EFFICACY OF SULFADOXINE-PYRIMETHAMINE WITH AND WITHOUT ARTESUNATE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN MOZAMBIQUE: A RANDOMISED CONTROLLED TRIAL

Elizabeth Allen
NTTELI002

Submitted to the University of Cape Town in partial fulfilment of the requirements of the degree of Master of Public Health

Faculty of Health Sciences
University of Cape Town (UCT)
7th May 2008

Supervisors:
Dr A Boulle, School of Public Health, UCT
Dr F Little, Department of Statistical Sciences, UCT

Trial Principal Investigator:
Assoc. Professor KI Barnes, Division of Clinical Pharmacology, UCT

TABLE OF CONTENTS

DECLARATION AND ACKNOWLEDGEMENTS

I, *...Elizabeth Allen.....*, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature:..... Date: .....

In addition to writing this report, I participated in the study design, provided day to day management of the study and conducted the statistical analysis.

I would like to first express my gratitude to the patients who volunteered for our SEACAT Evaluation studies, and my colleagues in Mozambique for their work on this study and for making my many, many trips there so enjoyable. I would like to acknowledge the work conducted at the Malaria Lead Programme, Medical Research Council, Durban; Amanda Jackson for facilitating data entry and Jaishree Raman for PCR amplification to enable SP mutational analysis and the differentiation of re-infection from recrudescence.

I would like to thank the UCT team, in particular Karen Barnes for giving me the opportunity to work in such an interesting field, for accessing funds to finance this degree and, most importantly, for her inspired mentorship for my chosen career path. Finally I would like to thank my supervisors; Andrew Boulle for his practical support and direction, and the ever-patient Francesca Little who has given me such valuable skills to enable me to better manage and analyse data.

ABBREVIATIONS

ACPR	Adequate clinical and parasitological response
ACT	Artemisinin-based combination therapy
AS	Artesunate
CDC	Centers for Disease Control
CI	Confidence interval
CRF	Case record form
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
EFT	Early treatment failure
GCP	Good clinical practice
GLURP	Glutamate rich protein
G6PD	Glucose 6 phosphate dehydrogenase
ICH	International conference on harmonisation
LCF	Late clinical failure
LTF	Late treatment failure
MSP	Merozoite surface protein
PCR	Polymerase chain reaction
Pf	*Plasmodium falciparum*
RCT	Randomised controlled trial
RDT	Rapid diagnostic test
SAE	Serious adverse event
SAMF	South African medicines formulary
SEACAT	South East African Combination Anti-malarial Treatment
SP	Sulfadoxine-pyrimethamine
WARN	World Anti-malarial Resistance Network
WHO	World Health Organisation
WHO-art	World Health Organisation Adverse Reaction Terminology

FIGURES AND TABLES

ABSTRACT

EFFICACY OF SULFADOXINE-PYRIMETHAMINE WITH AND WITHOUT ARTESUNATE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN MOZAMBIQUE: A RANDOMISED CONTROLLED TRIAL

Background and rationale

Malaria accounts for a large public health burden in Mozambique and a treatment policy with effective anti-malarials is a key component of their malaria control programme. Artemisinin-based combination therapies (ACTs) are now generally considered as the best treatment for uncomplicated *falciparum* malaria; the use of artesunate (AS) in combination with sulfadoxine-pyrimethamine (SP) is recommended by the World Health Organisation (WHO). Mozambique policy-makers recommended that an ACT be implemented and studied in 2003 therefore this RCT was conducted to compare SP monotherapy with AS, plus SP in order to provide further evidence of available treatment options in the region.

Trial design and methods

A prospective multi-centre, open-label, parallel-group randomised clinical trial (RCT) was conducted at 4 public health facilities in Maputo Province, Mozambique during the malaria seasons of 2003 - 2004 and 2004 – 2005. Eligible patients were aged over 1 year with body weight over 10kg and uncomplicated *Plasmodium falciparum* malaria (parasitaemia less than 500 000 asexual parasites/μl blood with axillary temperature less than or equal to 37.5°C or a history of fever). Patients were excluded if they took other anti-malarials or folate within 7 days, had moderately severe/severe malaria, history of G6PD deficiency or allergy to study drugs, or serious underlying disease. Patients were randomly assigned to sulfadoxine-pyrimethamine (SP): a single oral 25/1.25mg per kg dose on Day 0, with a maximum of 3 tablets), or artesunate (AS) plus SP: SP as above, plus single oral doses of 4mg/kg AS on Days 0, 1 and 2 with a maximum daily dose of 4 tablets).

The study aimed to compare the efficacy of SP monotherapy to SP in combination with AS as first line treatment of uncomplicated *falciparum* malaria. The primary objective was the comparison of the time to treatment failure (the relative hazard of treatment failure) between groups using standard WHO response to treatment definitions for low to moderate malaria

transmission areas, modified to a 42 day follow up. Randomisation was computer-generated with sequential allocation concealed in opaque sealed envelopes. Treatments were open-label, however laboratory staff responsible for parasite density measurements (in order to determine the primary efficacy end point) were blinded to treatment allocation.

Results

Four hundred and eleven subjects were randomised to take either SP (n= 199) or AS plus SP (n=212). 408/411 (99.3%) subjects were included in intention-to-treat analyses (SP 198, AS plus SP 210; 3 subjects were excluded as Day 0 parasitaemia could not be confirmed). Kaplan-Meier (KM) distributions and survival curves showed that AS plus SP had a significantly reduced relative hazard of treatment failure compared to SP monotherapy (KM estimates for achieving treatment success by Day 42 were 90.4% [95% CI 84.9%;93.9%] for SP and 98.0% [95% CI 94.8%;99.3%] for AS plus SP, log rank p= 0.0008). In a Cox Proportional Hazards Regression, treatment with AS plus SP was found to decrease the relative hazard of treatment failure by 80% (adjusted hazard ratio [HR] 0.2; 95% confidence interval [CI] 0.1;0.6). Other factors found to have an impact on treatment outcome included age over 7 years (which decreased the relative hazard of failure by 70% compared to younger age [HR 0.3; 95% CI 0.1:0.9]), body temperature on Day 0 (which decreased the relative hazard of failure by 50% for each additional °C [HR 1.5; 95% CI 1.1:2.2]), and having a quintuple *dhfr/dhps* mutation (which increased the relative hazard of failure 3.2 fold compared to fewer mutations [HR 3.2; 95% CI 1.3;7.5]). A per-protocol analysis including 354 subjects (SP 177, AS plus SP 177) confirmed these findings.

Conclusions

AS combined with SP significantly decreased the relative risk of treatment failure compared to SP monotherapy supporting the literature that combining AS with SP improves efficacy should the efficacy of SP be sufficiently high, as the drugs have independent modes of action. Age, body temperature and *dhfr/dhps* mutations were found to be important independent risk factors for treatment failure.

1 INTRODUCTION AND LITERATURE REVIEW

Malaria accounts for a large public health burden in the tropical regions of Africa where it is caused primarily by the *Plasmodium falciparum* (Pf) parasite transmitted by the anopheline mosquito vector.[1,2] Malaria presents on a continuum of disease severity depending on, among other factors, immunity of the individual to the infection, which itself appears to be partly dependent on transmission dynamics inherent within the regions affected.[3] Uncomplicated malaria is characterised by non-specific signs and symptoms including fever, chills, nausea and vomiting, malaise, headaches, body aches, splenomegaly, thrombocytopenia, diarrhoea, anaemia, coughing and convulsions, while the severe form of the disease is usually fatal if untreated.[4] Particularly vulnerable groups include those with reduced or no immunity such as young children, visitors to a malaria region or those who may have temporarily migrated from an endemic region and then returned, pregnant women, and those with concomitant disease such as HIV/AIDS. Diagnosis is made by assessing clinical signs and symptoms and confirmed using laboratory techniques (a rapid diagnostic test, RDT, or microscopy to detect parasites).

1.1 The burden of malaria in Mozambique

Mozambique is a country in Southern Africa, population *circa* 20,000,000. There is constant (hyperendemic) transmission of malaria, although prevalence throughout the country varies greatly according to the season, other environmental factors, and the use of vector control measures. The total number of malaria cases reported in Mozambique during 2004 was 5,610,884, although it is recognized that data surveillance methodologies in general may be weak due to under-reporting and misdiagnoses; malaria is usually clinically rather than definitively diagnosed.[2,5] Between 2003 and 2005 in several districts in Southern Mozambique (when and where this particular study took place) malaria prevalence was declining steeply due to an intensive intervention of indoor residual spraying (IRS), and ranged from between 4% (95% CI, 3%:6%) to 59% (95% CI, 49%;69%).[6]

1.2 Treatment of uncomplicated *Plasmodium falciparum* malaria

A treatment policy with an effective anti-malarial is a key component of malaria control, the primary aim of such treatment being prevention of the progression of uncomplicated malaria to severe malaria and, thereby, to preserve life.[7] A further aim of appropriate treatment is to reduce the malaria infectious reservoir by preventing its transmission from the human host to the mosquito vector. This is realised through a rapid effect on asexual parasites, preventing the development of gametocytes, the parasites' sexual stages and thereby reducing its infectivity.[8]

When considering treatment policies, governments take into account various factors such as cost, availability and accessibility, ease of use and tolerability of the drugs. Furthermore, resistance to anti-malarial drugs in the malaria parasites is now a critical component in decision-making. Chloroquine has been the most widely used treatment for malaria worldwide since the 1950s. However, due to resistance in the parasite to chloroquine in most regions, many governments have had to choose alternative drug policies.[9] As with other diseases such as HIV/AIDS, a treatment approach based on combining drugs with different modes of action is now advocated for malaria.[7]

1.2.1 Artemisinin-based combination therapies (ACTs)

Artemisinin-based combination therapies are now generally considered as the best treatment for uncomplicated *falciparum* malaria. This is primarily because they act against all 4 stages of the Pf parasite's development effecting a rapid cure and also reduce gametocyte carriage, thus transmission of malaria, and anti-malarial resistance.[10,11,12] Artesunate (AS) has been found to be particularly effective in the rapid treatment of uncomplicated malaria.[13] There is, as yet, no evidence of clinically relevant *in vivo* resistance to AS, and its use in combination with the longer-acting sulfadoxine-pyrimethamine (SP) is recommended by the World Health Organisation (WHO) as one of 4 ACTs because it is considered affordable compared to other ACTs. From a programmatic point of view, artesunate plus SP has the unique advantage of the full dose of the partner drug being administered under supervision when malaria is diagnosed.

