, 45% in Nigeria and 88% in Burkina Faso [1].

SMC is viewed as a potential gateway to future deployment in the population as a whole i.e. mass drug administration (MDA), as part of elimination campaigns. The selection of appropriate treatments for SMC must therefore considered ultimate deployment to the wider population, including pregnant women.

MMV carried out a review of the safety, tolerability, mechanism of action and potential for preventative efficacy (based on likely duration of prophylaxis) of currently approved products, with a view to identify a combination of two products that may be suitable for SMC in areas of SP resistance. A combination of the curative dose of atovaquone-proguanil (ATV-PG) and the SMC dose of amodiaquine (AQ) administered over 3 days was considered to have potential for this purpose. The efficacy of both AQ and ATV-PG has been demonstrated in the treatment of uncomplicated malaria in various clinical trials in different regions [6, 7], and the post-dose prophylactic period of the curative dose of ATV-PG [8, 9] and the SMC dose of AQ [7, 10] suggest that the target protective efficacy (PE) of >75% incident rate reduction [3] is likely to be achievable following 3-day treatment of the combination of ATV-PG plus AQ, on a monthly basis for 3 to 4 consecutive months. The combination of two products having distinct mechanisms of action [6, 7] is expected to reduce the rate of development of resistance, and in addition, neither product contains drugs with a positive developmental toxicity signal [6,7].

Since SMC is administered to apparently healthy children (who are either parasite-free or have circulating parasites but are asymptomatic) therefore, treatments must be very well tolerated. Although the tolerability of ATV-PG and AQ administered alone or as part of other combinations is established [6, 7], the tolerability when co-administered has not been explored.

This study in healthy adults is the first step towards establishing the tolerability and safety of the approved doses of ATV-PG and AQ when co-administered. If considered acceptable based on the findings of this study, the tolerability, safety and PE will subsequently be assessed, within the target geographical areas.

Mechanism of Action, Clearance and Pharmacokinetics

Amodiaquine (AQ) is a synthetic 4-aminoquinoline with schizonticidal activity against *Plasmodium* (P.) *falciparum*, *vivax*, *ovale* and *malariae*, resulting in killing of intra-erythrocytic forms. Although the mechanism of action is not fully understood it is accepted that 4-aminoquinoline derivatives penetrate infected red blood cells and prevent the parasite from polymerizing toxic haem to insoluble haemozoin, leading to parasite death. AQ is extensively metabolised to desethyl-amodiaquine (DEAQ), the principal active component, via CYP2C8 [7]. Both AQ and DEAQ inhibit CYP2D6, although no clinically relevant pharmacokinetic interaction has been documented [7].

In healthy subjects following administration of artesunate-amodiaquine (ASAQ), the median t_{max} (range) of AQ and DEAQ respectively was 0.79 (0.48-8) hours and 2 (1.33-8) hours [10]. Following administration to patients, the mean elimination half-life of AQ was 7.9 hours and the terminal elimination half-life of DEAQ was 211 hours [11]. When ASAQ was taken with a high fat meal, the C_{max} and $AUC_{(0-t)}$ of DEAQ increased 18% and 12% respectively, compared to fasting [7].

Malarone® is a fixed-dose combination of atovaquone (ATV) and proguanil (PG) hydrochloride which is active against the erythrocytic and pre-erythrocytic (hepatic) stages of *Plasmodium* species. ATV and PG interfere with 2 different pathways involved in the biosynthesis of pyrimidines required for nucleic acid replication. ATV is a selective inhibitor of parasite mitochondrial electron transport. PG is metabolised to the active metabolite cycloguanil (CG), which inhibits dihydrofolate reductase leading to disruption of deoxythymidylate synthesis [6]. In addition, PG acts synergistically with ATV [12].

ATV is eliminated largely unchanged in the faeces, while 40% to 60% of PG is excreted by the kidneys. PG is metabolised to CG primarily via CYP2C19 [6], and to a lesser extent CYP3A4, however CYP3A4 may play a larger role in conversion of PG to CG in CYP2C19 poor metabolisers [12], and it is noteworthy that ATV is an inhibitor of CYP3A4 [12].

Following a single administration of *Malarone* to healthy subjects, the median T_{max} (range) of ATV, PG and CG respectively was 3.25 (1.75-5.25) hours, 4.25 (3.00-5.50) hours, and 5.25 (4.50-8.25) hours. The median elimination half-life of ATV, PG and CG respectively was 87.2 (37.2-116) hours, 8.03 (4.28-29.5) hours and 10.2 (6.42-17.3) hours [12]. Concomitant treatment with tetracycline, metoclopramide, rifampin/rifampicin and rifabutin is known to reduce ATV plasma concentration [6].

Administration of *Malarone* with dietary fat increases the ATV AUC 2 to 3 times and C_{max} 5 times over fasting; the absolute bioavailability of atovaquone when taken with food was 23%. *Malarone* Tablets should be taken with food or a milky drink [6].

Potential for pharmacokinetic interaction between ATV and PG and between ATV-PG and AQ

There is no overlap in the clearance mechanisms and inhibition profiles of the components and metabolites of the two products which indicates a low potential for pharmacokinetic interaction between ATV-PG and AQ based on *in vitro* data [13]. There was no pharmacokinetic interaction between ATV and PG at the recommended dose [6].

1.1 Rationale for conducting study

Although the tolerability and safety of ATV-PG and AQ alone are well established, additive tolerability signals are possible on co-administration.

Four treatment arms will be used: ATV-PG + AQ placebo, ATV-PG placebo + AQ, ATV-PG placebo + AQ placebo, as well as ATV-PG + AQ. This will facilitate discrimination of the contribution of the treatments to any tolerability signal. In the event that ATV-PG + AQ is not considered sufficiently well tolerated, data on the individual agents will provide an indication of whether ATV-PG or AQ are suitable partner drugs for other combinations.

Thus, this study will evaluate in healthy adults, whether the tolerability and safety profile of once daily administration of ATV-PG + AQ for 3 days supports future use in SMC, which will entail administration on a monthly basis to an apparently healthy population of children of 3 months of age and older, for the duration of the malaria season.

This study will also determine the pharmacokinetics of ATV, PG, CG (active metabolite of PG), AQ and DEAQ (active metabolite of AQ).

Given the ultimate target population, the study will be carried out in healthy black subjects of sub-Saharan African origin (defined as subjects whose parents are both black and are of sub-Saharan African origin).

1.2 Risk-benefit evaluation

1.2.1 Potential Benefits

ATV-PG and AQ will be given to healthy subjects purely for research and development purposes and the subjects receiving the investigational medicinal products (IMPs) will experience no medical benefit.

1.2.2 Potential Risks

1.2.2.1 Amodiaquine (AQ)

For a summary of the tolerability and safety of amodiaquine hydrochloride (153 mg base) manufactured by Guilin, see amodiaquine (as hydrochloride) 153mg dispersible tablets SmPC [22].

The safety and tolerability of AQ was assessed in numerous clinical trials in uncomplicated malaria treatment. Adverse events were classified according to the rate of occurrence, in treated patients as: very common (>1 in 10); common (>1 in 100 but <1 in 10); rare (>1 in 10,000 but <1 in 1000); and very rare (< 1 in 10,000) [7].

Adverse events reported with AQ include: weakness, headache, dizziness, nausea, vomiting, anorexia, abdominal pain, and diarrhoea (very common), itching and itchy rash (common), neuromyopathy (rare) [7]. Very rare but clinically concerning events have been observed in multiple dose/repeated administration settings and include hepatotoxicity, agranulocytosis/neutropenia and irreversible changes of the retina [14]. The incidence of hepatic and haematological adverse events is higher when AQ is combined with other drugs with potential for liver and/or haematological toxicity.

Post marketing, extrapyramidal symptoms have been reported, even after administration of a single dose. These symptoms may include: abnormal movements of the head, tongue or neck (e.g. facial spasms, tongue protrusion, difficulties in speaking) and generalized rigidity [7, 25, 26]. In a study with ASAQ in Côte d'Ivoire, all cases resolved without sequelae after treatment with diazepam. Causality could not be proven since all patients had also taken non-prescribed

traditional medicines; furthermore, one patient was re-challenged with ASAQ without recurrence of symptoms [15].

Chloroquine, also a 4-aminoquinoline has been reported to cause seizures [7], and life-threatening cardiovascular complications have been reported after overdose, although there is no evidence of such complications with AQ [7].

Results from the WANECAM study have been published in 2018. This study was comparing several artemisinin-based combination therapies (ACTs) including ASAQ for the repeated treatment of acute *P. falciparum* malaria.

In this study, irrespective of the correction method, treatment with ASAQ resulted in a prolongation of the QTc interval. However, the magnitude of the effect with ASAQ varied from 30.9 ms for QTcF to 12.5 ms for QTcB. It is however advisable to continue cardiac monitoring for ASAQ because the amplitude of QTc prolongation with this drug is large [16, 17].

The amodiaquine (as hydrochloride) 153mg dispersible tablets SmPC [22] summarises the most significant reported safety problems with AQ as hepatitis, agranulocytosis and irreversible retinopathy, which have been reported especially at higher doses and/or during prolonged use. On the basis of the data submitted and public information on the use of AQ therapy in malaria, the team of assessors advised that AQ Hydrochloride Tablets, WHOPAR Part 4, are of acceptable quality, efficacy and safety to allow inclusion of the product in the list of prequalified medicinal products for the treatment of uncomplicated malaria, and was included in the list of prequalified medicinal products on 30 August 2007 [7].

1.2.2.2 Malarone (ATV-PG)

In clinical trials of *Malarone* in the treatment of malaria, the most commonly reported adverse reactions were abdominal pain, headache, anorexia, nausea, vomiting, diarrhoea and coughing. In clinical trials of *Malarone* for prophylaxis of malaria, the most commonly reported adverse reactions were headache, abdominal pain and diarrhoea (UK SmPC) [6].

Elevations of transaminases have been reported in malaria patients being treated with *Malarone*. In one phase III trial of malaria treatment in Thai adults, early elevations of ALT and AST occurred more frequently in patients treated with *Malarone* compared to active control drug. Rates for patients who had normal baseline levels were: Day 7: ALT 26.7% vs. 15.6% and AST 16.9% vs. 8.6%. By day 14 of this 28-day study, the frequency of transaminase elevations equalized across the 2 groups. In this and other studies in which transaminase elevations occurred, they were noted to persist for up to 4 weeks following treatment with *Malarone* for acute malaria. None were associated with untoward clinical events.

Rare cases of hepatitis, anaphylaxis and skin and subcutaneous tissue disorders have been spontaneously reported after usage of ATV-PG in post-marketing [6].

1.2.2.3 Amodiaquine and Malarone given in combination

There is the potential for additive effects of giving AQ and ATV-PG in combination.

Risk Assessment

The following aspects of the adverse event profiles of ATV-PG and AQ, plus possible class-effects of the structurally related 4-aminoquinoline chloroquine (CQ) were considered in determining the Inclusion and Exclusion criteria and in designing the tolerability and safety monitoring of this study:

- The overlapping adverse events profiles of ATV-PG and AQ in patients (e.g. nausea, vomiting, diarrhoea, dizziness and increases of AST and ALT) indicate a potential for additive side-effects [6, 7];
- AQ may concentrate in the liver [7];
- AQ has been associated with neutropenia and more rarely agranulocytosis;

- CQ is known to cause QT prolongation and literature review suggests AQ may also prolong QT;
- CQ has been reported to cause seizures, and life-threatening cardiovascular complications have been reported after overdose [7]
- There is no evidence of seizures, life-threatening cardiovascular complications, or psychiatric effects with AQ [7].

The most significant, clinically concerning adverse events (hepatotoxicity, agranulocytosis/neutropenia and irreversibly retinal changes) were all seen in a multiple dose/repeated administration setting, and in those who had malaria.

In a report of AQ-induced hepatotoxicity, all 7 cases of hepatitis occurred in those who had received AQ for a minimum of 5 weeks [18].

A report on SMC looked at children under 10 years old, who received a complete course of sulfadoxine-pyrimethamine plus AQ, once a month for 3 months, between 2008-2010. After 3 years 780,000 documented courses of SMC had been administered. There were no serious adverse events [19].

In this proposed study, the subjects will receive three single doses of ATV-PG and/or AQ and will be healthy.

Rescue Medications

Diphenhydramine:

Diphenhydramine can be given if there is airway compromise, or a subject is at risk of airway compromise. Emergency airway intervention maybe necessary if there are laryngeal and pharyngeal dystonic reactions, as they increase the risk of imminent respiratory arrest. This is a known risk of Amodiaquine. Onset of actions of diphenhydramine should be within minutes. Medication can be given as below:

a. Can be given orally (50mg).
b. Can be given IM/IV (50mg).
c. IM/IV dose (50mg) can be repeated once, if needed.

Diazepam:

Oral diazepam can be given to treat anxiety (with close clinical monitoring for side effects), or to help manage Extrapyramidal Symptoms (EPS). Two subjects experienced anxiety in Cohort 1 and it is felt that planned management would be prudent. Giving diazepam would be considered if Grade II (CTCAE) or higher anxiety is experienced. As possible drug-induced anxiety in this study would be expected to be a transient effect, efforts should be made to avoid using diazepam within 2 hours following the appearance of Grade 2 anxiety. Medication can be given as below:

a. Give a single 2mg oral dose initially.
b. Can give up to 30mg daily, in divided doses.
c. If oral route not possible (e.g. severe anxiety attack), can be given intramuscularly 10mg.
d. To be given at investigator's discretion.
e. If ongoing treatment is required, then escalating management to the local emergency department should be considered.

Indication for giving Diazepam:

- Interfering with ADLs (Activities of Daily Living).
- Affecting ability to remain in trial/on ward.
- Subject feeling significant discomfort.

- Possibly causing additional AEs.
- Doctor feels anxiety is significant enough to warrant intervention.

Risk-Management

This trial will be conducted at an accredited Phase 1 clinical trial unit by an experienced investigator and well trained medical and technical staff with ample experience in the conduct of early phase clinical trials. The trial has been designed to safely include suitable subjects, monitor, treat and communicate potential expected adverse reactions as well as potential unexpected adverse events. Additionally, a sentinel dosing strategy will be used (Please refer to protocol section 3.3.4).

Although not expected, we do need to be prepared for potential serious adverse events and will monitor for these closely.

Subjects with a risk of heart rhythm abnormalities, neurological or psychiatric conditions or with hepatic abnormalities will be excluded from participation in the study. Volunteers with LFT measurements outside of the normal ranges, or clinically significant haematology results, at screening or Day -1, will be rejected and not included. Close clinical monitoring will include LFT measurements (including AST, ALT, bilirubin and ALP), ECG measurements in triplicate (digital capture and storage), the Basic Neurological Examination and Profile of Mood (POM) Questionnaire. Subjects will remain in the unit under medical supervision until 24 hours following the final dose and will be followed up at least weekly up to 28 days after first dose, with final follow up at 36 days after first dose.

There are also special stopping rules (both individual and group) that apply to the most significant risks.

In applying the above risk management strategies, the overall risk to subjects in this study is considered low.

Guidelines on How to Deal With Extrapyramidal Symptoms (EPS)

Risk Assessment and Management [23, 24] for Extrapyramidal Symptoms

To be used as a guide for clinical judgment:

Not currently at risk of deterioration	Currently at risk of deterioration
Minimal help needed with ADLs	Needs help with all ADLs
No risk to airway	Possible risk of airway compromise
Painless/pain not requiring treatment	Painful involuntary movements warranting treatment
Symptoms improve on distraction	No improvement on distraction
Happy to leave subject for 1-hour before next assessment	Re-assessments more frequently than 1-hour intervals required
No cognitive impairment	Cognitive impairment present
Subject not distressed	Significant distress to subject

EPS Signs and Symptoms

Below are examples of EPS signs and symptoms to look out for:

- Tremor (shaking at rest/on moving)

- Impaired gait/posture (unable to walk or sit normally)
- Limbs/body feel rigid or stiff
- Abnormal/reduced facial expressions (reduced blinking/eye movements/lip smacking)
- Abnormal speech (slow/fast/slurred/hoarseness), breathing or swallowing
- Slowed voluntary movements
- Involuntary movements (jerking/twisting/spasms) of any body part (including eyes and tongue)
- Restlessness/fidgety/repetitive movements/cannot stay in one place
- Confusion
- Pain
- Anxiety

Guidelines for Clinical Trial Assistants (CTAs)

No Doctor in clinical unit:
Subject displaying EPS (see EPS signs and symptoms) or other clinical concerns.