In general, when widely used as monotherapy, SP has a relatively short useful therapeutic life.[14] However there is evidence of the superior efficacy of treatment regimens that add AS, to SP or other monotherapies.[15]

The tolerability profile of AS plus SP has been found to be similar to that for SP monotherapy, as AS is generally considered well tolerated except for a rare type 1 hypersensitivity reactions (urticaria).[16]

1.2.2 Treatment of uncomplicated *Plasmodium falciparum* malaria in Mozambique

While chloroquine was the treatment policy for uncomplicated malaria in Southern Mozambique, evidence of poor efficacy throughout tropical Africa since the 1980s signalled a need to explore alternatives.[9] Amodiaquine plus SP (a non-artemisinin combination) was subsequently selected as the Mozambique National Malaria policy for the treatment of uncomplicated *falciparum* malaria for implementation in 2005. However, it was recommended by policy-makers that an ACT be implemented and studied in parallel in a selected area of Southern Mozambique from 2003, as evidence regarding their efficacy and safety, while promising, was not yet well established in the region. The SEACAT (South-East African Combination Anti-malarial Treatment) Evaluation was tasked with this role at a SEACAT Evaluation/Ministry of Health meeting, Inhaca, Mozambique, Dec 2002.

The SEACAT Evaluation was initiated in 1999 as a comprehensive evaluation of the phased introduction of combination anti-malarial therapy and was nested in the Lubombo Spatial Development Initiative, a tripartite agreement between the governments of South Africa, Swaziland and Mozambique to improve the economic situation in the shared border area (Lubomo).[6] The SEACAT Evaluation's role included conduct of *in vivo* studies at selected sentinel sites in South Africa, Eastern Swaziland and Southern Mozambique to provide data to policy-makers regarding the efficacy, safety and emerging resistance patterns of monotherapy anti-malarials used in those regions and, subsequently, the combination anti-malarials chosen to replace them. Efficacy of SP monotherapy as an alternative to chloroquine, had been assessed by SEACAT during an *in vivo* study in Namaacha and Matatuine Districts, Southern Mozambique, during

2002. The data showed that cure rates had exceeded the 85% level which was considered appropriate for it to be considered for combination with an artemisinin-derivative to sustain its useful therapeutic life.[7]

1.3 Methodological issues in the analysis of malaria efficacy trials

It is widely accepted that RCTs be analysed using the 'intention-to-treat' (ITT) principle to preserve the random allocation of subjects to treatment group regardless of adherence, thereby distributing confounders (known and unknown) equally and providing an unbiased estimate of the effect. A problem with this method is how to manage missing outcomes. Often those subjects who do not complete a study are considered treatment failures, a 'worst case scenario'. This is impractical in malaria efficacy studies as they are frequently conducted in populations where migration is common and who may be difficult to follow to completion, and spurious results may be found if incorrect assumptions are made with regard to the many missing outcomes.

An alternative is to exclude all non-adherers (those who did not complete the study or who did not adhere to the protocol in terms of dose, missed critical observations, concomitant medications etc.) and conduct a 'per-protocol' (PP) analysis. This may, however, introduce bias as there could be systematic associations between protocol compliance and a treatment group. Where there are many missing data relating to the outcome and numerous protocol violations, problems with the analysis may be compounded by a decrease in statistical power if too many subjects are removed.

It is currently advocated that a survival analysis of time to treatment failure as the primary indicator of treatment response in malaria efficacy studies is most appropriate.[17,18,19] A benefit of survival analysis over other statistical methods such as logistic regression is that every enrolled subject is included, as per the ITT principle, and all available data for those who subsequently do not complete the study are included to the point where they left the study, whereupon the data are censored, removing the necessity to assume outcomes. The analysis may then be repeated excluding fully those subjects who were major protocol violators, as per the PP principle. However, data to the point of a violation may be included until censored increasing the available data overall.

2 RATIONALE FOR THE STUDY

During the malaria seasons of 2003 - 2004 and 2004 - 2005 this SEACAT Evaluation randomised controlled trial (RCT) was conducted in Southern Mozambique to compare SP monotherapy with the ACT, AS, plus SP in order to provide further evidence to policy makers of available treatment options in the region.

The study gave an opportunity to investigate objectives other than efficacy, such as safety, molecular markers of SP resistance, gametocyte carriage and pharmacokinetic parameters of SP. However, within the limits of a MPH mini-dissertation, only the efficacy analysis is presented here.

3 STUDY OBJECTIVES

The aim of the study was to compare the efficacy of SP monotherapy to that of SP in combination with AS as first line treatment of uncomplicated falciparum malaria in four clinical sites in Southern Mozambique to inform Mozambique policy-makers. In order to achieve this aim the following outcomes were developed:

Primary outcome

- To compare the time to, and risk of, treatment failure between treatment groups, adjusted by baseline characteristics.

Secondary outcomes

- To compare the time to, and risk of, parasite clearance between treatment groups, adjusted by baseline characteristics.
- To compare fever clearance between treatment groups.

4 STUDY DESIGN AND RESEARCH METHODS

This was a prospective multi-centre, open-label, parallel-group RCT conducted at Namaacha, Catuane, Boane and Magude public-sector health facilities in Maputo Province, Mozambique (Figure 1).

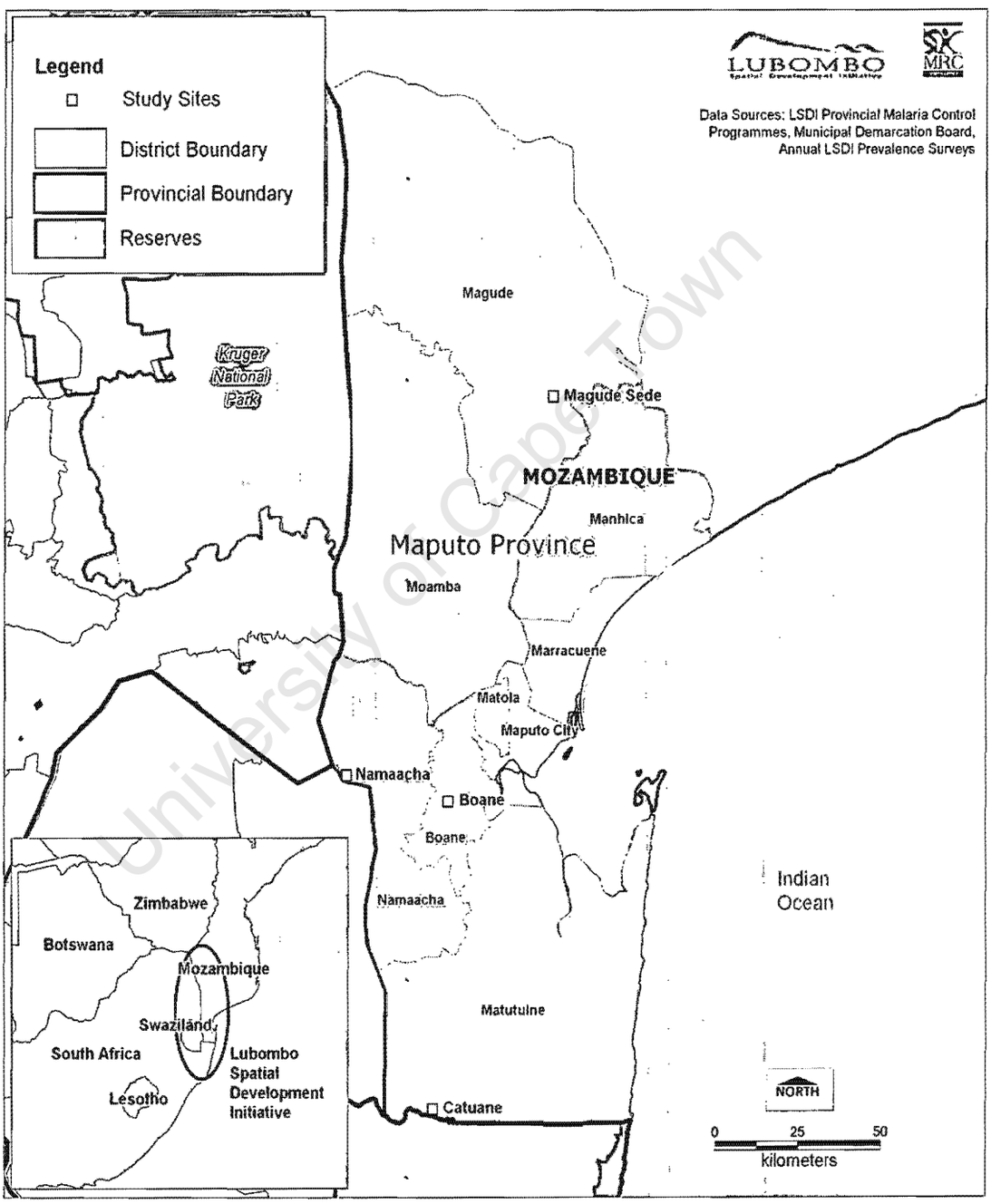


Figure 1: Geographical location of study sites

Namaacha and Boane are peri-urban settings while Magude and Catuane are rural settings. The study sites themselves were health centres or Health Posts which provide standard first-line treatment for uncomplicated malaria and refer severe/complicated cases to Matola or Maputo hospitals (Figure 2). Catuane is close to the South African border where population movement for work purposes (migration) is known to be prevalent.