1. Assess subject and ensure they are safe (including ABCDE assessment).
2. Call on-call doctor.
    a. If 2nd CTA available - 2nd CTA to perform vital signs and ECGs.
3. Follow on-call doctors' instructions.

Doctor in clinical unit:
Subject displaying EPS (see EPS signs and symptoms as mentioned above) or other clinical concerns.

1. Assess subject and ensure they are safe (including ABCDE assessment).
2. Call doctor to assess subject immediately.
3. All dosing stopped for all relevant subjects following the guideline as per the point 5 in Section 4.4) (if further dosing is scheduled for that cohort (i.e. the following day).

Guidelines for Research Physicians

1. Assess subject and ensure they are safe (including ABCDE assessment).
    - If airway at risk, consider giving diphenhydramine (effects should be seen within minutes of administration).
        - Oral 50mg
        - IM/IV 50mg, can repeat once if needed.
2. Perform full neurological examination.
3. Inform the P.I.
4. Consult risk assessment.

Not currently at risk of deterioration:

1. Move subject to bed nearest the nurses' station.
2. Reassess hourly.
3. Consider giving diazepam based on severity and duration of AE.
4. If symptoms improve:
    a. Reduce frequency of assessments until symptoms resolve.
5. If symptoms worsen:
    a. Follow "Significant" guidelines.

Currently at risk of deterioration:

1. Call ambulance.
2. ALS trained member of staff to stay with subject until care is taken over by ED or symptoms resolve (whichever happens first).

2. STUDY OBJECTIVES AND OUTCOMES

2.1 Objectives

2.1.1 Primary

- To assess the safety and tolerability of the approved curative dose of ATV-PG (once daily for 3 days) and the adult equivalent of the approved SMC dose of AQ (once daily for 3 days) when administered alone and in combination, in comparison with placebo.

2.1.2 Secondary

- To determine the pharmacokinetics (PK) of atovaquone (ATV), proguanil (PG) and cycloguanil (CG), and amodiaquine (AQ) and desethyl-amodiaquine (DEAQ) following administration of ATV-PG and AQ alone and in combination.
- To determine the relationship between AQ and DEAQ and ECG parameters and to evaluate any impact of the combination with ATV-PG on this relationship.

2.2 Endpoints

2.2.1 Primary

- Safety and tolerability as measured by the incidence of treatment-emergent adverse events (TEAEs) including the clinical signs, nausea, vomiting and diarrhoea, proportion of subjects with clinically relevant changes in laboratory safety tests (haematology, chemistry (in particular ALT, AST and bilirubin increases) and urinalysis), proportion of subjects with morphological and/or rhythm abnormalities on electrocardiogram (ECG), proportion of subjects with clinically significant changes in ECG time intervals (PR, QRS, QT and QTc intervals) and proportion of subjects with clinically significant changes in vital signs (systolic blood pressure, diastolic blood pressure and pulse rate).

2.2.2 Secondary

- PK parameters derived by non-compartmental methods including maximum observed plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from time zero to last detectable plasma concentration (AUC_{0-t}), area under the plasma concentration-time curve from time zero extrapolated to infinity (AUC_{0-inf}), terminal elimination rate constant (λz), terminal elimination half-life ($t_{1/2}$).
- Baseline corrected QTc (ΔQTc) as a function of the concentrations of AQ and DEAQ when administered alone, and when administered in combination with ATV-PG.

3. STUDY DESIGN

3.1 Overall study design

Study plan

The study is a randomized, double-blind, placebo controlled, parallel group study to determine the tolerability and safety of ATV-PG + AQ, ATV-PG + AQ placebo, ATV-PG placebo + AQ, and ATV-PG placebo + AQ placebo administered once daily for 3 days to healthy adult male and female subjects.

Fifty-two subjects will be enrolled and randomized to one of the four treatments in a ratio of 4:3:3:3 as described below:

- Treatment 1 (n=16) - ATV-PG 1000-400 mg + AQ 612 mg;
- Treatment 2 (n=12) - ATV-PG 1000-400 mg + AQ unmatched placebo;
- Treatment 3 (n=12) - ATV-PG unmatched placebo + AQ 612 mg;
- Treatment 4 (n=12) - ATV-PG unmatched placebo + AQ unmatched placebo.

Following the completion of dosing of the first 20 randomised subjects, Treatment 1 has been discontinued from further dosing in accordance with the Adverse Reaction (AR) rules in Section 4.4. Up to 24 further subjects will be included in the study, split in three cohorts of up to eight subjects.

The remaining three treatments will be re-randomised in such a way that across the whole study there will be still 12 subjects dosed with Treatment 2, 12 subjects with Treatment 3 and 12 Subjects with treatment 4. This is taking into account both the already dosed subjects with their originally randomised treatments and the newly randomised subjects yet to be included. Furthermore, within each cohort of up to eight subjects, each of the three treatments (2, 3, and 4) needs to be represented with a maximum of four and a minimum of one subject in the same cohort.

Subjects will be screened within 20 days prior to entering the study on Day -1. Each subject will receive verbal and written information followed by signing of the Informed Consent Form (ICF) prior to any screening procedures taking place. Subjects will be admitted to the study unit on Day -1 and will be discharged on Day 4.

All subjects will attend the unit for an outpatient visit on Days 8, 15, 22, 29 and a follow-up visit on Day 36 +/- 1 day (Figure 1). All the assessments performed during the study are detailed in the study schedule of assessments (Table 1 and Table 2). Study design features as well as number of subjects may be adapted according to the Adaptive Features (Table 3). This study will use a sentinel dosing strategy, for full details see Section 3.3.4.

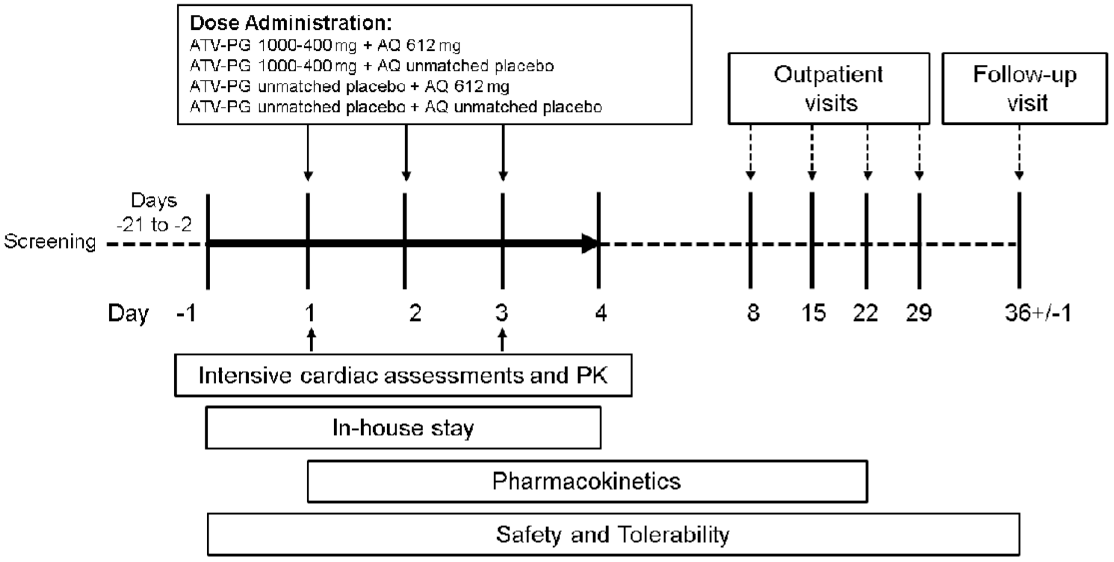


Figure 1. Study flow chart.

Table 1. Schedule of assessments.

Procedure	Scr D-21-D-2	D -1	D1	D2	D3	D4	D8	D15	D22	D29	Follow-up visit D36+/-1
Informed consent	X										
Demographic data	X										
Medical history and current medications	X	X[a]									
Urine drugs of abuse screen	X	X									
Breath alcohol test	X	X									
Serology HIV1&2, Hep B&C	X										
Serum pregnancy test[b]	X	X								X	X
FSH[c]	X										
Inclusion/Exclusion criteria	X	X									
Smoking history (pack/year) and current	X										
Study Residency		X	X	X	X						
Check-in		X									
Check-out						X					
Non-residential visit							X	X	X	X	X
Randomisation			X								
Study drug administration[d]			X	X	X						
Meals[e]		X	X	X	X	X					
Safety and tolerability:											
AE recording/ concomitant medication	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]			X[f]	X
Vital signs[g]	X		X	X	X	X					X
Telemetry[h]			X		X						
24h Holter ECG[i]	X										
12-lead ECG in triplicate[g]	X		X	X	X	X	X	X	X		X
Body height	X										
Body weight	X										
BMI	X										
Haematology/Biochemistry/ Coagulation[j]	X	X		X	X	X	X	X	X	X	X
Urinalysis	X	X								X	
Basic neurological exam			X	X	X	X	X			X	
Questionnaire Profile of Mood Scale (POM)			X			X	X			X	

Procedure	Scr D-21-D-2	D -1	D1	D2	D3	D4	D8	D15	D22	D29	Follow-up visit D36+/-1
CYP2C8 Genotyping Sample			X[k]								
Pharmacokinetic assessments											
PK blood sampling - ATV			X	X	X	X	X	X			
PK blood sampling - PG/CG			X	X	X	X					
PK blood sampling - AQ			X	X	X	X					
PK blood sampling - DEAQ			X	X	X	X	X	X	X		

a: Update only.
b: Females of childbearing potential only.
c: A hormonal test for FSH will be done for females at screening who believe they are post-menopausal. If female subjects are confirmed as post-menopausal then these subjects will not require any further serum pregnancy tests.
d: Dosing will be carried out in the fed state. On the morning of each dosing day (Days 1, 2 and 3), a standardised meal will be served 30 mins before dosing and must be finished 10 mins before dosing, firstly with the ATV-PG / placebo tablets, followed by the AQ dispersible/ placebo tablets.
e: Standard meals will be served at standard unit times. On Days 1 to 3, the 1st meal is breakfast, lunch given 5 hours post-dose and no other meals are given before completion of the 2-4 hours post-breakfast cardiac assessments. On Day 4 (dismissal day) meals will be optional.
f: Symptom directed examination.
g: Vital signs and 12-lead ECG to be carried out prior to any blood sampling (where time points coincide). Vital signs will be measured after rest of at least 5 minutes in supine position and in standing at screening only. From Cohort 2 onwards, standing blood pressure measurement will be performed at 2H and 5H post-dose (on study days 1, 2 and 3). In addition to these time-points, an unscheduled standing blood pressure measurement should also be performed if a subject wish to leave the bed between these time-points.
h: Continuous 12-lead telemetry will be recorded from at least 1-hour pre-dose to 24 hours post-dose on Day 1 and Day 3.
i: At screening Holter will be performed to exclude pre-existing ECG abnormalities.
j: Coagulation at screening and Day -1.
k: pre-dose. Samples will be analysed only if required as per the Adaptive Feature number 9.

Table 2. Detail schedule of assessments for in-house stay

Study Day	Time (h)	PK blood sampling	Meals	12-lead ECG[a]	Vitals[b]
D1	-3			X	
	-2.5			X	
	-2			X	
	-35 minutes	X			X
	-30 minutes		X[c]		
	0				
	1	X		X	X
	2	X		X	X[e]
	3	X		X	
	4	X		X	
	5	X	X[c]	X	X[e]
	6	X		X	
	8	X		X	X
	10		X		
	12	X		X	
D2	-35 minutes	X		X	X
	-30 minutes		X[c]		
	0				
	2				X[e]
	5				X[e]
D3	-3			X	
	-2.5			X	
	-2			X	
	-35 minutes	X			X
	-30 minutes		X[c]		
	0				
	1	X		X	X
	2	X		X	X[e]
	3	X		X	
	4	X		X	
	5	X	X[c]	X	X[e]
	6	X		X	
	8	X		X	X
	10		X		
	12	X		X	
D4	24	X	X[d]	X	X

a: 12-lead ECG will be measured in triplicate after a rest period of at least 10 min.
b: Vital signs will be measured after rest of at least 5 min in supine position.

c: Breakfast is the first meal and will be given at 30 minutes pre-dose. The 5-hour procedures are to be completed prior to lunch being given.
d: Optional breakfast after clinical assessments.
e: Standing blood pressure measurement will also be performed.

3.2 Adaptive Design

This study incorporates the use of an adaptive design. Study specific adaptive features and their limits are described in Table 3.

Adaptive features may be implemented only with the approval of the sponsor. Implementation of adaptive features affecting whole dose groups or cohorts, or the entire study, will be documented in a non-substantial amendment.

The exceptions to this are adaptive features **3 (limit I), 4 (limit I) and 5 (limit I),** which relate to individual subject safety. These may be implemented at the discretion of the investigator and recorded in that subject's source data.

Table 3. Adaptive protocol features.

Adaptive Study Design Areas	Features	Limits
Sentinel/subgroup dosing	1. Cohorts may be split into sub-groups.	I. A mandatory sentinel dosing strategy of dosing four subjects from treatment 1, and a minimum of two subjects each from treatments 2, 3 and 4 will be used. There will be a total of twelve subjects in the sentinel dosing. The remaining subjects can be dosed if safety and tolerability data up to 29 days post last dose is acceptable. II. The subjects in the sentinel group will all be dosed in parallel. If they are successfully dosed (i.e. it is deemed safe to dose the other subjects), the remaining 40 subjects may all be dosed at the same time, in parallel, or they may be divided into 2 or more dosing groups, with the groups being dosed on different days. Each dosing group would contain a minimum of two subjects from each of the 4 treatments, to maintain blinding and avoid bias.
Flexible Cohort Sizes	2. Withdrawn subjects can be replaced at the discretion of the sponsor and PI.	I. AR rules (Section 4.4)
Samples and Assessments (in-house duration and visit numbers)	3. The in-house stay or follow-up period may be prolonged or shortened if considered clinically necessary by the PI for individuals on a case-by-case basis.	I. A maximum extended in-house or follow-up period cannot be pre-defined as the extension will be as long as necessary to ensure the safety of the individual participant(s).
Samples and Assessments (safety)	4. Additional safety assessments may be performed on an individual subject if it is considered clinically necessary	I. For individuals, a maximum number of safety blood samples will be determined on a case-by-case basis and cannot be pre-defined as

Adaptive Study Design Areas	Features	Limits
	by the PI for individuals on a case-by-case basis.	investigations will be performed as necessary to ensure the safety of the individual participants. II. Study specific maximum blood volume will not be exceeded (Section 7.11).
	5. Specialist referrals (e.g. to a cardiologist) may be made (and may include all relevant assessments and investigations) if it is considered clinically necessary by the PI or sponsor or SRC for individuals on a case-by-case basis	I. A maximum for individuals will be determined on a case-by-case basis and cannot be pre-defined as investigations will be performed as necessary to ensure the safety of the individual participants.
Samples and Assessments (PK)	6. Additional or fewer blood PK assessments may be taken in accordance with evolving data and dosing schedule. This includes those for the analysis of metabolites. 7. The timing of blood PK, and/or any potential exploratory assessments may be adjusted in accordance with evolving data and dosing schedule. This includes those for the analysis of metabolites. 8. General metabolite profiling beyond the metabolite specified in the objectives may be undertaken on selected or pooled PK samples and reported separately. 9. CYP2C8 genotype analysis may be performed, if PK data suggests that it is relevant (i.e. that a subject may be a CYP2C8 poor metaboliser).	I. Minimum: sufficient PK samples to establish full protocol specific plasma PK profile. II. Study specific maximum blood volume will not be exceeded (Section 7.11).
Samples and Assessments (ECG assessments)	10. If PK sampling times are changed, then the matching ECG sampling times will be changed accordingly for exploratory QT/QTc analysis or intensive cardiac assessments. 11. If the meal times need to be changed, ECG times may also be changed to maintain the sampling schedule required to capture the food effect for the confirmation of assay sensitivity. 12. Telemetry data gathered during the study may be used in the exploratory analysis of drug	I. To confirm assay sensitivity, a minimum of four postprandial time points will be recorded in triplicate between 1-4h after the start of the meal.