Figure 2: Scenes from the study sites

Two protocols were developed (with input by the MPH candidate), as initially this RCT was nested within an ongoing open label single arm study protocol, but was subsequently extracted into its own protocol for clarity.[20,21]

4.1 Subject recruitment and enrolment

Inclusion and exclusion criteria were developed using a standard WHO protocol for assessment of efficacy for anti-malarials in low to moderate malaria transmission intensity areas, and contra-indications for treatments in terms of safety and/or for validity of the proposed efficacy analysis.[22,18]

Specifically; male and non-pregnant (or breast-feeding) female patients over the age of 1 year and over 10kg body weight with symptoms of malaria who presented routinely at the clinics were invited to give informed consent prior to sequential screening for *Plasmodium falciparum* infection by rapid diagnostic test (RDT: Immunochromatographic Test, ICT P.f®., SA Scientific). A physical examination assessed eligibility.

Those diagnosed with uncomplicated acute *P. falciparum* malaria parasitaemia of up to 500 000 asexual parasites/μl blood with an axillary temperature of greater than or equal to 37.5°C or a medical history of fever (defined as within the previous 24 hours) and who lived close enough to the study site for reliable follow up, were deemed suitable for inclusion.

However, patients who revealed during the medical history discussion that they had received any anti-malarial treatment in the past 7 days were excluded (including drugs with inherent anti-malarial properties though not used for treatment of malaria, specifically cotrimoxazole, trimethoprim, chloramphenicol or tetracyclines). In addition, patients who had received folate in the past 7 days (due to its role as a antagonist to the antimalarial activity of SP, subsequently published) or were likely to require any of the above mentioned drugs during the study period, or who were severely ill (according to WHO Criteria) or considered, in the opinion of the investigator or designee, to have moderately severe malaria (e.g. prostration, repeated vomiting, dehydration) or other danger signs, had a known history of G6PD deficiency, a history of allergy to any of the study drugs (including other sulphonamides or other artemisinin derivatives) or a serious underlying disease that in the opinion of the clinic team and/or Principal Investigator would make the patient unsuitable for the study in terms of their safety or study analysis, were excluded.[23,24]

4.2 Study treatments

4.2.1 Dosing schedule

Eligible subjects were randomised to receive a weight-appropriate dose of observed treatment with SP monotherapy (a single oral 25/1.25mg per kg dose on Day 0, with a maximum of 3 tablets) or AS plus SP (a single oral dose of SP as above on Day 0 and single oral 4mg/kg doses of AS on Days 0, 1 and 2, with a maximum daily dose of 4 tablets). The SP used was Fansidar® (Roche, Gauteng, South Africa) while the AS plus SP was co-packaged as Arsudar® (Sanofi, Gauteng, South Africa). Doses were to be given as whole tablets and administered with water.

A dosing schedule (Table 1) was determined by derivation of an optimal dosing regimen that allows for AS to be blister-packed with SP in whole tablets, from a CDC analysis of a 55,000 African malaria patient dataset which made recommendations to minimise over- and under-dosing (Personal communication A Terlouw, subsequently published)[7]:

Table 1: Doses of artesunate (AS) and sulfadoxine-pyrimethamine (SP)

Mass (kg)	Approximate age (years)	SP tablets (all subjects)	50mg artesunate tablets (AS plus SP group only)		
		Day 0	Day 0	Day 1	Day 2
10 – 20	1 – 6	1	1	1	1
21 – 35	7 – 13	2	2	2	2
> 35	14 +	3	4	4	4

4.2.2 Randomisation

A UCT statistician generated computerised random allocation schedules for each of the two protocols, which were withheld from the sites' study teams. Each clinic had a schedule which allowed for equal randomisation of subjects between SP and AS plus SP within blocks of ten. Each list (4 in total) of random allocations was divided sequentially into three sections to represent the three weight-based dosing levels such that, within each dose level, there was an equal chance of a subject being allocated SP or AS plus SP. Treatments were concealed in opaque padded envelopes which were packed, sealed and labelled with sequential numbers by the UCT Study Manager (MPH candidate). Once site staff selected the next available envelope within the appropriate dosing level, treatment allocation was open-

label as the manufacturer was not able to provide dummy tablets. Open-label spare treatments were available for re-dosing in case of vomiting within an hour of treatment.

4.3 Visit schedule

The visit schedule was chosen as recommended in the standard WHO protocol for the assessment of *in vivo* therapeutic efficacy of antimalarials, however with extension to a 42 day follow up due to the long elimination half life of SP.[22]

4.4 Study assessments

Enrolled subjects were seen on Day 0 and then asked to return to the clinic on Days 1, 2, 3, 7, 14, 21, 28 and 42 for assessments relating to clinical and parasitological end points: axillary temperature, asexual parasite density, haemoglobin concentration (the latter two samples by finger prick blood sample), and clinical signs and symptoms to capture adverse events, data regarding resolution of symptoms and concomitant medications.

In addition, dried blood samples (by finger prick sample) were taken at each visit to identify parasite mutations associated with resistance to SP such that, for those participants who experienced parasitological failure during the 42 day follow up period, polymerase chain reaction (PCR) could determine whether this was re-infection with a new parasite or recrudescence of the original infection.

Subjects were encouraged to return for unscheduled visits if they felt unwell. Further detail regarding efficacy assessments is given below:

4.4.1 Parasitology

Identification of *Plasmodium falciparum* and other Plasmodial parasites was performed according to a standardized WHO methodology.[25] Species identification was made from a thin smear, while asexual parasite density was calculated by counting the number of asexual parasites on a thick blood smear stained with Giemsa (assuming 8000 leukocytes per µl blood). All parasite counts were checked by a senior microscopist who made revisions if

necessary. Slides were labeled, air dried and stored in slide boxes at room temperature according to a defined process, prior to archive.

4.4.2 Rapid diagnostic tests, medical history, concomitant medication, adverse event data

Standard training in how site staff would elicit medical histories and the collection of concomitant medication and adverse event data was given prior to and during the study by the MPH candidate and/or UCT monitor. The correct method for using the rapid diagnostic tests was ensured by a senior microscopist.

4.4.3 Molecular analyses

Samples were labeled according to a defined process and were air dried away from dust, excessive humidity, insects & direct sunlight for 1 hour, prior to placement in a plastic zip-lock sample bag with desiccant. The plastic bags containing samples from each visit were kept in brown envelopes labeled with subject/visit details and stored in plastic boxes until collection by the monitor for dispatch to the laboratory.

This RCT used three highly variable proteins (MSPI, MSPII and GLURP) to differentiate re-infection form recrudescence, which is more accurate than the methodology used in some publications which quote only one or two proteins.[26]

SP mutational analysis was conducted by the Medical Research Council, Durban according to a standard methodology. Primers, PCR amplification conditions and restriction endonucleases were used to detect polymorphisms in the *dhfr* (codon 51, 59, 108, 164) and *dhps* (codon 436, 437, 540 and 581) genes (http://medschool.umaryland.edu/CVD/2002_pcr_asra.htm). Digestion products separated on a 2% agarose gel using electrophoresis were visualised and photographed using a MiniBIS documentation system and the genotype of each codon was classified as either pure wild, pure mutant or mixed (both mutant and wild genotypes present in an individual sample).

4.5 Withdrawal criteria

Those subjects who experienced clinical or parasitological failure, according to standard definitions (described under section 4.7), were withdrawn and given rescue treatment with quinine. Other reasons for withdrawal included:

- Any clinical deterioration other than treatment failure.
- Any serious adverse event requiring treatment withdrawal or resulting in inability to continue study assessments.
- Any allergic reaction or skin rash presenting during the treatment courses, whereupon the site staff were required to stop the study treatment, manage the allergic reaction, and counsel study subjects against repeated treatment with the same drugs in the future.
- Concomitant prescribing of any drugs that may interfere with the study analysis or present a safety concern for the study subject.

4.6 Sample size and power

The sample sizes required for each of the protocols independently were calculated assuming an adequate clinical and parasitological response (ACPR) rate of 75% for SP and 90% for AS plus SP (with a confidence interval of 95%, and 80% power) indicating that 100 per treatment group per protocol (400 subjects in total) were needed. Although the first protocol in 2003-2004 at Namaacha and Catuane clinics did not achieve the expected recruitment rate, the addition of data from the 2004-2005 protocol allowed the overall sample size projections to be exceeded.

4.7 Efficacy endpoints

The responses to treatment were standard WHO definitions relevant for low to moderate malaria transmission intensity areas, modified for a 42 day follow up (Table 2).[22]

Table 2: WHO classification of response to treatment modified for 42 day follow up

Early Treatment Failure (ETF)	• Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitemia; • Parasitaemia on Day 2 higher than Day 0 count irrespective of axillary temperature; • Parasitemia on Day 3 with axillary temperature ≥ 37.5 °C; • Parasitemia on Day 3 ≥ 25 % of count on Day 0.
Late Treatment Failure (LTF)	**Late Clinical Failure (LCF)** • Development of danger signs or severe malaria after Day 3 in the presence of parasitemia without previously meeting any of the criteria of early treatment failure • Presence of parasitemia and axillary temperature ≥ 37.5 °C (or history of fever) on any day from Day 4 to Day 42, without previously meeting any of the criteria of early treatment failure **Late Parasitological Failure (LPF)** • Presence of parasitemia on any day from Day 7 to Day 42 and axillary temperature < 37.5 °C, without previously meeting any of the criteria of early treatment failure or late clinical failure
Adequate Clinical and Parasitological Response (ACPR)	• Absence of parasitemia on Day 42 irrespective of axillary temperature without previously meeting any of the criteria of early treatment failure or late clinical failure or late parasitological failure.