Adaptive Study Design Areas	Features	Limits
	related QT/QTc interval changes.	
Screening	13. Screening assessments, including Holter ECG recordings performed at Richmond Pharmacology Ltd on volunteers screened (but not randomized) for another study can be used for this study to avoid unnecessary tests.	I. The assessments must meet protocol criteria (e.g. the method to be used). II. The assessments must be performed within the protocol defined screening window. III. The Holter ECG recordings are valid for a period of three months.

3.3 Rationale for study design, doses and control groups

This study will evaluate the tolerability and safety profile of the approved doses of ATV-PG and AQ when co-administered once daily for 3 days. The study is randomised and double blinded to minimise bias and includes placebo (unmatched) for ATV-PG and AQ to facilitate identification of effects related to treatment rather than the study procedures or situation.

3.3.1 Justification for the selected dose level

The approved adult malaria treatment dose of ATV-PG and the adult equivalent AQ dose that is used in children in combination with SP has been chosen for this study as these are the adult equivalent doses that will be used in SMC treatment of children.

The specified adult dose of ATV-PG is 1000 mg ATV + 400 mg PG once daily for 3 consecutive days [6] while the specified adult dose of AQ (for subjects over 13 years of age) is 600 - 612 mg once daily for 3 days (4 fold higher than the specified dose for 1-6-year olds) [7, 22].

3.3.2 Choice of subjects for study

Male and female subjects aged between 18 and 45 years considered healthy for their age are planned to be included in the study. The selection criteria are defined such that subjects selected for participation in the study are known to be free from any significant illness which potentially could confound the study results or put the subject at risk. Healthy subjects are also unlikely to require concomitant treatments which could interfere with the study drugs.

Considering that African population is at considerably higher risk of contracting malaria, this study will only recruit black subjects of sub-Saharan African origin (defined as subjects whose parents are black and are of sub-Saharan African origin).

3.3.3 Route and rate of administration

ATV-PG and AQ and placebos will be administered via the oral route.

3.3.4 Precautions to be applied for dosing between subjects within a cohort

A mandatory sentinel dosing strategy of dosing 12 subjects will be used to provide safety assessment in 4 subjects exposed to ATV-PG + AQ. The sentinel group will consist of 4 subjects from treatment 1, and a minimum of 2 subjects each from treatments 2, 3 and 4. Blinded safety data up to 29 days post last dose will be reviewed by a safety review committee (SRC) prior to dosing the remaining subjects.

If the sentinel group shows acceptable safety and tolerability, then the remaining subjects from all four Cohorts will be dosed.

The ratio of allocation to treatment group of the sentinel group has been chosen to ensure the safety of giving ATV+PG and AQ together, in a small number of subjects, while maintaining blinding.

The SRC will make a judgement (based on the minimum data requirements (Table 4) whether the drug administration to the remaining subjects can continue. See section 4.4 for Adverse Reaction (AR) rules.

3.3.5 Monitoring and communication of adverse events/reactions

AEs will be continuously monitored throughout the study from the signing of the informed consent form until the last follow up assessment. Each AE reported will be assessed by a trained Research Physician who will ensure that the event is dealt with as appropriate based on clinical need, study protocol, study operations manual and Richmond Pharmacology standard operating procedures (SOPs). AEs will be documented in the subjects' Case Report Forms (CRFs) and reviewed regularly by the Research Physicians and the investigator.

If any information relating to the study drugs in this study becomes available after the submission of a final protocol to the Competent Authority which may impact on the conduct of the study, including but not limited to the risk and benefit evaluations underpinning approvals and subject's consent, MMV shall notify RPL in writing as soon as practically possible and the parties will agree, in writing, what steps need to be taken if any.

3.3.6 Investigator Site Facilities and Personnel

This study will be conducted in a specialised early phase CPU with onsite resuscitation equipment and medication, in addition to access to an acute hospital with Critical Care facilities, thus ensuring direct access to equipment and staff for resuscitating and stabilising subjects in acute medical conditions and emergencies. The study is conducted by an experienced PI and well trained medical, nursing and technical staff with ample experience in the conduct of early phase clinical trials.

The study is designed to closely monitor, treat and communicate potential expected adverse reactions as well as potential unexpected adverse events.

3.3.7 Maximum Cohort Size

Following the reporting of an SAE in Cohort 1, it has been agreed between the SRC member to set the maximum cohort size at eight for all subsequent cohorts, with a minimum of one subject from each of the continuing treatment arms. A maximum of three subjects can be on the AQ + Malarone treatment arm within each cohort. This is to improve subject safety. Fewer subjects will ensure that all subjects can be assessed and managed (if required) properly, and everything can be documented properly.

Following the completion of dosing of the first 20 randomised subjects, Treatment 1 has been discontinued from further dosing in accordance with the Adverse Reaction (AR) rules in Section 4.4. Up to 24 further subjects will be included in the study, split in three cohorts of up to eight subjects.

The remaining three treatments will be re-randomised in such a way that across the whole study there will be still 12 subjects dosed with Treatment 2, 12 subjects with Treatment 3 and 12 Subjects with treatment 4. This is taking into account both the already dosed subjects with their originally randomised treatments and the newly randomised subjects yet to be included. Furthermore, within each cohort of up to eight subjects, each of the three treatments (2, 3, and

4) needs to be represented with a maximum of four and a minimum of one subject in the same cohort.

4. DECISION-MAKING, RULES AND LIMITS

4.1 Definitions

'Continuation' in the context of this protocol refers to either (1) continuing dosing in an individual subject or (2) continuing dosing in the study.

4.2 Rules and limits governing decision-making

The criteria and rules that will govern PI/SRC decisions are:

1. Minimum data requirements (Table 4).
2. Adverse reaction rules (section 4.4).
3. Adaptive features and their limits (section 3.2).

Table 4. Minimum data requirements to go from sentinel dosing to dosing remaining subjects.

STUDY PART			
Decision-making time point:	Person or body making the decision:	Minimum data to be looked at (in accordance with the CSP):	Method of documentation of decision and/or communication to sponsor (if applicable):
Continuation from the sentinel group to the remainder of the subjects in all cohorts	SRC	The SRC will review all available safety and tolerability data collected up to 29 days after first dose. All clinically significant findings will be taken into account by the SRC when determining whether the remaining subjects can be dosed as planned.	The SRC will document the decision on the dose continuation approval form.

4.3 Safety Review Committee

The SRC will consist of, as a minimum:

- Principal Investigator (RPL) or delegate
- MMV, Inc. Medical Advisor or delegate

Further internal or external experts such as a pharmacokineticist, and/or a statistician, may be consulted by the SRC as necessary. Any additional information, if required will be included in the study operations manual (SOM).

4.3.1 SRC Meeting

There is an option to have ad-hoc SRC meetings to discuss urgent issues should the need arise.

Prior to each SRC meeting, an interim safety report will be prepared, presenting the relevant safety and tolerability data. These will be signed by the investigator.

Initially the data will be reviewed blinded. If the SRC consider it necessary due to a safety concern, either individual subjects or the entire cohort may be unblinded to enable their decision-making. For non-emergency unblinding, before breaking the code, the potential decisions and actions should be determined and documented.

These decisions will be signed by one of the sponsor's representatives and by the investigator. For logistical reasons, the signature of the sponsor or investigator may be communicated via email.

The decision of the SRC will be taken in consensus between the members of the SRC. If consensus cannot be reached, then the most cautious approach will proceed.

4.4 Adverse Reaction (AR) rules

- An AR is any AE that is at least possibly related to Amodiaquine or *Malarone*.
- In this section, 'IMP' will refer any of the combinations of active drugs and/or placebos.
- When applying AR rules, ARs will be graded using CTCAE in accordance with Section 8.2 for the purpose of standardised recording and reporting.
- Seriousness will be assessed using the standard criteria outlined in Section 8.1.

These rules will only apply to AEs/SAEs that are at least possibly related to the IMP. We will assess every AE in the following order:

1) Whether blinding can continue for the individual

2) The impact on the individual subject, e.g. whether IMP administration can be continued.

3) a) (if the subject is unblinded) The impact on the cohort the individual subject is part of, e.g. in the following circumstances:

- If a cohort is due to receive further doses (i.e. a dose on the following day), whether that cohort can receive further doses as per the dosing schedule.
- If the individual(s) is/are part of the sentinel cohorts, the impact on the successive subjects of that cohort – i.e. whether the remaining subjects can be dosed or not.

3) b) (if the subject remains blinded) The impact on all remaining subjects, e.g. in the following circumstances:

- If subjects are due to receive further doses (i.e. a dose on the following day), whether remaining subjects can receive further doses as per the dosing schedule.
- If the individual(s) is/are part of the sentinel cohorts, the impact on all successive subjects – i.e. whether the remaining subjects can be dosed or not.

4) The impact on continuation or suspension of the overall study.

5) One subject from Cohort 1 experienced EPS. For safety reasons, it was felt that it was important to unblind this subject, to clarify which treatment he received. Unblinding revealed that he received both active IMPs (amodiaquine and Malarone). If any additional subject(s) experiences EPS (of any severity), then that/those subject(s) should be "unblinded" and depending on the treatment they received, the following actions should be taken:

a. Subject is on AQ + Malarone:
   i. Stop the AQ + Malarone treatment arm.
   ii. Consider stopping the Placebo + Malarone treatment arm*.
   iii. Continue the AQ + Placebo treatment arm**.
   iv. Continue the Placebo + Placebo treatment arm.

b. Subject is on AQ + Placebo
   i. Stop the entire trial.

c. Subject is on Placebo + Malarone
   i. Stop the AQ + Malarone treatment arm.
   ii. Stop the Placebo + Malarone treatment arm.
   iii. Continue the AQ + Placebo treatment arm**.
   iv. Continue the Placebo + Placebo treatment arm.

d. Subject is on Placebo + Placebo
   i. Temporarily halt the entire trial.
   ii. Placebo to be investigated

*Assuming that no safety concerns are observed in subjects dosed with Malarone + placebo, the Sponsor may decide to terminate this arm for strategic reasons. If the combination treatment AQ + Malarone is terminated for safety reasons, then assessing Malarone monotherapy may be unnecessary as the safety profile is already established.

**By contrast, AQ may be used in combination with other anti-malarial medications in future trials and further safety/tolerability data (CNS & QTc effects in particular) with this drug in a well-controlled Phase1 study are needed, so obtaining data from the AQ monotherapy arm would still be beneficial.

4.4.1 General AR rules:

The general AR rules refer to all adverse reactions, excluding those relating to the liver and haematology and QT interval prolongation. There is a greater anticipation of adverse reactions relating to these latter categories, and CTCAE may not give clear enough guidance and grading may not be appropriate for this trial in healthy volunteers; therefore special study-specific rules have been created.

Individual AR rules:

IMP administration for an individual subject will be stopped if they experience any CTCAE grade III (or higher) AR, or a serious AR irrespective of severity/CTCAE grade.

IMP administration for an individual subject will be stopped if they experience any CTCAE grade I or II AR that raises safety concern.

Group AR rules:

IMP dosing will be suspended in all subjects, or potentially only in an affected cohort (if unblinding occurs), if either of the following occur:

- One subject experiences a severe (CTCAE grade III) AR
- One subject experiences a serious AR (irrespective of severity/CTCAE grade)

Dosing can only continue with a substantial amendment to the protocol which has been approved by the MHRA and ethics committee.

4.4.2 Special individual AR rules:

Dosing in an individual will be suspended if they experience any of the following:

- LFT elevation
  - ALT or AST value >3x ULN together with bilirubin increase >2x ULN.
  - ALT or AST value >8x ULN.
  - ALT or AST value >3x ULN, and symptomatic.
- Haematology
  - Hb drop to an absolute value of 8g/dL (80g/L) or lower.
  - Clinically significant drop in neutrophil count.*
  - Platelet count drop of >50% from baseline or absolute value <80.000/mm^3 (Day -1 blood samples).
- QT interval prolongation
  - A prolongation of the uncorrected QT interval of greater than 500ms (using consistent, technically valid triplicate ECG).

*The special neutrophil rule has not been given a numerical value. This is because the subjects are of African origin and therefore a wide range of neutrophil values (including those that are outside the laboratory reference range. (i.e. Benign Ethnic Neutropenia and diurnal variation)) can be considered normal. These results will therefore be considered on a case by case basis.

4.4.3 Special group AR rules:

If one subject fulfils the special individual AR rules, and this could be reasonably attributed to one of the IMP medications, dosing for all subjects will be at least temporarily suspended and

a SRC meeting arranged. At this meeting, a decision will be made regarding the continuation of dosing in the remaining subjects.

If it is then confirmed that one subject fulfils Hy's Law criteria, dosing will be suspended in all subjects and can only continue with a substantial amendment to the protocol which has been approved by the MHRA and ethics committee.

If two or more subjects fulfil any of the other special individual AR rules, then dosing will be suspended in all subjects and can only continue if an SRC meeting is arranged (and a decision made that dosing can continue) and a substantial amendment is made to the protocol which has been approved by the MHRA and ethics committee.

5. SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Number and Source of Subjects

Fifty-two healthy adult subjects will be recruited to dose one of the four treatmentswhich will be randomized in a ratio of 4:3:3:3 as described below:

- Treatment 1 (n=16) - ATV-PG 1000-400 mg + AQ 612 mg;
- Treatment 2 (n=12) - ATV-PG 1000-400 mg + AQ unmatched placebo;
- Treatment 3 (n=12) - ATV-PG unmatched placebo + AQ 612 mg;
- Treatment 4 (n=12) - ATV-PG unmatched placebo + AQ unmatched placebo.

Following the completion of dosing of the first 20 randomised subjects, Treatment 1 has been discontinued from further dosing in accordance with the Adverse Reaction (AR) rules in Section 4.4. Up to 24 further subjects will be included in the study, split in three cohorts of up to eight subjects.

The remaining three treatments will be re-randomised in such a way that across the whole study there will be still 12 subjects dosed with Treatment 2, 12 subjects with Treatment 3 and 12 Subjects with treatment 4. This is taking into account both the already dosed subjects with their originally randomised treatments and the newly randomised subjects yet to be included. Furthermore, within each cohort of up to eight subjects, each of the three treatments (2, 3, and 4) needs to be represented with a maximum of four and a minimum of one subject in the same cohort.

5.2 Replacement subjects

Replacement subjects may be enrolled to ensure that a sufficient number of subjects complete the study. Replacement subjects must be agreed with the Sponsor.

5.3 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for enrolment in this study:

1. Black male or female, of sub-Saharan African origin (both parents are black and are of sub-Saharan African origin), aged ≥18 to ≤45 years at the date of signing informed consent which is defined as the beginning of the Screening Period. This inclusion criterion will only be assessed at the Screening Visit.

2. Female subjects of childbearing potential and male subjects with female partners of childbearing potential must be willing to follow the following contraception requirements. Contraception must start one complete menstrual cycle prior to the first day of dosing and continue until at least 90 days after the end of the systemic exposure of the study drug (90 days after the last study drug administration).