However, since the study was concluded the World Anti-malarial Resistance Network (WARN) has proposed an expanded description of possible outcomes for subjects enrolled in malaria efficacy studies so that data may be collated from numerous study sites in a standard format for a global database. While the WARN definitions are consistent with the above WHO guidelines in terms of the efficacy endpoints, it expands them and makes provision for how protocol violations, and other reasons for non-completion of a study, could be defined (Table 3).[27]

Table 3: WARN description of possible outcomes on the last day of follow up for patients enrolled in malaria efficacy studies

Variable code	Description
Patients who complete the study	
0	ACPR
1	ETF and death
2	ETF with severe malaria
3	ETF with danger signs
4	ETF with Parasitological Criteria (day 2 > day 0 or day 3 >25% day 0)
5	ETF with Clinical Criteria (documented fever and parasitaemia on day 3)
6	ETF not otherwise specified (for when details of why ETF classified not available)
7	LCF and Death
8	LCF with Severe Signs
9	LCF with Danger Signs
10	LCF with fever (either measured or subjective)
11	LPF
12	LPF/LCF Indistinguishable (for when details of LCF/LPF classification not available)
Patients who do not complete the study	
13	Adverse event requiring change in therapy prior to completion of full dose
14	Protocol violation
15	Death not due to malaria
16	Lost to follow-up
17	Use of other antimalarials outside of study protocol in the absence of parasitaemia
18	Withdrawal of consent by patient prohibiting further follow-up
19	Investigator initiated withdrawal from further follow-up
20	Patient who does not complete follow-up for any other reason not listed above
21	Enrolment Violations

To enable SEACAT studies to contribute to the global databases, data from this RCT were coded and described according to WARN guidelines. However, due to low numbers of subjects in some WARN categories in this study, data were grouped according to the WHO classification for subjects who completed the study (WARN variables 0 to 12) and in three further summary categories for subjects who did not complete (within WARN variables 13 to 21):

Patients who completed the study

- ACPR (WARN variable 0)
- ETF (WARN variables 1 to 6)
- LTF-LCF (WARN variables 7 to 10)
- LTF-LPF (WARN variables 11 and 12)

Patients who did not complete the study

- Adverse event requiring change in therapy prior to completion of full dose, protocol violation, death not due to malaria (WARN variables 13 to 15, 17, 20, 21)
- Lost to follow up (WARN variable 16)
- Withdrawal of consent by subject or investigator-initiated withdrawal (WARN variables 18 and 19)

4.8 Data collection and management

Baseline characteristics and data from study assessments were recorded on study-specific source templates (Appendix 1), designed by the MPH candidate, which supplemented any routine clinic notes. Key source data necessary for the analyses were transcribed onto case record forms (CRFs, Appendix 2), designed by the MPH candidate, which were 100% verified on-site by the UCT monitor and submitted for double data entry into an MS Access 2000 (Microsoft Corporation, Seattle, USA) database at the Medical Research Council Malaria Lead Programme in Durban. Written data queries were raised through both a manual review by the MPH candidate and by computerised validation checks specified by the MPH candidate. These were clarified with site staff in order to clean the database. Laboratory data from the MRC were recorded electronically (MS Word or MS Excel).

Data were imported from MS Access or MS Word/Excel into Stata/IC 10.0 (StataCorp LP, College Station, Texas, USA) and cleaned by the MPH candidate through cross-referencing relevant variables, thereby generating written queries which were resolved by reference to the source and/or CRF data prior to updating the STATA file.

Outcomes, time-to-event/censoring indicators and explanatory variables were programmed in STATA where necessary. Medical history and concomitant medication data were coded using the International Classification of Disease Version 10 (ICD10) and a study-specific coding dictionary developed using the South African Medical Formulary (SAMF) respectively.[28,29]

4.9 Handling of protocol violations and missing data

Major protocol violations were defined as:

- Subjects who missed study Days 1, 2, 3 (AS plus SP) or 2,3 (SP)
- Subjects who took a concomitant medication with antimalarial activity or folic acid
- Subjects whose Day 42 visit was more than 3 days late
- Incorrect dose administered
- Repeat dose due to vomiting (although directions for managing this were provided in the protocols)

Except for Day 42 (as indicated above), visit windows were not applied as actual dates of assessments were used in the survival analyses. Similarly, data were not censored due to missed visits as it is assumed that intensive AE monitoring would detect any history of malaria symptoms or treatment since the last visit.

For safety reasons an attempt was made to follow all subjects to completion of the actual Day 42 visit or the last assessment prior to loss to follow up despite protocol violations that may have impacted on the efficacy analysis.

Individual missing data fields were automatically or manually dropped from the analyses where appropriate. Three subjects' Day 0 parasitology slides were lost after a positive RDT for malaria, but prior to a parasite density measurement being documented, and these subjects were excluded from all efficacy analyses.

4.10 Ethical considerations

Both protocols and related documentation were approved by the University of Cape Town Research Ethics Committee and the Ethics Committee for the Mozambique Ministry of Health prior to study commencement (Appendix 3). The study was conducted in accordance with the South African Clinical Trials Guidelines 2000 which are based on the principles of the Declaration of Helsinki and the International Conference on Harmonisation for Good Clinical Practice Guideline (ICH GCP).[30]

Underpinning good clinical practices are the ethical principles of autonomy (respect for the dignity of the person), beneficence (benefit) and non-maleficence (absence of harm) to the research subject, and justice (equal distribution of risks and benefits between communities).[31] These principles were integral to the study design and its management.

In terms of autonomy, written informed consent was obtained from each subject or their guardian. Study teams were given extensive training in how to request informed consent from subjects who could be considered vulnerable because they had limited financial resources, may have been illiterate or unfamiliar with their rights regarding clinical research, and who also needed to make a quick decision whether to enroll in the study while they were unwell. Illiterate patients marked 'X' in the presence of an independent literate witness who signed the consent documentation. The team was aware that informed consent was an ongoing process throughout the study and that the subjects could leave the study at any time with no detrimental impact on their obtaining future health care at the health centre. The importance of ensuring confidentiality regarding subjects' participation and their personal data was also stressed.

While the study was designed expecting that the efficacy of AS plus SP would be different to that of SP monotherapy, this was not established in the region. SP had already been found to have acceptable efficacy there, which was far superior to chloroquine, the first line treatment policy at that time. Both study treatment groups were followed closely to ensure that possible harms relating to adverse drug reactions or lack of efficacy would be detected promptly and managed appropriately.

The study sites were selected, after discussions with policy-makers in Mozambique, to be where there was a significant malaria risk and, if better treatment policy could be established as a result of the study, the benefit to the communities involved may be significant. Community involvement was sought to establish dialogue between the research teams and community leaders, and to convey the possible impact of the results on future treatment policy. In addition an overall objective of the SEACAT Evaluation was to build capacity in the regions involved, whether in terms of research capabilities of

the teams and/or the clinics or by improving infrastructure. Over and above the training in research methodology and ethics provided to teams during the study, several post-graduate study opportunities were made available. During the study, useful equipment or services (such as microcopy for parasitological diagnosis of malaria and Hemacue® equipment for the rapid accurate determination of haemoglobin) were made available at the clinics which did not usually have them and, after the study was completed, some of this equipment was donated to the Ministry of Health.

5 STATISTICAL ANALYSIS METHODS

Although the primary outcome was analysed using survival methods, the overall treatment response of subjects who completed the study were also summarized by treatment group and proportions compared using Chi-square tests.

5.1 Demographic and clinical characteristics

Some characteristics were prospectively identified as potential risk factors and/or confounders: age, haemoglobin, presence of anaemia/severe anaemia (WHO 2001), parasite *dhfr/dhps* mutations, gender, parasite density, body temperature, body weight, mg/kg dose of pyrimethamine (as a measure of both pyrimethamine and sulfadoxine doses), mg/kg dose of artesunate, study site. Other characteristics were identified retrospectively based on a subsequent literature review: recent history of diarrhoea or vomiting, diarrhoea in the first 24 hours or during days 2 to 7, recent history of vomiting combined with fever on Day 0, vomiting within 1 hour of treatment, concomitant therapy with a drug having antimalarial properties.[27,59]

Categorical variables were summarised using frequencies and percentages. Continuous variables were summarised using means and standard deviations if normally distributed, or alternatively using medians and inter-quartile ranges if non-parametric, and distributions were examined using histograms. Correlations were tested using Pearson's product moment correlation coefficient (or Spearman's rank correlation if not normally distributed).

5.1.1 Differences between groups and across study sites

To explore associations between baseline characteristics and treatment group (in order to demonstrate successful randomization), baseline data were compared using Chi-square or Fisher's exact tests (if categorical) and Student's two sample t-tests or the Wilcoxon rank sum test (as appropriate, if continuous). Differences between the four study sites were explored using Chi-square or Fisher's exact tests (if categorical) and Kruskal-Wallis (if continuous). All tests were two-sided.

5.1.2 Association with completion status

To further investigate selection bias, the baseline characteristics of subjects who did not complete the study (i.e. were neither successfully treated nor considered treatment failures) were compared with those who did complete the study using Chi-square or Fisher's exact tests (if categorical) and Student's two sample t-tests or the Wilcoxon rank sum test (as appropriate, if continuous).

5.2 Survival analysis methodology

Survival analysis is currently recommended for analysing malaria efficacy studies (section 1.3) therefore the Kaplan-Meier (KM) method was used to generate time to event (treatment failure or parasite clearance) distributions and survival curves for the two treatment strategies, and these were compared using a log rank test.

Associations between response and baseline characteristics (other potential risk factors) were estimated using Cox's Proportional Hazards Regression. These risk factors were modelled in a univariate fashion and the variable with the most significant association chosen on which to build the model. Thereafter, remaining explanatory risk factors were added manually in a forward stepwise manner for comparison with the last best model by evaluating the likelihood ratio (LR) statistic and Aikaike's Information Criterion (AIC). Finally, the main effect (treatment group) was included. Automated stepwise models were compared with those obtained from the manual process. Interaction terms were generated where appropriate and interpreted for significance. Models were interpreted using hazard ratios.

Cox-Snell residuals were generated to assess overall fit of the models and outliers/influential observations were identified using rescaled Martingale (deviance) and ESR residuals. The assumption for proportional hazards in each model was tested using Schoenfeld residuals.[32]

5.3 Primary outcome analysis: time to treatment failure

5.3.1 Intention-to-treat analysis

For the intention-to-treat analysis the time to treatment failure was defined as the time from Day 0 until the actual day of treatment failure due to recrudescence (ETF, LTF-LCF or LTF-LPF). Subjects were considered as not yet failed treatment if they achieved an ACPR by actual Day 42 or were censored at the last evaluable assessment due to loss to follow up, withdrawal or re-infection with other malaria parasites.

Subjects' use of concomitant therapy with another drug having inherent antimalarial properties, and a repeat dose of study drug due to vomiting within 1 hour of treatment, were both included as potential risk factors. The protocols also included provision for a repeat half dose if vomiting occurred within ½ an hour but due to low numbers any repeat dosing occurrences were combined for the analyses.

5.3.2 Per-protocol analysis

For the per-protocol analysis, the time to treatment failure was again defined as the time from Day 0 until the actual day of treatment failure due to recrudescence (ETF, LTF-LCF or LTF-LPF). Subjects were considered as not yet failed treatment if they achieved an ACPR by actual Day 42 (± 3 days) or were censored at the last evaluable assessment due to loss to follow up, withdrawal or re-infection.

Data were excluded fully for subjects who missed a study dose and/or Days 2 and/or 3 (days where key protocol withdrawal criteria were applied) or who did not otherwise take the dose according to the protocol, including those who had a repeat dose of study drug within 1 hour, due to vomiting. Furthermore, data were censored at the last evaluable visit if subjects took a prohibited concomitant treatment with antimalarial properties or when Day 42 was more than 3 days late (i.e. data would be censored at Day 28 if that was the last visit before a Day 42 visit that was more than 3 days late). Since this study was concluded it was recommended that erythromycin be prohibited in malaria efficacy studies due to its weak anti-malarial activity, therefore erythromycin was added retrospectively to the pre-defined list of prohibited concomitant therapies.[27]

5.4 Secondary outcome analyses

5.4.1 Time to parasite clearance

For time to parasite clearance the data were analysed both on an intention-to-treat and a per-protocol basis using the same datasets as described above for the primary outcome. The definition of time to parasite clearance was the time from Day 0 to the first of 2 consecutive zero parasite density readings.

5.4.2 Fever clearance

For fever clearance, data were restricted to a subset of subjects who were documented as having a fever on Day 0. Fever clearance time was defined as the time from this Day 0 fever to the first temperature reading of less than 37.5°C.

6 RESULTS

6.1 Study subjects

6.1.1 Enrolment and follow-up

Four hundred and eleven subjects were enrolled between the 4 study sites from January 2003 to January 2005 and were randomised to take either SP (n= 199) or AS plus SP (n=212) (Figure 3). All subjects took at least one dose of study medication although 1 subject (SP) could not tolerate the dose and was withdrawn for parenteral treatment with quinine. 332 (80.8%) subjects were followed to study completion despite protocol violations such that some data were ultimately excluded from, or censored in the analyses. By nominal Day 42, 50 subjects overall (12.2%) were lost to follow up (the main reason reported to be movement out of the study area due to work) while 2 (0.5%) were withdrawn due to adverse events. The lost to follow up percentages were similar between treatment groups (SP, 11.6% AS plus SP, 12.7%). A further 27 subjects were rescued for malaria according to the protocol, but were found to have a re-infection on PCR analysis of MSP1, MSP2 and GLURP.

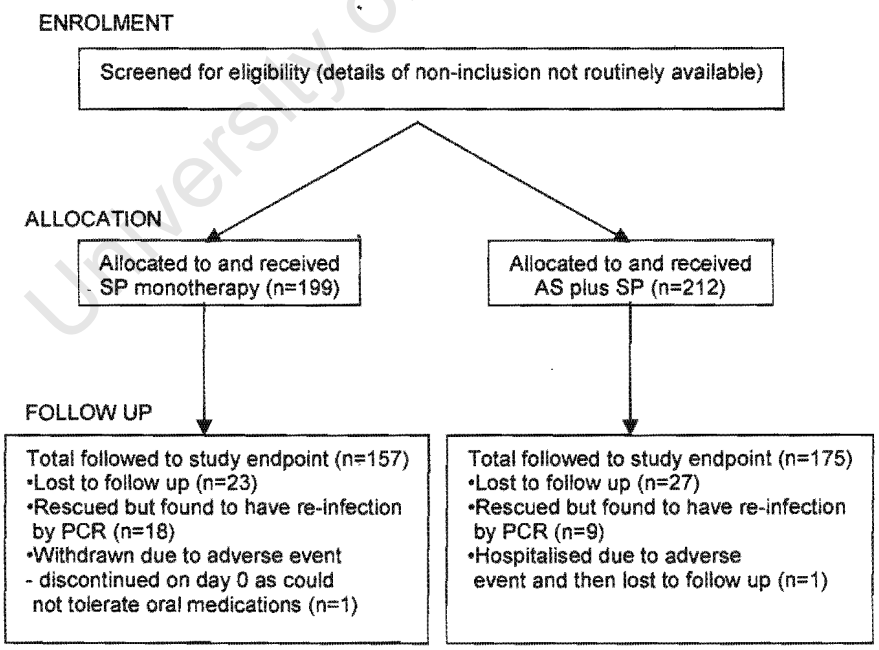


Figure 3: Subject flow

6.1.2 Protocol violations

The following protocol violations were found (subjects may have had more than 1 violation). There were no significant differences between groups:

Table 4: Protocol violations by treatment

Violation	SP (no.)	AS plus SP (no.)
Day 0 parasitology slides lost	1	2
Dose incorrect	4	4
Missed a study dose and/or visits on days 2/3	9	15
Disallowed medication (folic acid)	2	1
Disallowed medication (chloroquine)	0	1
Disallowed medication (cotrimoxazole)	2	3
Disallowed medication (doxyxycline)	1	0
Disallowed medication (erythromycin)*	18	18
Repeat dose of study drug due to vomiting	7	13

* Added to prohibited medication list retrospectively

6.2 Overall treatment response

Three RDT positive subjects were removed from all analyses as Day 0 slides were lost. Of 329 subjects followed to completion 87.9% in the SP arm and 97.7% in the AS plus SP arm achieved an ACPR (P=0.0008). There were 6.7% recrudescent treatment failures and 7.6% were withdrawn for treatment failure yet subsequently found by PCR to have been re-infected. There were differences between groups for all categories except for ETF while the difference between treatments for re-infection was not significant. (Table 5):

Table 5: Overall treatment response by treatment

Response	SP n(/%)	ASSP n(/%)	Total n(/%)	P*
ACPR	138/156 (87.9)	169/173 (97.7)	307/329 (93.3)	0.0008
Treatment failure	18/156 (11.5)	4/173 (2.3)	22/329 (6.7)	
ETF	5/156 (3.2)	3/173 (1.7)	8/329 (2.4)	0.39
LCF	4/156 (2.6)	0/173	4/329 (1.2)	0.03
LPF	9/156 (5.8)	1/173 (0.6)	10/329 (3.0)	0.006
Re-infection**	18/174 (10.3)	9/182 (4.9)	27/356 (7.6)	0.054

*2-sample test of proportions **denominator includes those found to be re-infected by PCR

ACRP: adequate clinical and parasitological response, ETF: early treatment failure, LCF: late clinical failure, LPF: late parasitological failure

6.3 Primary outcome analysis: time to treatment failure

6.3.1 Intention-to-treat (ITT) analysis

408/411 (99.3%) subjects were included in the intention-to-treat analyses (3 subjects were excluded completely as malaria could not be confirmed. Figure 4).

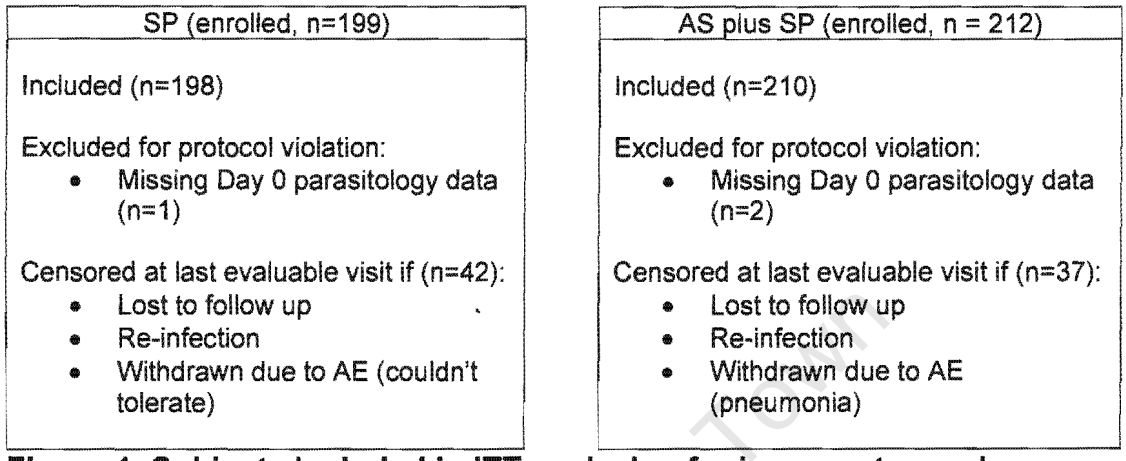


Figure 4: Subjects included in ITT analysis of primary outcome by treatment

Histograms of data from all subjects show haemoglobin, temperature, mg/kg dose of SP and AS to be normally distributed while age, duration of malaria symptoms, parasite density and weight were not. Parasite density was $\log_{10}$ transformed when it took a bimodal distribution indicating a possible interaction with (an)other variable(s) (Figure 5).

As anticipated, there was correlation between age and weight (Spearman 0.9 $p<0.001$) indicating they would not both be able to be brought into a Cox regression model.