   Female subjects who are documented as being of non-childbearing potential are exempt from contraception requirements. Documentation of non-childbearing potential must include at least one of the following criteria:

   - Postmenopausal – evidence of menopause based on a combination of amenorrhea for at least one year and increased serum follicle-stimulating hormone (FSH) level (> 30 IU/L), or
   - Surgical sterilization – evidence of hysterectomy and/or bilateral oophorectomy.

   All female subjects of childbearing potential and all male subjects with female partners of childbearing potential, who are pregnant or breastfeeding must practice highly effective or acceptable methods of contraception (defined below) when having heterosexual intercourse.

   Sexual abstinence, defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug treatment can be considered a highly effective method of contraception for female and male subjects. Reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical study and the preferred and usual lifestyle of the subject.

   Contraception methods for female subjects in this study:

   - Hormonal contraception:
     - Combined i.e. oestrogen- and progestogen-containing (oral, intravaginal or transdermal)
     - Progestogen-only (oral, injectable or implantable)
     - Intrauterine hormone-releasing system (IUS)
   - Intrauterine device (IUD) or
   - Bilateral tubal occlusion
   - Male partner vasectomised (with documented evidence of azoospermia if possible)
   - (Male partner uses) male condom with or without spermicide
   - Cap, diaphragm, or sponge with spermicide

   Contraception methods for male subjects in this study:

   - Male condom with or without spermicide
   - Vasectomy (with documented evidence of azoospermia if possible)

3. Subjects must agree not to donate sperm or ova from the time of the first administration of study medication until 3 months after the end of the systemic exposure of the study drugs.

4. Subjects must have a body weight of at least 50 kg and a body mass index (BMI) between 18-25.0 kg/m² inclusive at screening.

5. Satisfactory medical assessment with no clinically significant or relevant abnormalities as determined by medical history, physical examination, vital signs, 12-lead ECG, and clinical laboratory evaluation (haematology, biochemistry, coagulation, and urinalysis) that is reasonably likely to interfere with the subject's participation in or ability to complete the study as assessed by the Investigator.
6. Ability to swallow 8 capsules/tablets at a time or consecutively.
7. Ability to provide written, personally signed, and dated informed consent to participate in the study, in accordance with the ICH Good Clinical Practice (GCP) Guideline E6 (R2) (2016) and applicable regulations, before completing any study-related procedures.
8. An understanding, ability, and willingness to fully comply with study procedures and restrictions.

5.4 Exclusion Criteria

Subjects will be excluded from enrolment in this study if they meet any of the following criteria:

1. Current or recurrent disease (e.g., cardiovascular, haematological, neurological, endocrine, immunological, renal, hepatic or gastrointestinal or other conditions) that could affect the action, absorption, or disposition of ATV-PG or AQ, or could affect clinical assessments or clinical laboratory evaluations.
2. Any significant history of seizures or epilepsy.
3. Current or relevant history of physical or psychiatric illness that may require treatment or make the subject unlikely to fully comply with the requirements or complete the study, or any condition that presents undue risk from the investigational product or study procedures.
4. Any other significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study may influence the result of the study, or the subject's ability to participate in the study.
5. Documented retinopathy.
6. History of photosensitivity.
7. History of malaria.
8. Subjects have travelled to malaria endemic regions for more than a total of 4 weeks within the past 12 months (as per Global Malaria Risk: https://www.malariasite.com/malaria-risk/ [20]-)
9. The history or presence of any of the following cardiac conditions: known structural cardiac abnormalities; family history of long QT syndrome; cardiac syncope or recurrent, idiopathic syncope; exercise related clinically significant cardiac events.
10. Any clinically significant abnormalities in rhythm, conduction or morphology of resting ECG or clinically important abnormalities that may interfere with the interpretation of QTc interval changes. This includes subjects with any of the following (at screening or Day -1):
    - Sinus node dysfunction.
    - Clinically significant PR (PQ) interval prolongation.
    - Intermittent second or third degree AV block.
    - Complete bundle branch block.

- Sustained cardiac arrhythmia's including (but not limited to) atrial fibrillation or supraventricular tachycardia; any symptomatic arrhythmia with the exception of isolated extra systoles.
- Abnormal T wave morphology which may impact on the QT/QTc assessment.
- QT interval corrected using the Fridericia's formula (QTcF) > 450 ms (males and females) on any individual ECG (unless obviously an anomaly).
- Any other ECG abnormalities in the standard 12-lead ECG and 24-hour 12 lead Holter ECG or an equivalent assessment which in the opinion of the Investigator will interfere with the ECG analysis.

Subjects with borderline abnormalities may be included if the deviations do not pose a safety risk, and if agreed between the appointed Cardiologist and the PI.

11. Has vital signs outside of the following normal range at screening or Day -1:
    a. Blood pressure (BP):

       Supine BP (after at least 5 minutes of supine rest):

       - Systolic blood pressure: 90 - 140 mmHg.
       - Systolic blood pressure drop of >20mmHg on standing (at the time-points specified in Table 1)
       - Diastolic blood pressure: 40 - 90 mmHg.

    b. Pulse rate (after at least 5 minutes of supine rest):
       - Less than 40 or greater than 100 beats per minute
12. Positive test for Hepatitis B surface antigen (HBsAg), Hepatitis C antibody (HCV Ab), or human immunodeficiency virus antibody (HIV Ab) at screening;
13.
    a. AST, ALT or bilirubin measurement above the laboratory reference ranges, at screening or Day -1.
    b. Haemoglobin or platelet count outside of the laboratory reference ranges.
    c. or any other clinically significant abnormal haematological measurement (per the Investigator's discretion).
14. Positive test results for alcohol or drugs of abuse at screening or Day -1.
15. Female subjects who are pregnant (including a positive serum pregnancy test at screening and on Day-1) or breastfeeding.
16. History or clinical evidence of substance and/or alcohol abuse within the 2 years before screening. Alcohol abuse is defined as regular weekly intake of more than 14 units (for both males and females), using the following NHS alcohol tracker http://www.nhs.uk/Tools/Pages/drinks-tracker.aspx.
17. Use of tobacco in any form (e.g., smoking or chewing) or other nicotine-containing products in any form (e.g., gum, patch, electronic cigarettes) within 3 months prior to the planned first day of dosing.
18. Has used any other prescription medication (excluding hormonal contraception, hormone replacement therapy) within 14 days or 10 half-lives (whichever is longer) prior to Day 1 of the dosing period that the Investigator judges is likely to interfere with the study or pose an additional risk in participating.

19. Has used any over-the-counter medication (including multivitamin, herbal, or homeopathic preparations; excluding paracetamol - up to 1g paracetamol per day permitted) during the 7 days or 5 half-lives of the drug (whichever is longer) prior to Day 1 of the dosing period, that the Investigator judges is likely to interfere with the study or pose an additional risk in participating.
20. Has used any medication listed on the Flockhart table (http://medicine.iupui.edu/clinpharm/ddis/main-table/) that is either a moderate or strong inhibitor or inducer of CYP450 within 30 days or 5 half-lives (whichever is longer) prior to the planned first day of dosing.
21. Has received an investigational product or been treated with an investigational device within 90 days or 5 half-lives (whichever is the longer) prior to firstdrug administration
22. Known or suspected intolerance or hypersensitivity to the investigational product, atovaquone, proguanil or amodiaquine, any closely related compound, or any of the stated ingredients.
23. History of significant allergic reaction (anaphylaxis, angioedema) to any product (food, pharmaceutical, etc).
24. Has donated or lost 400 mL blood (excluding plasma) or more within the last 16 weeks preceding the first day of dosing.
25. Has a mental incapacity or language barriers precluding adequate understanding, co-operation, and compliance with the study requirements
26. An inability to follow a standardised diet and meal schedule or inability to fast, as required during the study.
27. Subjects have veins unsuitable for intravenous puncture or cannulation on either arm (e.g. veins that are difficult to locate access or puncture veins with a tendency to rupture during or after puncture).
28. Prior screen failure (where the cause of the screen failure is not deemed to be temporary), randomisation, participation, or enrolment in this study. Subjects who initially failed due to temporary non-medically significant issues are eligible for re-screening once the cause has resolved.

5.5 Subject Restrictions

Subjects will have to comply with the restrictions described in Table 5.

Table 5. Subject restrictions.

Items subjects must not consume or do:	When subjects must stop:	When subjects can re-start:
Tobacco in any form (e.g., smoking or chewing) or other nicotine-containing products in any form (e.g., gum, patch, electronic cigarettes).	From three months prior to the planned first day of dosing.	After study completion/last visit.

Items subjects must not consume or do:	When subjects must stop:	When subjects can re-start:
Meals/snacks/water	Whenever subjects are confined in the ward, only the drinks and meals provided by the trial personnel will be allowed. Standard meals will be provided (except for breakfast on dosing days) at the standard unit times as stated in the study plan, and meals should be completed each time. On the evening prior to each dosing day, subjects will be required to fast overnight of all food and drink except water (minimum of 8 hours). Dosing will be carried out in the fed state. On the morning of each dosing day (Days 1, 2, and 3), subjects will fully consume a breakfast of porridge. It will be given at 30 min prior to dosing and completed at 10 minutes prior to study drug administration. Details will be provided in the SOM. Lunch will be provided following clinical sampling/measurements at 5 hours post dose. Water will be provided ad libitum until one hour pre-dose and from 1 hour post-dose.	Standard meals will be given at regular intervals throughout the in-house stay.
Caffeine-containing or Xanthine-containing products.	48 hours before the planned first study drug administration and each outpatient/follow-up visit.	After study completion/last visit.
Energy drinks or drinks containing taurine, glucuronolactone (e.g. Red Bull).	48 hours before the planned first study drug administration and each outpatient/follow-up visit.	After study completion/last visit.
Alcohol.	48 hours before the planned first study drug administration and each outpatient/follow-up visit. On other days: less than 14 units a week and less than three units in one day is permitted.	After study completion/last visit.
Poppy seeds	24 hours before screening and the planned first study drug administration and each outpatient/follow-up visit.	After study completion/last visit.
Strenuous physical activity.	48 hours before the planned first study drug administration and each outpatient/follow-up visit.	After study completion/last visit. [Subjects should not start new physical training activities during

Items subjects must not consume or do:	When subjects must stop:	When subjects can re-start:
Activity	Subjects will be requested to remain in a semi-supine position for a period of time (to be specified in the SOM) after dosing on each day, except to use the bathroom. Subjects may then be ambulatory, but should not engage in strenuous activities and should rest semi-supine for at least 5 minutes prior to any Vital Signs or at least 10 minutes prior to ECG measurement.	the study until study completion (last visit)]
Any prescription medication. For details, including exceptions see exclusion criteria 16.	14 days or 10 half-lives (whichever is longer) before the planned first study drug administration.	After study completion/last visit.
Any over-the-counter (OTC) medication. For details, including exceptions see exclusion criteria 17	7 days or 5 half-lives (whichever is longer) before the planned first study drug administration.	N.B: If subjects have a medical need to take any medication or have any medications prescribed to them by a doctor, they should follow the medical advice but inform the Investigator as soon as possible afterwards. Subjects should be informed not to stop taking any medication that has been prescribed by their GP or other doctor
Any herbal remedy or dietary supplement containing St John's Wort.	Within 3 weeks before the planned first study drug administration.	After study completion/last visit.
Not consume any other substances known to be potent inhibitors or inducers of CYP P450s. This includes food or drink products containing cranberry, pomegranate, star fruit, grapefruit, pomelos, exotic citrus fruits or Seville oranges (including marmalade and juices made from these fruits).	Within 3 weeks before the planned first study drug administration.	After study completion/last visit.
Avoid excessive UV radiation exposure. Subjects should use sun protective measures and limit exposure to natural sunlight (including occupational exposure to the sun or sunbathing) and avoid artificial sunlight (tanning beds or phototherapy). Ideally, outdoor activities should be scheduled outside the hours that ultraviolet radiation is most intense, or should be performed in the shade. Subjects should be advised to use sun protective measures (such as a hat, sunglasses, protective clothing, and sunscreen).	From admission (Day -1).	At least one week after last dose administration.

Items subjects must not consume or do:	When subjects must stop:	When subjects can re-start:
Blood donation.	Within 16 weeks before the planned first study drug administration.	Three months after study completion/last visit

5.6 Criteria for withdrawal

The investigator or designee may withdraw a subject from the study if the subject:

- Is in violation of the protocol;
- Has an AE warranting withdrawal;
- Becomes pregnant;
- Meets individual stopping criteria;
- Use of/need for a prohibited medication which in the opinion of the sponsor or investigator may jeopardize the study results or represent a risk to the participant;
- Requests to be withdrawn from the study (subject withdrawal of consent);
- Is found to be considerably non-compliant with the protocol-required dosing visits;
- In the investigator's opinion, is unable to continue study participation;
- Is withdrawn from the study upon the request of sponsor or the SRC, including if sponsor terminates the study.

5.6.1 Handling of Withdrawals

In the event a subject withdraws or is withdrawn from the study, the investigator will inform the sponsor immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the investigator for protocol-specified safety follow up procedures.

Should any of the subjects be withdrawn from the study (by the investigator and/or sponsor) after being dosed, all the relevant assessments in relation to last dose should be completed as per protocol. The investigator and/or sponsor may decide to perform additional (or fewer) assessments in accordance with Table 3 – Adaptive Features.

Should a subject withdraw themselves from the study, every effort should be made to conduct and complete an Early Termination (ET) visit at an appropriate time-point. The procedures required for the final "follow up visit" should be performed at this visit. The investigator and/or sponsor may request additional (or fewer) assessments in accordance with Table 3 **– Adaptive Features**, with the subject's agreement.

A subject who fails to return for final evaluations will be contacted by the site in an attempt to have the subject comply with the protocol in accordance with the site SOPs.

When a subject withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the case report form (CRF).

6. STUDY AND CONCOMITANT TREATMENTS

The study treatments and concomitant therapies are categorised as described below and shown in Figure 2 below.

An **Investigational Medicinal Product (IMP)** is defined as a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorisation but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form.

- The term "**test IMP**" is used to indicate only the experimental product under study or development. Note: This term is more specific than "investigational medicinal product" which includes comparators and placebos.
- The term "**reference IMP**" is used to indicate any medicinal product being used as a comparator or reference substance (including placebo). Note: This term is more specific than "investigational medicinal product" which includes the experimental product and placebos.

The **non-IMP (NIMP)** is defined as any medicinal products intended for research and development trials, which does not fall within the definition of an IMP. NIMPs include:

- Rescue medication (for ineffective treatment, anticipated adverse reactions, or anticipated emergency situations): e.g. analgesic rescue medication or laxative in opioid studies, naltrexone.
- Challenge agents, e.g. skin prick tests.
- Medicinal products used to assess end-points in the clinical trial, e.g. any diagnostic agents used to assess the disease under study.
- Concomitant medicinal products systematically prescribed to the study subjects, e.g. cancer treatment in an opioid trial with cancer patients who all get the same cancer treatment according to the protocol.
- Background treatment, standard care that all patients receive in addition to the IMP and for the same indication, e.g. standard chemotherapy in addition to a new oncological product to be tested.