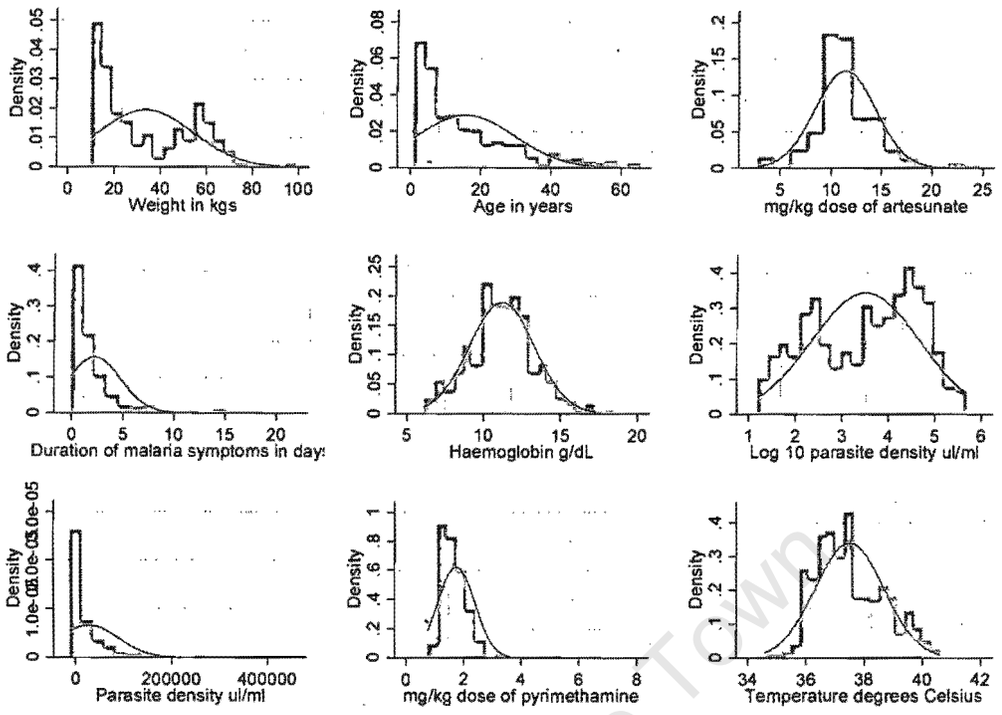


Figure 5: Histograms of continuous baseline characteristics, all subjects

There were no differences between the treatment groups in terms of baseline and clinical characteristics, including the 3 subjects subsequently removed from all analyses (Table 6a). Age was further explored to find whether a binary variable would be more informant and if so to determine an optimal cut-off for a categorical variable. In areas of moderate to high intensity malaria transmission younger children are at greater risk of treatment failure generally compared with older children and adults.[33]

Differences in haemoglobin, temperature, mg/kg dose of SP and AS, age, weight, parasite density, duration of malaria symptoms, history of vomiting (alone and combine with fever) and mutations were found between study sites (Table 6b). The lower Day 0 $\log_{10}$ parasite density for Catuane subjects compared to other sites may have contributed to the bimodal distribution found for the whole dataset (Figure 6).

Table 6a Baseline and clinical characteristics by treatment, all subjects

Continuous variables	SP (N=199)	AS SP (N=212)	Total (N=411)	p-value
Haemoglobin (g/dL) (mean/SD)	11.0(2.1)	11.4(2.1)	11.2(2.1)	0.07*
Temperature (°C) (mean/SD)	37.6(1.2)	37.4(1.2)	37.5(1.2)	0.251*
Dose of pyrimethamine (mg/kg) (mean/SD)	1.7(0.7)	1.8(0.6)	1.7(0.6)	0.645*
Dose of artesunate (mg/kg) (mean/SD)	N/a	11.4(3.0)	11.4(3.0)	N/a
Age (years) (median/IQR)	10.0(5.0-22.0)	12.0(5.0-24.0)	11.0(5.0-23.0)	0.237#
Weight (kg) (median/IQR)	25.0(15.0-52.0)	28.0(15.0-55.0)	25.8(15.0-54.0)	0.508#
Parasite density (per µl) (geometric mean/95% CI)	3630.7(2464.8; 5348.1)	2885.2(2028.4; 4104.0)	3225.6(2486.3; 4184.8)	0.284#
Log parasite density (per µl) (median/IQR)	3.8(2.5-4.5)	3.6(2.5-4.5)	3.7(2.5-4.5)	0.315#
Duration malaria symptoms (days) (median/IQR)	1.0(1.0-3.0)	2.0 (1.0-3.0)	2.0(1.0-3.0)	0.347#
Categorical variables (n/%)				
Gender Male	76 (38)	94 (44)	170 (41)	0.206^
Age in years 7 or under	84 (42.2)	79 (37.3)	163 (39.7)	0.306^
Anaemic (haemoglobin < 11g/dL)[1]	94 (47)	94 (44)	188 (46)	0.556^
Severely anaemia (haemoglobin < 7g/dL)[1]	6 (3)	2 (1)	8 (2)	0.129^
History of vomiting	20 (10)	17 (8)	37 (9)	0.472^
History of vomiting, with fever on day 0	9 (5)	6 (3)	15 (4)	0.361^
Vomited within 1 hour of any dose	7 (4)	12 (6)	19 (5)	0.301^
Diarrhoea in first 24 hrs post dose	14 (7)	7 (3)	21 (5)	0.086^
Diarrhoea between days 2 and 7	7 (4)	5 (2)	12 (3)	0.485^
Quintuple mutation	45 (22.6)	39 (18.4)	84(20)	0.289^

[1] WHO 2001
N/a Not applicable
* Student's two sample t-tests #Wilcoxon rank sum test ^Chi-square or Fisher's exact tests

Table 6b: Baseline and clinical characteristics by study site, all subjects

Continuous variables	Boane (N=104)	Catuane(N=50)	Magude(N=179)	Namaacha(N=78)	p-value
Haemoglobin (g/dL) (mean/SD)	11.5(2.4)	11.7(1.8)	11.0(1.9)	11.0(2.4)	0.024"
Temperature (°C) (mean/SD)	37.3(1.1)	37.8(0.7)	37.3(1.2)	37.8(1.3)	<0.001'
Dose of pyrimethamine (mg/kg) (mean/SD)	1.6(0.6)	1.9(0.7)	1.8(0.5)	1.8(0.8)	<0.001'
Dose of artesunate (mg/kg) (mean/SD)	10.5(0.3)	12.4(0.6)	11.7(0.3)	11.5(0.6)	0.014"
Age (years) (median/IQR)	19.0(8.0-28.0)	8.0(6.0-13.0)	10.0(5.0-20.0)	5.0(3.0-23.0)	<0.001'
Weight (kg) (median/IQR)	50.0(20.5-60.0)	23.1(16.4-36.6)	25.0(15.0-50.0)	17.2(12.4-55.0)	<0.001'
Parasite density (per µl) (geometric mean/95% CI)	4252.7(2701.7; 6694.1)	717.2(412.2; 1247.8)	2261.2(1468.6; 3481.6)	12475.1(7537.5; 20647.3)	<0.001'
Log parasite density (per µl) (median/IQR)	3.8 (2.8-4.5)	2.6 (2.2-3.3)	3.5 (2.2-4.5)	4.3 (3.6-4.8)	<0.001'
Duration malaria symptoms (days) (median/IQR)	1.0(1.0-3.0)	1.0(0.0-2.0)	2.0(1.0-3.0)	2.0(1.0-3.0)	<0.001'
Categorical variables (n/%)					
Gender Male	42(25)	24(14)	67(39)	37(22)	0.351^
Age in years 7 or under	24(15)	22(14)	71(43)	46(28)	<0.001'
Anaemic (Haemoglobin < 11g/dL)[1]	44(23)	15(8)	90(48)	39(21)	0.055^
Severely anaemic (Haemoglobin < 7g/dL)[1]	1(13)	1(13)	2(25)	4(50)	0.152^
History of vomiting	14(38)	0	19(51)	4(11)	0.012^
History of vomiting, with fever on day 0	7(47)	0	8(53)	0	0.036^
Vomited within 1 hour of any dose	5(26)	2(11)	8(42)	4(21)	0.985^
Diarrhoea in first 24 hrs post dose	8(38)	1(5)	5(24)	7(33)	0.078^
Diarrhoea between days 2 and 7	5(42)	0	3(25)	4(33)	0.156^
Quintuple mutation	23(27)	4(5)	35(42)	22(26)	0.039^

[1] WHO 2001
N/a Not applicable
* Student's two sample t-tests #Wilcoxon rank sum test ^Chi-square or Fisher's exact tests "Kruskal-Wallis test

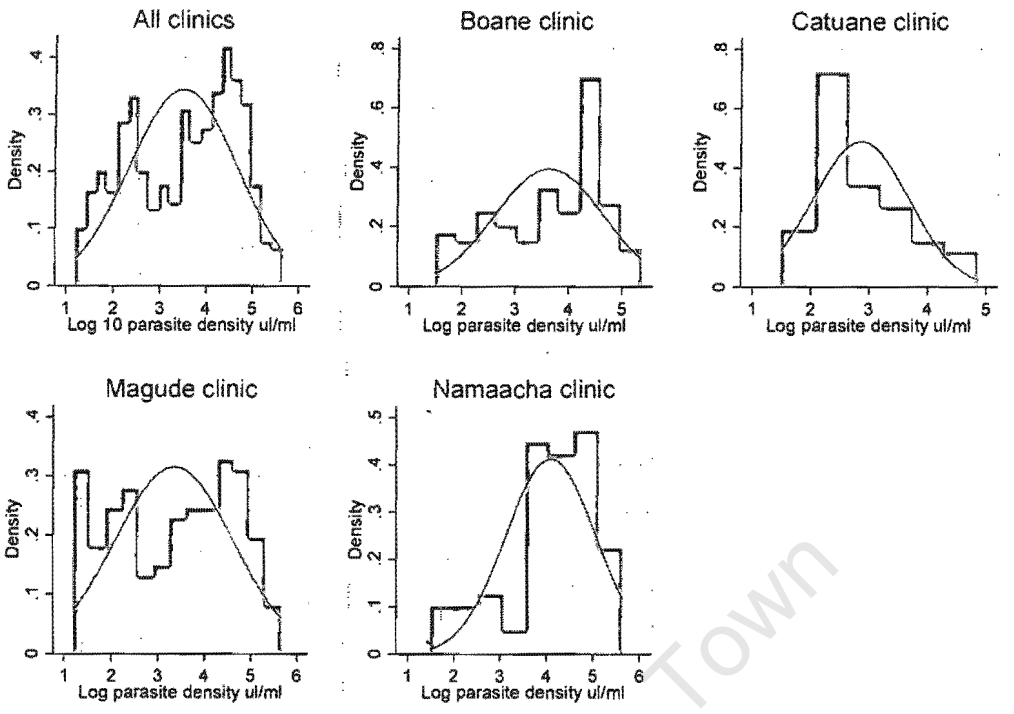


Figure 6: Histograms of log parasite density day 0 by site, all subjects

When data were explored in terms of associations between the baseline characteristics and whether or not subjects completed the study, no differences were found.