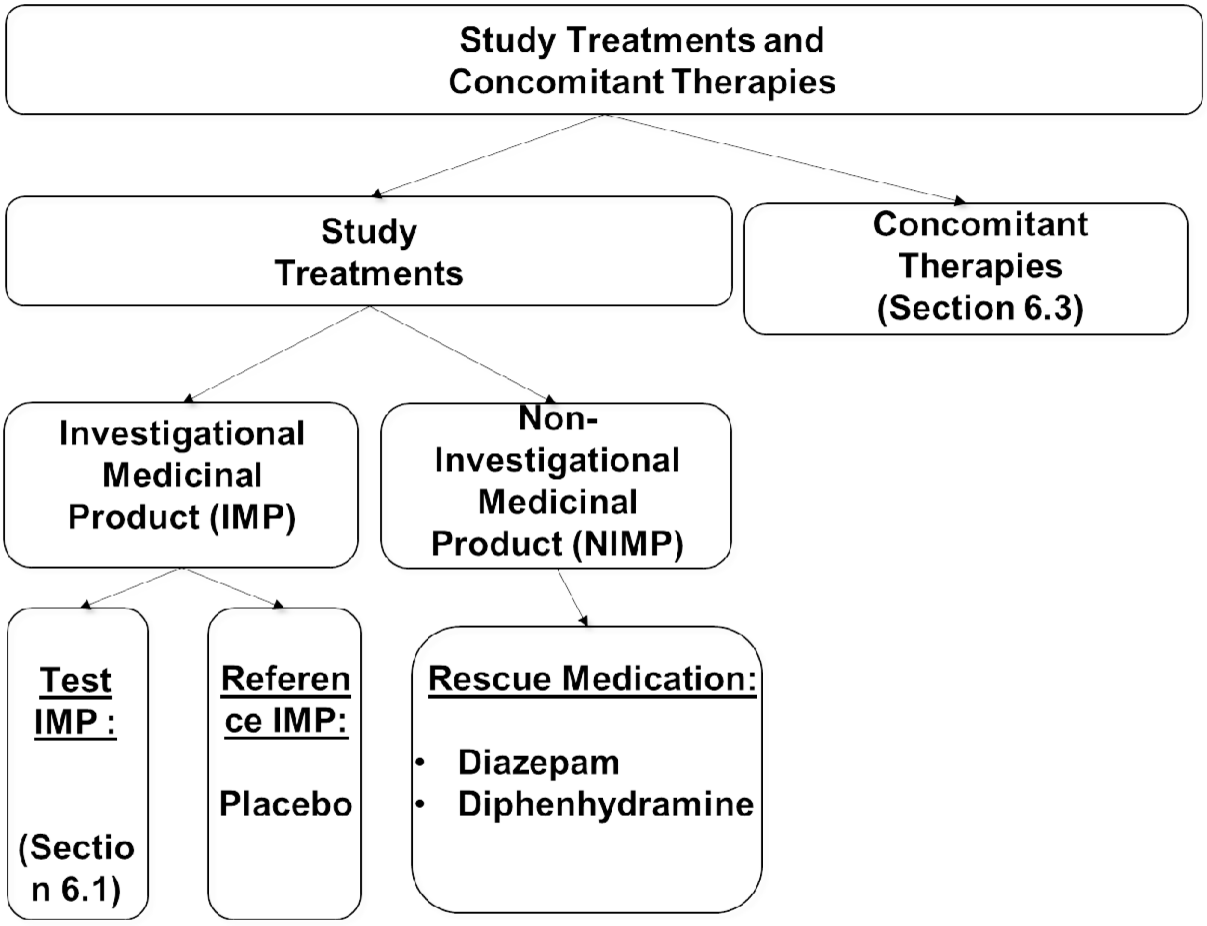


Figure 2. Study treatments and concomitant therapies

6.1 Investigational Medicinal Products (IMPs):

The following IMPs will be used in this study:

- Atovaquone-proguanil (ATV-PG) – the test IMP;
- Amodiaquine (AQ) – the test IMP;
- ATV-PG unmatched placebo– the reference IMP;
- AQ unmatched placebo – the reference IMP.

One or a combination of the following IMP strengths may be used in this study:

- Atovaquone-proguanil (ATV-PG) – 250mg-100mg tablets;
- Amodiaquine (AQ) – 153 mg dispersible tablets;
- ATV-PG unmatched placebo tablets;
- AQ unmatched placebo tablets.

ATV-PG (*Malarone*) will be sourced from the UK market from RPL's approved supplier. Amodiaquine will be sourced from outside the EU and will be imported & QP certified by RPL. Unmatched placebos will be sourced from the EU from RPL's approved supplier. Prior to being used on the clinical trial, the IMPs will be re-packaged by Richmond Pharmacology's clinical trials pharmacy. The packaged individual subject doses will be QP certified and dispensed by the site's pharmacy staff.

6.1.1 Packaging and labelling of IMPs

The labelling of the study drugs will be in compliance with Good Manufacturing Practice (GMP) specifications, as described in The Rules Governing Medicinal Products in the European Union, Volume 4, Annex 13, Investigational Medicinal Products, and any other or local applicable regulations.

Sample label(s) will be submitted to the UK health authorities according to the submission requirements.

6.1.2 Drug administration

ATV-PG, AQ and placebo are planned to be administered orally as detailed in the Table 6.

Table 6. IMP or placebo for each anticipated dose level.

Treatment	IMP*/Placebo Dispensed per dosing day		Tablet number per dosing day		Administration
	250 mg-100 mg ATV-PG tablets	Placebo tablets for ATV-PG	153 mg AQ dispersible tablets	Placebo tablets for AQ	
1000-400 mg ATV-PG + 612 mg AQ*	4	0	4	0	Doses will be administered with subjects in the sitting position. Doses will be administered by oral route with 240 mL of water. If required, an additional 240mL of water will be allowed to enable all the tablets to be swallowed.
1000-400 mg ATV-PG	4	0	0	4	
612 mg AQ	0	4	4	0	
Placebo	0	4	0	4	

* The combination treatment will not be given for future cohorts i.e. from Cohort 3 onwards

Doses of ATV-PG, AQ or placebo will be verified by a Research Physician and the details of dosing will be recorded in the CRF. The dosing will be carried out by an unblinded member of the investigator's staff. Dosing will take place behind a curtain and during IMP administration the subjects will be blindfolded. ATV-PG (or placebo) will always be given first, then AQ (or placebo).

Subjects who vomit or regurgitate the tablets within 30 minutes of dosing should be re-administered ATV-PG (or placebo) or ATV-PG (or placebo) plus AQ (or placebo) (if vomiting occurs after both drugs have been administered). They will remain in the trial and can receive subsequent doses on subsequent days..

Subjects who vomit after 30 minutes from the time of dosing should not be re-dosed. Subjects can only be re-dosed once a day.

Subjects who vomit after 30 minutes will remain in the trial and can receive doses on subsequent days.

Detailed instructions for dose administration will be included in the SOM.

6.1.3 Storage of IMPs

ATV-PG, AQ and placebo will be stored in accordance with the labelling instructions as defined in the SmPCs. The IMPs will be stored securely in a temperature-controlled pharmacy with authorised access only.

6.1.4 Drug Accountability

The designated pharmacy staff at the clinical study site will maintain accurate records of receipt and the condition of all study drugs, including dates of receipt. In addition, accurate records will be kept by the pharmacy staff of when and how much study drug is dispensed and used by each subject in the study. Any reason for departure from the protocol dispensing regimen must also be recorded.

Drug accountability records and inventory will be available for verification by the sponsor or designee. At the completion of the study, there will be a final reconciliation of all study drugs.

Study drug must not be used for any purpose other than the present study. Remaining study drug will be returned to the sponsor or its agent or its destruction arranged by the clinical study site according to applicable regulations and only after receipt of written authorization from the sponsor.

6.2 Treatment Allocation and Blinding

6.2.1 Subject Randomisation

Subjects in this study will be assigned to a treatment regimen according to a randomisation schedule generated by a statistician using PROC Plan. Details regarding the unique screening and subject number will be included in the SOM.

Eligible subjects will be randomly assigned on Day 1. According to the original protocol subjects were randomly assigned to one of four treatments in a 4:3:3:3 ratio as described below.

- Treatment 1 (n=16) - ATV-PG 1000-400 mg + AQ 612 mg;
- Treatment 2 (n=12) - ATV-PG 1000-400 mg + AQ unmatched placebo;
- Treatment 3 (n=12) - ATV-PG unmatched placebo + AQ 612 mg;
- Treatment 4 (n=12) - ATV-PG unmatched placebo + AQ unmatched placebo.

As per the stipulations of the non-substantial amendment No 05 (V1.0, dated 29JUL2019), following the completion of dosing of the first 20 randomised subjects, Treatment 1 has been discontinued from further dosing in accordance with the Adverse Reaction (AR) rules in Section 4.4. Up to 24 further subjects will be included in the study, split in three cohorts of up to eight subjects.

The remaining three treatments will be re-randomised in such a way that across the whole study there will be still 12 subjects dosed with Treatment 2, 12 subjects with Treatment 3 and 12 Subjects with treatment 4. This is taking into account both the already dosed subjects with their originally randomised treatments and the newly randomised subjects yet to be included.

Furthermore, within each cohort of up to eight subjects, each of the three treatments (2, 3, and 4) needs to be represented with a maximum of four and a minimum of one subject in the same cohort.

6.2.2 Methods for ensuring blinding

This study will be conducted in a double-blind fashion whereby subjects and clinical study site staff are blinded.

Because the placebo tablets are not an exact match for the active treatment, tablets will be administered by an un-blinded member of clinical study staff, who will protect the blinding from both subjects and all other site staff. Dosing will take place behind a curtain and during IMP administration the subjects will be blindfolded.

The pharmacy staff preparing the investigational products will not be blinded to study drug assignment. During the study, the individual randomisation codes will be kept in the site's clinical trials pharmacy, accessible to the pharmacy personnel only. Upon completion of the study, after the database lock and after the blind is revealed, the randomisation list will be filed in the Trial Master File.

Sponsor staff involved in clinical decision-making (such as those involved in SRC decisions) will be blinded to study drug assignment.

6.2.3 Methods for unblinding the study

In the event of an emergency, an envelope for each subject containing his/her study drug(s) assignment will be available in the pharmacy at the clinical study site. Unblinding should only be considered for the safety of the subject. If unblinding is deemed necessary by the Investigator, the Investigator or designee can unblind the subject's treatment allocation using the envelope available from the pharmacy. The Investigator or designee must note the date, time and reason for unblinding and inform the sponsor of unblinding as soon as practicably possible.

6.3 Concomitant medications/Permitted medications

The use of hormonal contraception and hormone replacement therapy is permitted. Subjects using stable doses of prescription or over-the-counter medications for stable conditions may be enrolled at the discretion of the Investigator. During the study, other prescription or over-the-counter medications may be permitted at the discretion of the Investigator. The need for other medication may lead to subject's withdrawal from the study. In any case, the Investigator will inform the sponsor about the concurrent medication given.

Paracetamol should be used for rescue pain relief only. Use of paracetamol for other indications should be avoided and should only be given with the Principal Investigators' approval. The dose and reason for paracetamol use in AEs other than as rescue medication must be recorded in the CRF and source record. While outside of the unit, a maximum of 1 g paracetamol a day is permitted. During in-house stays, a maximum of 4 g per day is permitted (under investigator supervision), but this will be limited as much as possible and administered case by case.

Details of all other prior and concomitant medications should be recorded by the Investigator on the CRF and source record.

6.4 Rescue Medication

Diphenhydramine:

Diphenhydramine can be given if there is airway compromise, or a subject is at risk of airway compromise. Emergency airway intervention maybe necessary if there are laryngeal and pharyngeal dystonic reactions, as they increase the risk of imminent respiratory arrest. This is a known risk of Amodiaquine. Onset of actions of diphenhydramine should be within minutes. Medication can be given as below:

a. Can be given orally (50mg).
b. Can be given IM/IV (50mg).
c. IM/IV dose (50mg) can be repeated once, if needed.

Diazepam:

Oral diazepam can be given to treat anxiety (with close clinical monitoring for side effects), or to help manage Extrapyramidal Symptoms (EPS). Two subjects experienced anxiety in Cohort 1 and it is felt that planned management would be prudent. Giving diazepam would be considered if Grade II (CTCAE) or higher anxiety is experienced. As possible drug-induced anxiety in this study would be expected to be a transient effect, efforts should be made to avoid using diazepam within 2 hours following the appearance of Grade 2 anxiety. Medication can be given as below:

a. Give a single 2mg oral dose initially.
b. Can give up to 30mg daily, in divided doses.
c. If oral route not possible (e.g. severe anxiety attack), can be given intramuscularly 10mg.
d. To be given at investigator's discretion.
e. If ongoing treatment is required, then escalating management to the local emergency department should be considered.

Indication for giving Diazepam:

- Interfering with ADLs (Activities of Daily Living).
- Affecting ability to remain in trial/on ward.
- Subject feeling significant discomfort.
- Possibly causing additional AEs.
- Doctor feels anxiety is significant enough to warrant intervention

7. STUDY METHODOLOGY

7.1 Medical history

All clinically significant medical history (including any significant surgical procedures) must be recorded for each subject. Each subjects' full medical history will be obtained through direct questioning and the medical assessment at screening and updated at Day -1. If any clinically relevant observations are made prior to dosing, they will be counted as medical history.

7.2 Meals

Standardised meals will be provided during the study period according to the timings described in the Schedule of assessments (Table 1 and Table 2). Details of the meals will be contained in the SOM. Restrictions relating to meals/water apply, see Table 5.

7.3 Clinical laboratory assessments

Laboratory parameters to be measured are presented in Table 7.

Table 7. Laboratory parameters.

Haematology
- Platelets
- Haemoglobin
- Haematocrit
- White blood cells
- Neutrophils
- Eosinophils
- Basophils
- Lymphocytes
- Monocytes
- Red blood cells
- Mean cell haemoglobin (MCH)
- Mean corpuscular haemoglobin
- concentration (MCHC)

Biochemistry
- Aspartate aminotransferase
- Alanine aminotransferase
- Alkaline phosphatase
- BUN
- Gamma GT
- Total bilirubin
- Conjugated bilirubin
- Creatinine
- Urea
- Total Serum Proteins
- Albumin + Alpha1 Glycoprotein (AGP)*
- HbA1c**
- Sodium
- Potassium
- Calcium
- Corrected calcium
- Magnesium
- Chloride
- Bicarbonate
- Amylase
- Total cholesterol
- Triglycerides
- Serum pregnancy test***
- FSH
- Oestradiol#
- Progesterone#
- Testosterone#

Urinalysis
- Leukocytes
- Nitrite
- Urobilinogen
- Protein
- pH
- Blood
- Specific gravity
- Ketones
- Bilirubin
- Glucose
- Urine microscopy

Urine Screen for Drugs of Abuse
- Benzodiazepines
- Opiates
- Amphetamines
- Methadone
- Cocaine
- Cannabinoids
- Barbiturates

Serology
- Hepatitis B surface antigen (HBsAG
- Hepatitis B core antibody (anti-HBC IgG + IgM, if IgG positive)
- Hepatitis C antibody (anti-HCV)
- HIV I and II antibodies

Coagulation
- aPPT
- PT
- INR
- Fibrogen

***AGP to be measured on Day -1 only, and not required for subject eligiblity**
**** Measured only at screening**
*****Measured at the times presented in the Schedule of assessments (Table 1)**
#Measured for all female volunteers on Day 3 only

7.3.1 Haematology, biochemistry and coagulation

Blood samples for determination of biochemistry, haematology and coagulation parameters will be taken at the times given in the Schedule of assessments (Table 1). The date and time of collection will be recorded on the appropriate CRF pages. The analyses will be done using routine methods. Further details will be described in the SOM.

7.3.2 Serology

Serology will be performed at Screening as detailed in the Schedule of assessments (Table 1). At the screening visit all subjects will be tested for the parameters listed in Table 7. This is done for the safety of the study personnel and the result from the tests will not be entered into the study database.

If a subject is found to be confirmed positive in any of these tests, they will be referred for further examination/treatment and will not be included in the study, with the exception of subjects with a confirmed positive anti-HBc IgG and negative anti-HBc IgM and negative

HBsAg, indicative of natural immunity due to a past infection without active chronic or acute infection.

The serology tests will be analysed in the same blood sample used for biochemistry.

7.3.3 Urinalysis

Urine samples for determination of urinalysis parameters will be taken at the times given in the Schedule of assessments (Table 1). If deemed necessary, based on a clinically significant positive test, microscopic examination of urine will be performed. Microscopic examination result will supersede the urine dip result.