When Kaplan-Meier distributions and survival curves were generated, 10 subjects' observations ended before entering the analysis, as they were censored immediately after Day 0. The Kaplan Meier estimates for achieving an ACPR by Day 42 were 90.4% (95% CI 84.9%;93.9%) for SP and 98.0% (95% CI 94.8%;99.3%) for AS plus SP. AS plus SP showed a significantly reduced relative hazard of treatment failure compared to SP monotherapy (log rank p= 0.0008) (Figure 7 and Table 7).

There was a greater difference observed between treatments by Day 45; ACPR following SP, 83.4% (95% CI 63.9%;92.9%), AS plus SP, 98% (95% CI 94.8%;99.3%) however the numbers of subjects analysed after Day 42 was small.

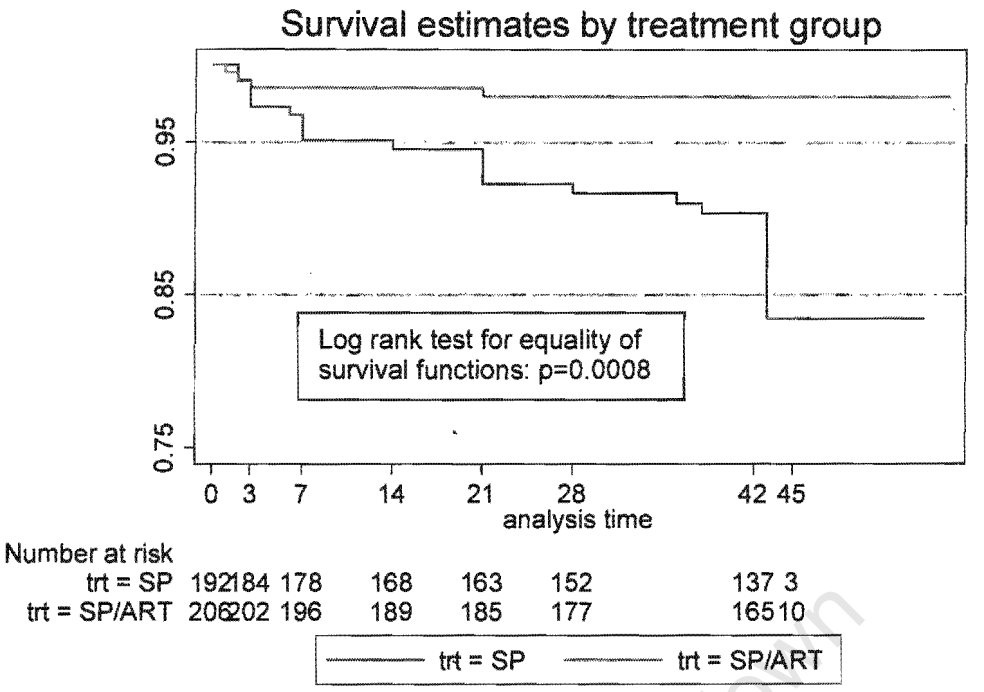


Figure 7: Kaplan Meier survival curves for time to treatment failure, ITT dataset

Table 7: Kaplan Meier survival estimates for time to treatment failure, ITT dataset

Days	SP		AS plus SP	
	N	Survivor function (% and 95% CI)	N	Survivor function (% and 95% CI)
0	0	100	0	100
1	192	100	206	99.5 (96.6 – 99.9)
2	188	99.0 (95.8 – 99.7)	205	99.0 (96.2 – 99.8)
3	184	97.3 (93.7 – 98.9)	202	98.5 (95.5 – 99.5)
7	178	95.2 (90.9 – 97.5)	196	98.5 (95.5 – 99.5)
14	168	94.6 (90.2 – 97.1)	189	98.5 (95.5 – 99.5)
21	163	92.3 (87.3 – 95.3)	185	98.0 (94.8 – 99.3)
28	152	91.7 (86.5 – 94.9)	177	98.0 (94.8 – 99.3)
42	137	90.4 (84.9 – 93.9)	165	98.0 (94.8 – 99.3)
45	3	83.4 (63.9 – 92.9)	10	98.0 (94.8 – 99.3)

A univariate Cox regression model aimed at estimating the unadjusted treatment effect, showed a decrease of 80% in the relative risk of treatment failure for AS plus SP compared to SP (Hazard ratio 0.2, 95% CI 0.1;0.6, p=0.003). The following univariate relationships were found for the other risk factors (Table 8):

Table 8: Time to treatment failure univariate analyses of potential risk factors, ITT dataset

Variable	Hazard ratio	95% CI	p
Age > 7 years vs. age ≤ 7 years	**0.3**	**0.1-0.8**	**0.010**
Anaemia, presence vs. absence	1.8	0.8-4.1	0.188
Severe anaemia, presence vs. absence	2.6	0.4-19.2	0.355
AS total dose (mg/kg)	0.7	0.5-1.7	0.187
Pyrimethamine total dose (mg/kg)	1.1	0.6-1.9	0.809
Diarrhoea in the first 24 hours post-dose, presence vs. absence during that time	0.9	0.1-7.5	0.891
Diarrhoea between days 2-7 presence vs. absence on these days	1.5	0.2-11.3	0.681
Duration of malaria symptoms before Day 0 (days)	1.0	0.9-1.2	0.753
Gender, male vs. female	1.4	0.6-3.3	0.404
Haemoglobin Day 0 (g/dL)	0.9	0.8-1.1	0.478
Log_{10} parasite density (per µl blood)	**1.7**	**1.1-2.7**	**0.013**
Presence of a quintuple mutation vs fewer mutations	**3.3**	**1.4-7.5**	**0.006**
Site Catuane vs Boane	Unable to estimate due to no treatment failures at Catuane		
Magude vs Boane	1.3	0.4-4.1	0.705
Namaacha vs Boane	3.0	0.9-9.8	0.065
Namaacha (compared to all other sites)	**3.1**	**1.3-7.2**	**0.010**
Temperature Day 0 (°C)	**1.7**	**1.2-2.4**	**0.002**
History of vomiting vs. no history of vomiting	1.1	0.2-4.5	0.952
History of vomiting combined with fever Day 0 vs. no such combination	1.3	0.2-9.7	0.793
Repeat dose of study drug due to vomiting	0.93	0.1-6.9	0.944
Weight (kg)	**0.97**	**0.95-1.0**	**0.039**
Use of other drug with anti-malarial activity	1.2	0.4-4.0	0.792

Statistically significant variables in bold type

Using these univariate models as a starting point, several multivariate models were considered and compared to find the best subset of combined risk factors that are predictive of the relative hazard of treatment failure (Table 9). The variables relating to concomitant medication with another drug having inherent antimalarial activity and repeat dose of study drugs due to vomiting, which were only tested in the ITT analysis, were both not significantly associated with the hazard of treatment failure.

Table 9: Time to treatment failure best model without treatment, ITT dataset

Variable	Hazard ratio	95% CI	p
Age > 7 years vs. age ≤ 7 years	0.4	0.2-0.9	0.033
Presence of a quintuple mutation vs fewer mutations	3.6	1.5-8.3	0.003
Temperature Day 0 (°C)	1.6	1.1-2.3	0.011

This table shows a significant negative association between age and the time to treatment failure, indicating that those over 7 years have a 60% decreased relative hazard of failure compared to younger children. There were significant positive associations for both a presence of a quintuple mutation and baseline temperature with the outcome, suggesting that those subjects with a quintuple mutation have a greater than 3-fold increased relative hazard of failure compared to those with fewer mutations. There was a 60% increased hazard of failure for every 1° increase in body temperature.

Table 10 presents the model with treatment group added to the selected subset of risk factors presented in Table 9. The addition of treatment did not significantly change the previous model, and the association between treatment and failure, when modelled with the explanatory variables, was almost identical to that found when unadjusted. Interaction terms between all final variables selected were tested and no significant interactions were found, indicating that the treatment effect is the same for both the age and mutations categories and for all temperatures.

Table 10: Time to treatment failure final model, ITT dataset

Variable	Hazard ratio	95% CI	p
Treatment with AS plus SP vs SP	0.2	0.1-0.6	0.004
Age > 7 years vs. age ≤ 7 years	0.3	0.1-0.9	0.033
Presence of a quintuple mutation vs fewer mutations	3.2	1.3-7.5	0.009
Temperature Day 0 (°C)	1.5	1.1-2.2	0.020

The above results show that AS plus SP decreases the relative hazard of treatment failure by 80% compared with SP monotherapy. Other important factors that also have an impact include being aged over 7 years (which decreases the relative hazard of failure by 70%), the body temperature on Day 0 (which increases the relative hazard of failure by 50% for each

additional 1°C) and having a quintuple mutation (which increases the relative hazard of failure 3.2 fold compared to fewer mutations).

Temperature on Day 0 was chosen during model-building due to the strictly systematic method used (temperature had a greater univariate statistical significance). However parasite density, which is also a known indicator of disease severity, has been found to be a significant predictor of treatment failure in other studies.[34] In our study $\log_{10}$ parasite density had a similar univariate association with the relative hazard of treatment failure as for temperature therefore the final model was tested substituting parasite density for temperature, whereupon it was not found to be a significant risk factor and also did not improve the model overall.