7.3.4 Drugs of Abuse

Urine will be tested for the drugs of abuse as described in the Schedule of assessments (Table 1). If a subject fails the drugs of abuse screen, they will be excluded from the study. A repeat drug screen can be done where methodological reasons are believed to have led to a false positive. If subjects are suspected to be positive due to medication e.g. flu/cold remedies, they may undergo a repeat drug screen.

7.4 Alcohol Breath Test

An alcohol breath test will be done using an alcometer as described in the Schedule of Assessments (Table 1). If a subject tests positive to the test they will be excluded from the study.

7.5 Vital Signs

7.5.1 Blood pressure, pulse rate, respiratory rate and tympanic temperature

Vital signs will be measured at the time points as detailed in the Schedule of Assessments (Table 1). Blood pressure and pulse rate will be measured in supine position after the subject has rested comfortably for at least 5 minutes using automated blood pressure monitors. Respiratory rate will be measured by manual counting for one minute. Temperature will be measured using tympanic thermometers.

7.6 Electrocardiographic (ECG) Measurements

7.6.1 Recording of 12-lead ECGs

12-lead ECGs will be recorded at the time-points described in Schedule of assessments (Table 1 and Table 2) using a GE Marquette MAC1200® /MAC1200ST® recorder connected via a fixed network connection to the MUSE® Cardiology Information System (MUSE). ECGs recorded during screening will be stored electronically on the MUSE information system. Only ECG recorded electronically will be valid ECG for any purpose other than safety assessment. ECG printouts may be filed in the subject's CRF for medical safety reviews.

Each ECG recorder will be set up to the required technical specifications and containing the information required to identify the records. Each ECG recording will be clearly identified (Subject ID, visit date, and the actual times of ECG recordings).

12-lead ECG recordings will be made after the subjects have been resting in a supine position for at least 10 minutes. The subjects will avoid postural changes during the ECG recordings and clinical staff will ensure that subjects are awake during the ECG recording.

At each time point, the ECG will be recorded in triplicate, to reduce variance and improve the precision of measurement. The triplicates will be performed at approximately 1-minute intervals. Each ECG recording (trace) will last 10 seconds. Repeat ECG will be performed until at least three 10-second ECG records per scheduled time-point meet the quality criteria set out in the SOM and the applicable SOP to enable reading and analysing at least 5 complexes per derivation.

7.6.2 Safety review of 12-lead ECGs

All recorded ECG will be reviewed by a Research Physician and the review be documented in the CRF. If a subject shows an abnormal ECG, additional safety recordings (including the use of 5 or 12 lead Holter equipment) may be made and the abnormality be followed to resolution if required.

7.6.3 24-hour Holter ECG

Holter recording will be performed at screening as described in Table 1. Each electronic Holter ECG file will be downloaded onto the GE Gatemed Holter Analysis system.

7.6.4 Real time display (ECG telemetry)

A 12-lead real time ECG will be recorded as described in the Schedule of Assessments (Table 1). ECG telemetry will be monitored by the investigator or qualified member of clinical staff.

The system will be managed according to local working practices. The ECG telemetry reports will be archived with study documents.

7.6.5 Analysing and over-reading 12-lead ECG for the purpose of intensive cardiac assessments

Each electronic ECG will contain the ECG data as well as the result of the automated ECG analysis performed by the Marquette® 12SL™ ECG Analysis Program (MEAP), a program resident in each of the ECG machines. All ECG and their associated automated interval measurements will subsequently be reviewed by qualified Cardiologists in accordance with the ICH E14 Guidance for Industry document and ICH E14 Implementation Working Group Questions and Answers document before any of the ECG are used for the thorough ECG analysis. The manual adjudication process applied in this study is also referred to in the ICH guidance and relevant literature as “manual over-read”, “computer-assisted” or “semi-automated” ECG measurements. The following parameters on each ECG will be assessed by a cardiologist using the commercially available MUSE® in its latest version:

- QT interval
- RR interval
- Heart rate (HR)
- PR interval
- Presence or absence of U-wave
- Quantitative and qualitative ECG variations

Manual on-screen over-reading using electronic callipers in MUSE® will be performed by a small and select group of cardiologists with extensive experience with manual QT measurement (including on-screen measurement with electronic callipers). For all study ECG the over-reading cardiologists will be blinded to time, date, treatment and any data identifying the subject. All ECG of a given subject will be over-read by the same cardiologist (or cardiologists in case manual adjustments of the automated measurement are necessary).

7.7 Physical Examination, Height and Weight

The full physical examination performed at screening will include an assessment of the following: general appearance, skin, eyes, ears, nose, neck, lymph nodes, throat, heart, lungs,

abdomen, musculo-skeletal system and extremities. The timings of the physical examinations are described in the Schedule of assessments (Table 1).

Height will be measured in centimetres and weight in kilograms. Measurements should be taken with subjects wearing light clothing and without shoes using calibrated scales for all measurements. BMI will be calculated from the height and weight. Full details will be described in the SOM.

7.8 Profile of Mood Scales questionnaire

Subjects will be asked to complete a self-report questionnaire for the assessment of mood as described in the Schedule of assessments (Table 1). Details will be described in the SOM.

7.9 Basic neurological exam

A neurological examination will be carried out as described in the Schedule of Assessments (Table 1). This is will include (as a minimum) assessment of tone, power, coordination and proprioception of all limbs; gait; examination of the cranial nerves; and assessment of mental status. Details will be described in the SOM.

7.10 Pharmacokinetic Assessments

7.10.1 PK blood samples

For timing of individual samples refer to the Schedule of assessments (Table 1 and Table 2). The date and time of collection will be recorded on the appropriate CRF.

Plasma samples for determination of atovaquone, proguanil, cyloguanil, amodiaquine and desethyl-amodiaquine concentrations will be analysed by Swiss BioQuant using a validated method. Full details of the analytical methods used will be described in a separate bioanalytical report. All sample handling procedures, including the time of each sample collection, the time of placement into frozen storage (at the end of the sample workup), and the date of transfer or shipment of the samples to the responsible analyst will be documented in detail in the SOM.

7.11 Volume of Blood Sampling

The maximum total blood volume collected from subjects participating in this study will not exceed 500 mL. Details will be described in the SOM.

8. ADVERSE EVENTS

The collection, evaluation and reporting of adverse events/reactions arising from this clinical study will be performed in accordance with:

- Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3') (2011/C 172/01).
- International Conference on Harmonization (ICH) harmonised tripartite guideline on clinical safety data management: "Definitions and standards for expedited reporting" E2A.
- ICH harmonised tripartite guideline on development safety update report: E2F.
- ICH guideline E2F "Note for guidance on development safety update reports (DSUR)".

It is the investigator's responsibility to document and report all adverse events occurring in the clinical trial. The period of observation for collection of adverse events extends from the signing of the informed consent form up to the final visit. Additionally, spontaneously reported serious adverse events (SAEs) will be collected up until 30 days after the final study visit. SAEs

experienced after this 30-day period will only be reported if the investigator suspects a causal relationship with the study drug.

8.1 Definitions

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 medicinal product, whether or not considered related to it.

An AE may be:

- A new symptom or medical condition;
- A new diagnosis;
- An inter-current illness or an accident;
- A worsening of a medical condition/diseases existing before the start of the clinical trial;
- The recurrence of a disease;
- An increase in frequency or intensity of episodic diseases;
- A change in a laboratory or other clinical test parameter.
    - The criteria for determining whether an abnormal test result should be reported as an AE/ADR are as follows:
        - The test result is associated with accompanying symptoms, and/or
        - It requires additional diagnostic testing or medical/surgical intervention
- It leads to a change in trial dosing outside of protocol-stipulated dose adjustments, or discontinuation from the trial, significant additional concomitant drug treatment, or other therapy, and/or
- It is considered to be an adverse event by the investigator or sponsor.

An AE does not necessarily include the following:

- An abnormal test that needs repeating, in the absence of any of the above conditions. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.
- Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE/ADR. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs, if the condition leading to the measure was present before inclusion in the trial and has not worsened. In the latter case, the condition should be reported as medical history.

A **serious adverse event (SAE)** is defined as any adverse event that fulfils any of the following criteria:

- it results in death;
- it is life-threatening (the term "life-threatening" in the definition of "serious" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe);

- it requires a hospitalisation* or prolongs existing hospitalisation;
- it results in persistent or significant disability/incapacity;
- it is a congenital abnormality/birth defect;
- it is considered medically important (medical and scientific judgement should be exercised in deciding whether other AE/ADRs are to be considered serious, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias; convulsions that do not result in hospitalisation; development of drug dependency or drug abuse).
- for the purpose of this protocol an AE will be considered as serious should the AE constitute a possible Hy's Law case (defined as a subject with any value of ALT or AST greater than or equal to 3x upper limit of normal (ULN) together with an increase in bilirubin to a value greater than 2xULN (>35% direct) and NOT associated to an ALP value greater than 2xULN.

*'Hospitalisation' in the context of SAEs does not include the following: rehabilitation or nursing facility; hospice; presentation to emergency departments or other urgent care centres; admissions to hospital in-patient facilities for logistical reasons only that did not result in any therapeutic intervention (e.g. lack of senior medical staff, investigations or transport home overnight); admissions to hospital in-patient facilities for investigation alone and where no significant abnormality was identified and/or no therapeutic intervention was necessary; same-day surgery; admissions for pre-existing condition not associated with the AE; protocol-specified admission; pre-planned admission.

"Hospitalisation" will include hospital visits where the subject remains an inpatient for >24 hours (from when the decision to admit is made, until the decision to discharge is made)

An **Adverse Reaction (AR)** is a response to a medicinal product which is noxious and unintended, and which occurs at any dose (in pre-approval clinical experience) or at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function (in post-approval clinical experience). The term "reaction" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility. This means that there are facts (evidence) or arguments to suggest a causal relationship.

A **serious adverse reaction** is any adverse reaction that fulfils the criteria of seriousness, as defined above.

A **suspected unexpected serious adverse reaction (SUSAR)** is a serious adverse reaction that is unexpected. The 'expectedness' of a serious adverse reaction is assessed in the light of the reference safety information.

8.2 Classification

Severity:

The investigator will assess the severity of AEs. All AE/ARs will be graded for severity with guidance from CTCAE v5.0, published 27 Nov 2017.

Changes in the severity of an AE/AR should be documented to allow an assessment of the AE/AR duration at each level of severity.

Application of CTCAE:

Background

CTCAE terminology and grading was developed by the US National Cancer Institute (NCI) to achieve standardised classification of adverse drug reactions in cancer therapy. It is the most comprehensive set of standardised criteria available and is updated regularly. The current version 5.0 was released in November 2017.

The CTCAE displays AE Grades I through V with detailed clinical descriptions of severity for individual conditions and symptoms. The standard grade definitions are described as follows:

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

Grade II: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*.

Grade III: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL**.

Grade IV: Life-threatening consequences; urgent intervention indicated.

Grade V: Death related to AE.

*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.

The NCI CTCAE criteria and their interpretation are consistent with the standard intensity grading for AEs during clinical trials: Grade I: mild, Grade II: moderate, Grade III: severe or medically significant but not immediately life-threatening.

Limitations

The CTCAE grading is now used in many clinical trials outside of oncology, where the terminology and grading may not always be accurate or applicable for non-cancer patients and healthy volunteers. In some cases it can be too limited in terms of available grades for specific diagnoses, and at other times it may lack the specificity to achieve appropriate grading for a listed diagnosis. CTCAE does not permit grading based on deviation from individual patients' baseline conditions, with few exceptions. There is also a significant overlap between listed severe Grade III and serious adverse events.

Standard toxicity terminology and grading according to NCI CTCAE, version 5.0 will therefore be used as a guide, rather than a rigid rule, to assess AEs and abnormal measurements.

Further details can be found in the SOM.

Causality:

The causality assessment of an AE to the IMP will be rated as follows by the investigator:

Reasonable Possibility: An AE which can be reasonably explained that the study drugs caused the AE by available facts (evidence) or arguments. For example, the occurrence of

the AE cannot be explained by other causative factors, but can be explained by pharmacological effect of the study drug such as:

- Temporal relationship to IMP exposure.
- Event is known to be associated with the IMP drug class.
- Event improved on discontinuation or dose reduction of IMP.
- Event reoccurred on re-challenge of IMP.
- Biological plausibility.
- Other (must be specified).

No Reasonable Possibility: An AE which cannot be reasonably explained by available facts (evidence) or arguments that the study drug caused the AE. For example, the occurrence of the AE can be explained by other causative factors, such as:

- Event attributed to concomitant medication (provide details of the concomitant medication).
- Event attributed to the concurrent disease (s) / condition(s) (provide details of the disease/condition).
- Event attributed to a non-investigational medicinal product (NIMP) (specify the NIMP).
- No reasonably temporal relationship associated with IMP administration.
- Event is expected in the study indication and/or target population.
- Negative de-challenge and/or negative re-challenge.
- Other (must be specified).

The investigator should also comment on the adverse event page of the Case Report Form whether an adverse event is not related to the study treatment but is related to study participation (e.g. study procedures, wash-out periods etc.).

Expectedness (reference safety information):

The expectedness of an adverse reaction is determined by the sponsor. An adverse reaction, the nature or severity of which is not consistent with the applicable reference safety information (IB for drugs in clinical development or SmPC for marketed drugs) is unexpected. Reports which add significant information on the specificity, increase in the occurrence or severity of a known and already documented serious adverse reaction are unexpected events. This should be done from the perspective of events previously observed, not on the basis of what might be anticipated from the pharmacological properties of a medicinal product.

For this protocol, the reference document for the assessment of expectedness is the *Malarone* U.K. SmPC and amodiaquine (as hydrochloride) 153mg dispersible tablets SmPC.

Adverse events of special interest (AESI):

An AESI is an adverse event of scientific or medical concern specific to the sponsor or the particular product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate. It may require further investigation in order to characterise and understand them. It could be serious or non-serious and could include events that might be potential precursors or prodromes for more serious medical conditions in susceptible individuals.

The AESIs for this protocol have been pre-defined:

A. Hepatic
   1. Possible Hy's law case: defined as a subject with any value of ALT or AST above 3x ULN together with an increase in bilirubin to a value higher than 2x ULN and not associated with an ALP value higher than 2x ULN*.
   2. Any ALT or AST above 8x ULN.
   3. Any AST or ALT above 3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil percent or count above the ULN).

B. Cardiac
   1. QTcF prolongation from baseline of >60 msec.
   2. QTcF at any time >480 msec.
   3. Complete bundle branch block.
   4. Any arrhythmia.

C. Haematological
   1. Hb drop >25% or >2.0 g/dL from baseline.
   2. Clinicallly significant drop in neutrophil count .
   3. Platelets count <100x10^9/L

D. Clinical signs of possible Cutaneous reactions such as:
   1. Urticaria
   2. angioedema
   3. Rash (erythematous, macular, macculo-papular, popular, pruritic, pustular, vesicular)
   4. Discoloration
   5. Dermatitis

E. Pregnancy
   1. Pregnancy
   2. Pregnancy in partner of a male volunteer

8.3 Recording of adverse events and follow-up

All (serious and non-serious) adverse events detected by the investigator or spontaneously notified by the subject at each visit/examination must be reported on the special section of the CRF.

The following information should be reported for each adverse event, whether or not it can be attributed to trial drug:

- Description of adverse event
- Date of onset/date of resolution
- Characteristics of the event (seriousness, intensity)
- Actions taken (treatment required or dose adjustments must be reported in the CRF)

- Outcome
- Relationship with trial drug (causality assessment) and/or study participation

All adverse events must be documented and followed up until the event is either resolved or a satisfactory explanation is found, or the investigator considers it medically justifiable to terminate the follow-up. The reason(s) will be recorded in the CRF when the AE follow-up is terminated.

Borderline abnormal laboratory findings and other objective measurements should NOT be routinely captured and reported as AEs, as they will be collected and analysed separately. However, abnormal laboratory findings or other objective measurements that meet the criteria for a SAE, result in discontinuation of the Investigational Medicinal Product, require medical intervention or are judged by the Investigator to be clinically significant changes from baseline values should be captured and reported in the CRF.

When recording an abnormal laboratory finding in the CRF, a clinical diagnosis should be provided rather than the abnormal value itself, if this is available (for example, “anemia” rather than “decreased red blood cell count” or “hemoglobin = 10.5 g/dL”).

8.4 Reporting of serious adverse events and AESI

Detailed reporting procedures will be contained in the pharmacovigilance plan.

If an SAE or AESI occurs, the investigators will take appropriate action immediately and will strive to identify the causes of the events.

All SAEs and AESI will be notified by the investigator to the pharmacovigilance provider (Prime Vigilance) within 24 hours of awareness by email or by Fax to:

Email: MMV@primevigilance.com

Fax: +44 (0)800 471 5694

using the “SAE Report Form” or "AESI Report Form" for SAEs and non-serious AESI respectively. The pharmacovigilance provider will, within 1 working day after the receipt of the form notify the sponsor and ICON Medical.

The follow-up observation period, for the concerned subjects, will be jointly decided by the investigator, sponsor and/or SRC.

The initial SAE report will be followed up by a full written report within three working days or five calendar days, whichever comes first unless no further information is available. In this case, the follow-up report will be provided as soon as new information becomes available. Further follow-up reports will be provided as and when new information becomes available. Photocopies of relevant CRF pages, such as demography, medical history, concomitant medications, as well as test results, consultant report(s), a summary of the outcome of the reaction will accompany the SAE form if and when available.

The sponsor will also perform an evaluation of all SAEs.

SUSARs will be notified to the Competent Authority and to the relevant RECs by the Prime Vigilance within 7 (for fatal and life-threatening SUSARs) or 15 days (all other SUSARs).

Annual safety reporting to the national Competent Authority and the Ethics Committee will be in agreement with ICH guideline E2F “Note for guidance on development safety update reports (DSUR)”.

8.5 Pregnancy

Any female subject who becomes pregnant during the study and the following 3 month after the last dosing should be followed through delivery or termination of the pregnancy. Pregnancy is reported to Prime Vigilance (acting on behalf of MMV) by completion of the pregnancy form.

Subjects who become pregnant during the study will be offered counselling and must be followed up until delivery. In pregnancies that progress to term, any congenital abnormalities/birth defects in the offspring of a patient who received the study medication should be reported on an SAE. The pregnancy outcome and the presence or absence of a congenital abnormality will be documented by completion of a pregnancy form.

If a male subject's female partner becomes or is found to be pregnant while the subject was being treated or exposed to study drug, the investigator must submit the "Pregnancy Reporting and Outcome/Breast Feeding Form" to RPL and MMV via the same method as SAE reporting.

Male subjects may continue in the study if an accidental pregnancy of their female partner occurs despite adequate contraception.

The male subject's partner should be followed until the outcome of the pregnancy is known (spontaneous miscarriage, elective termination, normal birth or congenital abnormality), even if the male subject discontinues study drug(s) or discontinues from the study. When the outcome of the pregnancy becomes known, the form should be updated and returned to RPL and MMV. If additional follow-up of the female partner is required, the investigator will be requested to provide the information.

Pregnancy in itself is not regarded as an AE, unless there is a suspicion that investigational product may have interfered with the effectiveness of a contraceptive medication. However, complications of pregnancy and abnormal outcomes of pregnancy are AEs, and many may meet criteria for an SAE. Complications of pregnancy and abnormal outcomes of pregnancy, such as ectopic pregnancy, spontaneous abortion, intrauterine foetal demise, neonatal death, or congenital anomaly, would meet the criteria of an SAE and therefore should be reported as an SAE. Elective abortions without complications should not be handled as an AE.

9. QUALITY ASSURANCE AND QUALITY CONTROL

9.1 Quality Assurance and Quality Control

A regulatory inspection of this study may be carried out by regulatory agencies. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the PI and RPL agree to allow the auditor/inspector direct access to all relevant documents and to allocate their time and the time of their staff to the auditor/inspector to discuss any findings or relevant issues.

Quality Control (QC) procedures at the RPL will be implemented to ensure data recorded into the CRFs are accurate. QC checks will be carried out on an ongoing basis and according to the relevant SOPs. Records of QC checks will be documented and available for review.

9.2 Monitoring

All aspects of the study will be carefully monitored by the sponsor, or designee, for compliance with applicable government regulations with respect to Good Clinical Practice (GCP) and current standard operating procedures.

The monitoring of this study will be performed by the sponsor's monitor(s) or a designee in accordance with the principles of GCP as laid out in the International Conference on Harmonisation (ICH) Good Clinical Practice Guideline E6(R2) (2016).

The clinical monitor, as a representative of the sponsor, has an obligation to follow the study closely. In doing so, the monitor will visit the investigator and site periodically as well as maintain frequent telephone and email contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and staff. Further details will be described in the SOM.

10. STATISTICAL ANALYSIS

10.1 Statistical Analysis Plan

A statistical analysis plan (SAP) containing detailed statistical methodology will be written and signed off before the DB hard lock. The plan may be updated to reflect adaptive features of the study as appropriate.

10.2 Analysis sets

The analysis of data will be based on different analysis sets according to the purpose of analysis. Subject eligibility for each analysis set will be finalised before the DB hard lock. A subject who withdraws prior to the last planned observation in a study period will be included in the analyses up to the time of discontinuation.

Safety set

The Safety set will consist of all randomised subjects who received at least one dose of the IMP. The safety set will be used for the safety analyses.

PK set

The PK set will consist of those subjects in the safety set with a sufficient plasma profile to allow AUC to be derived. The PK set will be used for the presentation of the PK analyses.

ECG set

The ECG set will consist of those subjects in the safety set that have at least one valid pre-dose ECG assessment and one valid post-dose assessment. An ECG assessment will be considered valid if it is based on at least two evaluable replicates with measurable QTc.

The analysis set for intensive cardiac assessment will be based on the intersection of the PK set and the ECG set. In addition, subjects on placebo will be included with plasma concentrations set to 0. Individual QTc/concentration pairs will be excluded from this set if the time of ECG and the time of blood sampling are too far apart. Details will be defined in the SAP.

10.3 Statistical analysis of safety

Individual subject demographics (age, gender and race) and body measurement data (height, weight and BMI) at screening will be listed. These demographic characteristics and body measurements will be summarised by treatment group and overall (mean, median, standard deviation, minimum, maximum), using the safety analysis set. Other baseline characteristics will be listed only.

AE, AESI and SAE data will be listed and summarised using descriptive statistics: the number (and %) of subjects who had any AEs and the number of AE episodes will be summarised for each dose. All AEs will be summarised and listed by using system organ class (SOC) and preferred term (PT) assigned to the event using Medical Dictionary for Regulatory Activities

(MedDRA). Furthermore, these events will be summarised by the maximum intensity. The number of subjects who had drug-related AEs will also be summarised. Any AESI, SAEs and/ or AEs that led to withdrawal will be summarised and listed.

Vital signs data (SBP, DBP, pulse rate, Respiratory Rate, Temperature) will be listed and summarised, along with changes from baseline, using descriptive statistics (mean, median, standard deviation, minimum, maximum). Out-of-reference-range values will be flagged as high (H) or low (L) and as being clinically relevant or not: the number of subjects presenting out-of-range and clinically relevant values will be summarised.

All safety clinical laboratory data will be listed. Laboratory test results will also be compared to laboratory reference ranges and those values outside of the applicable range will be flagged as high (H) or low (L) and as being clinically relevant or not: the number of subjects presenting out-of-range and clinically relevant values will be summarised. The quantitative laboratory data, along with changes from baseline will be summarised using descriptive statistics (mean, median, standard deviation, minimum, maximum). Change from baseline values at each assessment will be calculated as the assessment value minus the baseline value. The qualitative urinalysis data will be listed only.

Results from the POMs questionnaire will be listed and along with changes from baseline will be summarised using descriptive statistics (n, mean, median, standard deviation, minimum, maximum).

Basic Neurological Examination data will be listed only.

ECG analyses will be performed on two sets of ECGs: all ECGs prior adjudication and selected triplicates from each time-point after adjudication.

All un-adjudicated ECG data (PR, QRS, QT, QTcB, QTcF and HR) and overall ECG evaluation will be listed. ECG data, along with changes from baseline will be summarised using descriptive statistics (mean, median, standard deviation, minimum, maximum).

Furthermore, categorical analysis of QTcF data will be presented as follows:

- Absolute QTcF interval prolongation
  - QTcF interval > 450 ms
  - QTcF interval > 480 ms
  - QTcF interval > 500 ms
- Change from baseline in QTcF interval
  - QTcF interval increases from baseline > 30 ms
  - QTcF interval increases from baseline > 60 ms

Mean value of QTcF parameters will be plotted by dose group and time point. Placebo values will be pooled.

Likewise, categorical analyses of other quantitative ECG parameters will be given. More specifically, an increase in PR from predose baseline >25% to a PR>200 ms; an increase in QRS from predose baseline >25% to a QRS >120 ms; a decrease in HR from predose baseline >25% to a HR <50 bpm or an increase in HR from predose baseline >25% to a HR >100 bpm will be summarised.

10.4 ECG Analysis

Intensive cardiac assessments will be performed using food effects on the ECG to establish assay sensitivity. Analysis of drug related QT/QTc interval changes relative to plasma PK concentrations will be conducted. The principles of this analysis follow the statistical methods

described by Garnett et al., 2018 [21]. The ECG utilised for this analysis require adjudication by qualified cardiologists in accordance with principles set out in the ICH E14 guideline and subsequent Q&A documents. All ECG recordings are in triplicate and will be compliant with the correct recording and manual adjudication of ECG in thorough QT/QTc studies.

Two complementary sets of analyses will be performed: a per timepoint analysis and a concentration-QTc analysis. While the first is based on minimal assumptions on the nature of the data, it has limited power to show the absence of a prolonging effect. On the other hand, the concentration-QTc analysis has shown to have this power, but it is based on more restrictive assumptions.

10.4.1 Heart rate correction

The analysis of drug related QTc-interval changes will be based on Fridericia-corrected QT (QTcF) unless one of the treatments induces a change in heart rate exceeding 10 bpm. In this case, an individual correction (QTcI) will be established based on the pre-drug data and the analyses described below will be repeated with this QTcI.

10.4.2 Per timepoint analysis

For all quantitative ECG parameters, descriptive statistics will be given for the change from baseline. In addition, for QTc, a linear model will be fitted for each timepoint and the difference between each of the active treatments and placebo will be estimated based on this model and two-sided 90 % confidence intervals be given.

Details of the model selection procedure and the concentrations to be included in the best fitting model will be given in the statistical analysis plan.

10.4.3 Concentration-QTc analysis

This analysis will be based on the change of QTc from baseline and will use baseline QTc and the concentrations of AQ, DEAQ and relevant analytes/metabolites (when AQ administered alone and in combination with ATV-PG) as covariates. Treatment and time will be used as discrete fixed effects. A series of linear models including one or more of the concentrations will be fitted and the most appropriate one will be selected based on the AIC (Akaike Information Criterion) and the size of the fixed treatment effect. A significant treatment effect (based on an F-test) will be considered an indication for model misfit. For the best fitting linear model, predictions of the effect on QTc will be made at the mean concentrations of the moieties involved at the t_{max} for each of these moieties.

If the per-timepoint analysis described above excludes an effect exceeding 10 ms – i.e. the all confidence intervals are below this value – the best fitting linear model will be accepted. If this is not the case and the best fitting linear model has a significant treatment effect, nonlinear e-max models will also be considered, and predictions will be made based on the best fitting model. In this case, hysteresis between QTc and the concentrations of the relevant analytes and metabolites will be investigated graphically.

Absence of an effect of concern of the IMP on QTc will be concluded if the predictions based on the best-fitting model at the concentrations described above exclude an effect exceeding 10 ms, i.e. the upper limit of the two-sided confidence interval for these predictions is below 10 ms.

10.4.4 Assay sensitivity

Assay sensitivity will be assessed based on the estimates of the time effect of the best fitting. More specifically, for each time point in the window 2-4 hours after a meal model (breakfast on

Days 1 and 3 and lunch on Days 1 and 3) the change from the relevant time point before the meal (i.e. pre-dose baseline for breakfast of Days 1 and 3, the average of the 4 and 5 hours values for lunch) will be tested on the one-sided 5 % level. If this change is significantly negative (point estimate around -5 ms or below and the 95 % CI <0), assay sensitivity will be considered shown. Details and considerations on multiplicity will be given in the SAP.

10.5 Pharmacokinetics

10.5.1 Evaluation of Pharmacokinetic Parameters

Non-compartmental analysis will be used for estimation of pharmacokinetic parameters.

The following pharmacokinetic parameters will be calculated for atovaquone (ATV), proguanil (PG), cyloguanil (CG), amodiaquine (AQ) and desethyl-amodiaquine (DEAQ):

C_{max}	Maximal plasma concentration
t_{max}	Time at which the maximum plasma concentration occurs
$t_{1/2}$	Terminal elimination half-life
AUC_{0-t}	Area under the plasma concentration curve from time zero up to the last quantifiable concentration
AUC_{0-inf}	Area under the plasma concentration-time curve from time zero extrapolated to infinity
λz	Terminal elimination rate constant

For individual plasma concentration data, the actual time of ATV-PG and AQ administration and actual blood sampling time will be used in the derivation of the PK parameters. If there is any doubt in the actual time a sample was taken, then the scheduled time will be used.

AUC values will be calculated using the linear/log trapezoidal method, applying the linear trapezoidal rule up to C_{max} and the log trapezoidal rule for the remainder of the curve. Samples below limit of quantification (LOQ) prior to the first quantifiable concentration will be set to zero. Samples with concentrations below LOQ after the first quantifiable concentration will be set to 'missing' and omitted from the analysis. Other pharmacokinetic parameters will be calculated according to standard equations.

Details will be provided in SAP.

10.5.2 Statistical Analysis on PK Parameters

Plasma concentrations will be listed and summarised by time point (N - the number of subjects, n – the number of samples, n(LLOQ) - the number of samples <LLOQ, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum). The PK parameters will be listed for each subject and summarized for each treatment group using descriptive statistics (N - the number of subjects, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum).

10.6 Handling of Missing and Incomplete Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) for handling missing data may be reassessed at the data review prior to database lock.

10.7 Determination of sample size

This is an exploratory study to evaluate tolerability and pharmacokinetics of each treatment group and is not based on formal statistical considerations. The numbers assigned to each treatment are deemed adequate to describe the tolerability, safety and pharmacokinetics.

11. DATA MANAGEMENT

Data Management will be performed by the Data Management department of RPL. The data management process will be described in detail in the Data Handling Protocol (DHP).

The RPL Data Management department will be responsible for developing and maintaining the DHP; setting-up and validating the clinical study database; programming validation checks; entering data into the clinical study database; reviewing data for accuracy, completeness and consistency between the CRF and the database; and verifying adherence to the clinical pharmacology study protocol and the DHP.

Safety laboratory data will be loaded into the database as an electronic data transfer file according to transfer specification document.

Clinical data queries will be generated and resolved according to the DHP. They are documented individually on a Data Clarification Form (DCF) which is generated in Oracle Clinical. The DCF is a form designed to maintain an audit trail of modifications of the data in the clinical study database and the justification for those modifications. Clinical data queries are resolved with the assistance of RPL clinical staff.

After all clinical data queries are resolved, final error rate is confirmed, and QC checks are acceptable the database will be locked.

Standard SAS® datasets are generated from the final study database ready for analyses. Datasets may be converted to CDISC standard datasets SDTM and ADAM thereafter. Associated documents such as define.xml may also be generated as per the agreement with sponsor.

A complete audit trail of all corrections in the database will be available for inspection.

Medical coding will be performed by RPL. AEs, diagnoses from Medical History and procedures from Surgical History will be classified according to MedDRA. Concomitant medication will be coded using WHODRUG.

SAEs in the clinical database will be reconciled with the safety database.

Final raw SAS® datasets will be transferred to statistician and sponsor (as applicable) according to the Data Structures Document (DSD).

11.1 Case Report Forms

A source data agreement will be signed by the sponsor and investigator to define what constitutes source data for all types of data captured.

Case Report Forms will be used to record data in the study. Data should be recorded legibly onto the CRFs in black ballpoint pen. Correction fluid or covering labels must not be used.

The monitor will check data at the monitoring visits to the study site. The PI will ensure that the data in the CRFs are accurate, complete, and legible.

Data from the completed CRFs will be entered into RPL's clinical study database and validated under the direction of the Data Manager. Screening failures (subjects who signed consent to take part in the study but were not randomised) as well as admission data for Reserves will not be entered into the clinical study database. Any missing, impossible (inconsistent with human life), or inconsistent recordings in the CRFs will be referred back to the PI using a DCF and be documented for each individual subject before clean file status is declared.

12. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

12.1 Sponsor's Responsibilities

12.1.1 GCP compliance

MMV and any third party to whom aspects of the study management or monitoring have been delegated will undertake their roles for this study in compliance with all applicable regulations and ICH GCP Guidelines.

Visits to investigator sites will be conducted by representatives of MMV to inspect study data, subjects' medical records, and CRFs in accordance with current ICH Good Clinical Practice Guideline E6 (R2) (2016) and the respective local and national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by competent authorities.

12.1.2 Regulatory approval

MMV (or delegate) will ensure that Local Competent Authority requirements are met before the start of the study.

12.1.3 Indemnity/liability and insurance

MMV will adhere to the recommendations of the Association of British Pharmaceutical Industry (ABPI) Guidelines. A copy of the Indemnity document will be supplied to the investigator before study initiation.

MMV will ensure that suitable insurance cover is in place prior to the start of the study. An insurance certificate and a statement of insurance will be supplied to RPL.

12.1.4 Protocol management

All protocols and amendments will be prepared by MMV and/or RPL. If it becomes necessary to issue a protocol amendment during the course of the study, MMV will notify the investigator and collect documented Investigator Agreement to the amendment.

12.1.5 End of trial notification

RPL on behalf of MMV will submit an end of trial notification to the competent authority of the Member State within 90 days of the end of the trial in accordance with EU Directive 2001/20/EC. The PI will be responsible for submitting these to the REC within 90 days of the end of the trial.

For the purposes of this notification, the end of the trial will be defined as the last subject/last visit.

12.1.6 Posting or submission of summary of clinical trial report to competent authorities of member states concerned and RECs

MMV or delegate will post result-related information on this clinical trial to the European Database (which is considered as the submission of a summary of the clinical trial report) within one year of the end of the complete trial to the competent authority of the Member State concerned as required by the regulatory requirement and to comply with the Community guideline on Good Clinical Practice. RPL on behalf of MMV will submit a summary of the clinical trial report to the concerned REC.

12.2 Investigator's Responsibilities

12.2.1 GCP compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guidelines, EU Directive 2001/20/EC, and the applicable regulatory requirements.

It is the investigator's responsibility to ensure that adequate time and appropriate resources are available at the study site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a record of appropriately qualified persons to whom the investigator has delegated significant trial-related tasks. An up-to-date copy of the *curriculum vitae* for the investigator, sub-investigator(s), and essential study staff will be provided to MMV (or designee) before starting the study.

Agreement with the final Clinical Study Report will be documented by the dated signature of the PI, in compliance with Directive 75/318/EEC, Directive 2001/83/EC, and ICH E3.

12.2.2 Protocol adherence and investigator agreement

The PI and delegates must adhere to the CSP as detailed in this document. The PI will be responsible for including only those subjects who have met CSP eligibility criteria. The PI will be required to sign an investigator Agreement to confirm acceptance and willingness for themselves and delegates to comply with the CSP.

12.2.3 Documentation and retention of records

After completion of the study, all documents and data relating to the study will be kept in an orderly manner and securely by the PI in a secure file and/or electronically. The data will be available for inspection by MMV or their representatives. Essential documents must be retained for 2 years after the final marketing approval in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of study drug. The PI or delegate must contact MMV before destroying any study-related documentation and it is the responsibility of MMV to inform the investigative site of when these documents can be destroyed. In addition, all subject records and other source documentation will be kept for a longer period if required by the applicable regulatory requirements.

12.3 Ethical Considerations

This protocol complies with the principles of the World Medical Assembly (Helsinki 1964) and subsequent amendments.

12.3.1 Informed consent

The informed consent is a process by which a subject voluntarily confirms his/her willingness to participate in a clinical study. It is the responsibility of the PI or delegate to obtain written informed consent from subjects. All consent documentation must be in accordance with applicable regulations and the ICH Good Clinical Practice Guideline E6 (R2) (2016). Each subject is requested to sign the ICF after they have received and read the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. Signed ICFs must remain on file and must be available for verification by Study Monitors at any time. Another signed original of the ICF must be given to the subject or the subject's legally authorized representative. The PI or delegate will provide the sponsor with a copy of the REC approved consent forms, and a copy of the REC written approval, prior to the start of the study.

12.3.2 Research Ethics Committee (REC) approval

It is the responsibility of the PI to submit this CSP, the informed consent document (approved by MMV), relevant supporting information, and all types of subject recruitment information to the REC for review, and all must be approved prior to the start of subject screening. In addition, advertisements must be approved by the REC prior to use at the site. Prior to implementing changes in the study, MMV and the REC must also approve any substantial amendments to the CSP and corresponding updates to informed consent documents. For non-substantial protocol amendments (that do not require REC approval) and subsequent updates of the ICF all changes will be done in agreement with MMV and RPL.

12.4 Confidentiality

For the purposes of this Section 12.4, "Applicable Data Protection Law" shall mean (a) the Data Protection Act 1998; or (b) from 25 May 2018, the General Data Protection Regulation ((EU) 2016/679), and any applicable legislation that supersedes or replaces the General Data Protection Regulation in the UK.

Data collected during this study may be used to support the development, registration, or marketing of medicinal product. MMV will control all data collected during the study and will abide by the Applicable Data Protection Law. For the purpose of the Applicable Data Protection Law, MMV will be the data controller. To the extent that RPL processes personal data on behalf of MMV, in relation to such data RPL shall only act in accordance with the terms of this protocol and MMV's reasonable written instructions and RPL shall take appropriate technical and organisational measures against the unauthorised or unlawful processing of such personal data.

After subjects have consented to take part in the study, their medical records and the data collected during the study will be reviewed by MMV and/or its representatives. These records and data may, in addition, be reviewed by the following: independent auditors who validate the data on behalf of MMV; national or local regulatory authorities, and the REC which gave its approval for this study to proceed.

Although subjects will be known by a unique number, their date of birth will also be collected by RPL and used to assist MMV to verify the accuracy of the data, for example, that the results of study assessments are assigned to the correct subject. The results of this study containing the unique number, date of birth, and relevant medical information including ethnicity may be recorded and transferred to and used in other countries throughout the world, which may not afford the same level of protection that applies within the EU. The purpose of any such transfer would be to support regulatory submissions made by MMV in such countries.

The Parties agree to comply with the relevant provisions of the Applicable Data Protection Law and any directions issued by the UK Information Commissioner in its processing of such Personal Data. All nominative information in the subject's medical record will be kept in strict confidentiality. Nominative information shall mean the name, the address and all other personally identifiable information associated with a subject's name. MMV access to subject's data shall be performed in such a way that no subject could be identified by such data.

If there are any contradictions in terms of confidentiality requirements, the requirements of Applicable Data Protection Law will prevail.

[In addition to the terms of this Section 12.4, from 25 May 2018, to the extent that RPL processes personal data on behalf of MMV, the terms of **Appendix 1** shall also apply.]

12.5 Publication Policy

If the sponsor and RPL agree that it will be desirable to publish the results of this study; both parties will liaise in good faith to publish the results, RPL agree to obtain the sponsor's prior written approval of such publications.

13. REFERENCES

1. World Malaria Report 2017, https://www.who.int/malaria/publications/world-malaria-report-2018/report/en/
2. UNICEF Data: Malaria, https://data.unicef.org/topic/child-health/malaria/
3. WHO Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for *Plasmodium falciparum* malaria control in highly seasonal transmission areas of the Sahel sub-region in Africa, March 2012. https://www.who.int/malaria/publications/atoz/smc_policy_recommendation_en_032012.pdf?ua=1
4. Naidoo I and Roper 2013. Mapping 'partially resistant', 'fully resistant', and 'super resistant' malaria. Trends Parasitol; 29: 505–15. https://www.cell.com/trends/parasitology/fulltext/S1471-4922(13)00136-0?
5. Meremikwu MM, *et al*, 2012. Intermittent preventive treatment for malaria in children living in areas with seasonal transmission; Cochrane Databaseof Systematic Reviews, Issue 2. Art. No.: CD003756.DOI: 10.1002/14651858.CD003756.pub4. www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD003756.pub4/epdf/abstract
6. Malarone UK SmPC. https://www.medicines.org.uk/emc/product/947/smpc
7. Amodiaquine hydrochloride (150mg base) manufactured by Guilin, Prequalification Programme, WHO PUBLIC ASSESSMENT REPORT (WHOPAR 048) https://extranet.who.int/prequal/sites/default/files/MA045part4.pdf
8. Shanks GD, *et al,* 1999., Time to reappearance of malaria parasites following various drug treatment regimens in a holoendemic area of western Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene 93,304-305
9. Polhemus, ME, *et al*, 2008., Malaria Treatment with Atovaquone-Proguanil in Malaria-Immune Adults: Implications for Malaria Intervention Trials and for Pre-Exposure Prophylaxis of Malaria, AAC Apr., p. 1493–1495 Vol. 52, No. 4.
10. Artesunate amodiaquine Winthrop Product Label http://www.wipo.int/export/sites/www/research/en/data/sanofi/marketed_products/artesunate_and_Amodiquine.pdf
11. Jullien, V. et al, 2010. Population pharmacokinetics & pharmacodynamic considerations of amodiaquine and desethylamodiaquine in Kenyan adults with uncomplicated malaria receiving artesunate-amodiaquine combination therapy, AAC 54 (6), pp2611-2617
12. Thapar MM, 2004, Pharmacokinetics and dynamics of atovaquone and proguanil (Malarone R); https://openarchive.ki.se/xmlui/handle/10616/43294
13. Abla et al., PAGE conference, Montreux, May 2018; https://www.page-meeting.org/pdf_assets/65-Poster_PAGE%202018_Abla.pdf
14. Wittes R, 1987, Adverse Reactions to Chloroquine and Amodiaquine as Used for Malaria Prophylaxis: A Review of the Literature Can. Fam. Physician Vol. 33: Nov, 2644-2649.
15. Assi S-B, 2017, Safety of a fixed-dose combination of artesunate and amodiaquine for the treatment of uncomplicated Plasmodium falciparum malaria in real-life conditions of use in Côte D'Ivoire. Malar J. Vol.16, No. 8
16. West African Network for Clinical Trials of Antimalarial, D. Pyronaridine-artesunate or dihydroartemisinin-piperaquine versus current first-line therapies for repeated treatment of uncomplicated malaria: a randomised, multicentre, open-label, longitudinal, controlled, phase 3b/4 trial. Lancet 391, 1378-90 (2018).

17. Funck-Brentano C, et al., 2019. Evaluation of the effects on the QT-interval of 4 artemisinin-based combination therapies with a correction-free and heart rate-free method. Nature; Scientific Reports 9, No. 883.

18. Larrey D, et al. 1986. Amodiaquine-induced hepatitis. A report of seven cases. Ann Intern Med.;104(6):801-3.

19. NDiaye J et al. Safety of Seasonal Malaria Chemoprevention (SMC) with Sulfadoxine-Pyrimethamine plus Amodiaquine when Delivered to Children under 10 years of Age by District Health Services in Senegal: Results from a Stepped-Wedge Cluster Randomized Trial.

20. Global Malaria Risk, https://www.malariasite.com/malaria-risk/

21. Garnett et al. 2018. Scientific white paper on concentration-QTc modeling. J Pharmacokinet Pharmacodyn.;45(3):383-397. doi: 10.1007/s10928-017-9558-5

22. Amodiaquine (as Hydrochloride) 153mg dispersible tablets SmPC.

23. D'Souza R S, Hooten, WM: Extrapyramidal symptoms (EPS). StatPearls; https://www.ncbi.nlm.nih.gov/books/NBK534115/;

24. Chouinard H, Margolese HC: Manual for the Extrapyramidal Symptom Rating Scale (ESRS). Schizophrenia Research 76 (2005); 247 – 265.

25. Adjei G O, et al. 2009. Amodiaquine-associated adverse effects after inadvertent overdose and after a standard therapeutic dose.; Ghana Medical Journal 43(3): 133-138

26. Olabode Akindele M, et al. 1976: Amodiaquine-induced involuntary movements; British Medical Journal; 214-215

14. APPENDIX 1
[DATA PROCESSING SCHEDULE TO THE PROTOCOL]

1 Where RPL processes personal data on behalf of MMV, RPL shall:

process the personal data only in accordance with the documented instructions of MMV**;**

implement appropriate technical and organisational measures to protect the personal data against unauthorised or unlawful processing and against accidental loss, destruction, damage, alteration or disclosure. These measures shall be appropriate to the harm and risk which might result from any unauthorised or unlawful processing, accidental loss, destruction or damage to the personal data and having regard to the nature of the personal data which is to be protected and shall include inter alia as appropriate:

the pseudonymisation and encryption of personal data;

the ability to ensure the on-going confidentiality, integrity, availability and resilience of systems and services processing personal data;

the ability to restore the availability and access to personal data in a timely manner in the event of a physical or technical incident; and

a process for regular testing, assessing and evaluating the effectiveness of technical and organisation measures for ensuring the security of processing;

In order to enable RPL to implement appropriate technical and organisational measures, MMV shall provide to RPL any information reasonably required by RPL to enable it to assess the appropriateness of such measures.

only employ or appoint personnel to process the personal data who have given binding undertakings of confidentiality or are under a statutory obligation of confidentiality;

remain entitled to appoint third party sub-processors. Where RPL appoints a third party sub-processor, it shall:

ensure that the third party is subject to, and contractually bound by, at least the same obligations as RPL;

provide to MMV copies of any documentation to demonstrate compliance with the obligations under this Section 0; and

remain fully liable to MMV for all acts and omissions of the third party;

notify MMV without undue delay after becoming aware that it has suffered a data breach;

at MMV's cost, permit MMV (subject to reasonable and appropriate confidentiality undertakings), to inspect and audit RPL's data processing activities to enable MMV to verify and/or procure that RPL is in full compliance with its obligations under the protocol;

taking into account the nature of the processing, assist MMV by appropriate technical and organisational measures, insofar as this is possible, for the fulfilment of MMV's

obligation to respond to requests from data subjects exercising their rights under Applicable Data Protection Law;

unless applicable law requires otherwise, upon termination of the protocol:

at the option of MMV comply or procure the compliance with the following:

return to MMV all personal data and any other information provided by MMV to RPL; and/or

permanently delete all personal data provided by MMV to RPL;

cease to process the personal data.

Notwithstanding the foregoing, RPL shall be entitled to retain personal data to the extent that it is required to do so pursuant to the law of the European Union or the law of a member state of the European Union;

where the laws of the country where RPL is established require RPL to transfer the personal data to a third country or an international organisation, inform MMV as soon as reasonably possible of that legal requirement unless that law prohibits such communication on important grounds of public interest.