6.3.2 Per-protocol (PP) analysis

354/411 (86.1%) subjects were included in the per-protocol analysis (Figure 8). There were no differences between the treatment groups in terms of baseline and clinical characteristics (Table 11).

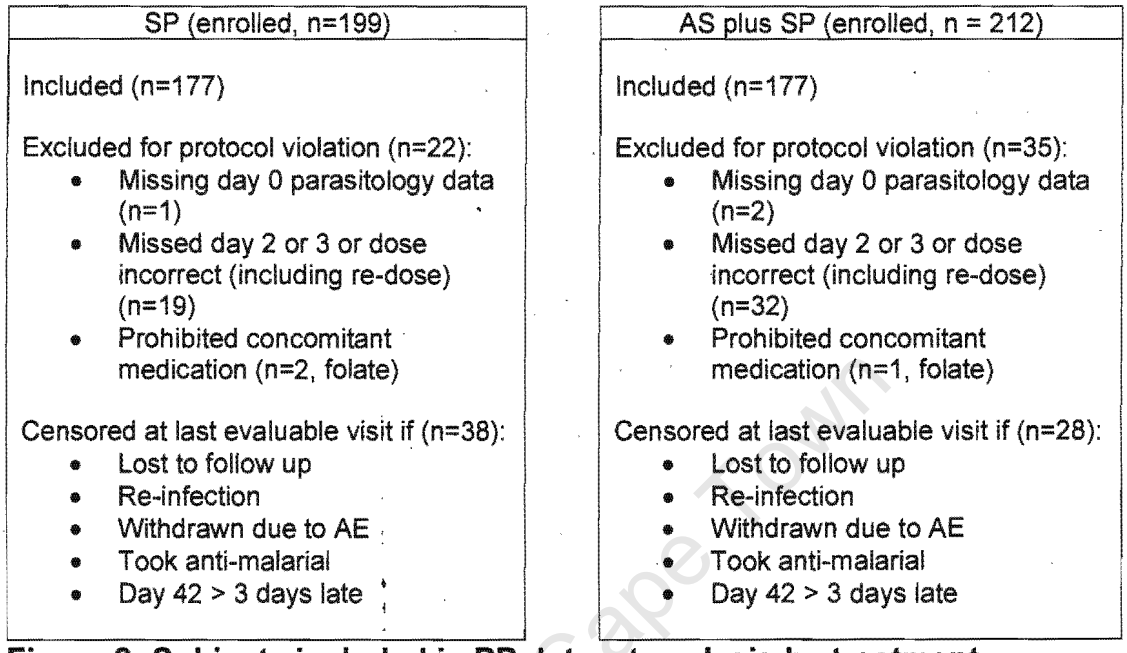


Figure 8: Subjects included in PP dataset analysis by treatment

Table 11: Baseline and clinical characteristics by treatment, PP dataset

Continuous variables	SP (N=177	AS plus SP(N=177)	Total (N=354)	p-value
Haemoglobin (g/dL) (mean/SD)	11.0(2.1)	11/4(2.1)	11.2(2.1)	0.07*
Temperature (°C) (mean/SD)	37.5(1.1)	37.4(1.2)	37.5(1.2)	0.2346*
Dose of pyrimethamine (mg/kg) (mean/SD)	1.6(0.4)	1.7(0.4)	1.6(0.4)	0.639*
Dose of artesunate (mg/kg) (mean/SD)	N/a	11.1(2.5)	11.1(2.5)	N/a
Age (years) (median/IQR)	10.0(5.0-24.0)	13.0(6.0-26.0)	12.0(5-25)	0.1816#
Weight (kg) (median/IQR)	25.0(15.0-54.0)	31.1(15.0-55.0)	27.0(15.0-55.0)	0.4472#
Parasite density (per µl) (geometric mean/95% CI)	3315.8(2203.5; 4989.7)	2534.8(1727.2; 3720.0)	2899.1(2192.8; 3833.0)	0.274#
Log parasite density (per µl) (median/IQR)	3.7(2.5-4.5)	3.5(2.4-4.3)	3.6(2.4-4.5)	0.2738#
Duration malaria symptoms (days) (median/IQR)	1.0(1.0-3.0)	2.0(1.0-3.0)	2.0(1.0-3.0)	0.6085#
Categorical variables (n/%)				
Gender Male	69(39)	83(47)	152(43)	0.133^
Age in years 7 or under	72(41)	60(34)	132(37)	0.187^
Anaemic (haemoglobin < 11g/dL)[1]	81(46)	75(42)	156(44)	0.521^
Severely anaemia (haemoglobin < 7g/dL)[1]	5(3)	1(1)	6(2)	0.215^
History of vomiting	18(10)	11(6)	29(8)	0.245^
History of vomiting, with fever on day 0	7(4)	4(2)	11(3)	0.542^
Vomited within 1 hour of any dose	9(5)	3(2)	12(3)	0.139^
Diarrhoea in first 24 hrs post dose	12(7)	4(2)	16(5)	0.07^
Diarrhoea between days 2 and 7	7(4)	5(3)	12(3)	0.77^
Quintuple mutation	39(22)	36(20)	75(21)	0.696^

[1] WHO 2001
N/a Not applicable
* Student's two sample t-tests #Wilcoxon rank sum test ^Chi-square or Fisher's exact tests

When Kaplan-Meier distributions and survival curves were generated, 29 subjects' observations ended before entering the analysis, as they were censored immediately after Day 0. Treatment with AS plus SP was found to prolong the time to treatment failure compared to SP monotherapy (log rank p= 0.005) as shown by Kaplan-Meier curves (Figure 9) and survival estimates (Table 12).

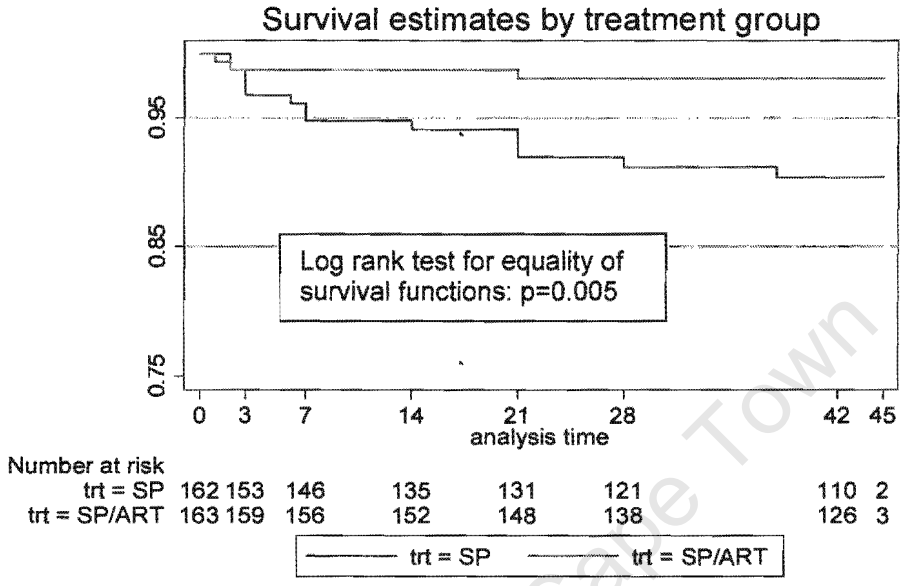


Figure 9: Time to treatment failure KM survival curve, PP dataset

Table 12: Time to treatment failure KM survival estimates, PP dataset

Days	SP		AS plus SP	
	N	Survivor function (% and 95% CI)	N	Survivor function (% and 95% CI)
0	0	100	0	100
1	162	100	163	99.4 (95.7 – 99.9)
2	158	98.7 (95.0 – 99.7)	161	98.8 (95.2 – 99.7)
3	153	96.8 (92.5 – 98.7)	159	98.8 (95.2 – 99.7)
7	146	94.8 (89.9 – 97.4)	156	98.8 (95.2 – 99.7)
14	135	94.1 (89.0 – 96.9)	152	98.8 (95.2 – 99.7)
21	131	92.0 (86.3 – 95.4)	148	98.1 (94.2 – 99.4)
28	121	91.2 (85.3 – 94.8)	138	98.1 (94.2 – 99.4)
42	110	90.4 (84.3 – 94.2)	126	98.1 (94.2 – 99.4)
45	2	90.4 (84.3 – 94.2)	3	98.1 (94.2 – 99.4)

The data show that the Day 42 ACPR estimates were 90.4% (95% CI 84.4%;94.2%) for SP and 98.1% (95% CI 94.2%;99.4%) for AS plus SP. These were very similar to those found in the ITT dataset. There was no

change in these estimates observed by Day 45, unlike the greater difference at Day 45 seen in the ITT dataset.

A univariate Cox regression model aimed at estimating the unadjusted treatment effect showed that the relative hazard of treatment failure decreased by 80% on treatment with AS plus SP compared to SP monotherapy (Hazard Ratio 0.2, 95% CI 0.1;0.7, p=0.011), as was found in the intention-to-treat analysis.

The following univariate relationships were found for the other risk factors (Table 13):

Table 13: Time to treatment failure univariate analyses of potential risk factors, PP dataset

Variable	Hazard ratio	95% CI	P
Age > 7 years vs. age ≤ 7 years	**0.2**	**0.1-0.6**	**0.005**
Anaemia, presence vs. absence	2.1	0.8-5.4	0.137
Severe anaemia, presence vs. absence	3.7	0.5-27.6	0.208
AS total dose (mg/kg)	**0.5**	**0.3-0.8**	**0.004**
Pyrimethamine total dose (mg/kg)	1.1	0.3-3.6	0.883
Diarrhoea in the first 24 hours post-dose, presence vs. absence during that time	1.2	0.2-8.8	0.881
Diarrhoea between days 2-7 presence vs. absence on these days	1.6	0.2-12.3	0.637
Duration of malaria symptoms before Day 0 (days)	1.0	0.8-1.2	0.810
Gender, male vs. female	1.1	0.4-3.0	0.788
Haemoglobin Day 0 (g/dL)	0.9	0.7-1.1	0.161
Log_{10} parasite density (per µl blood)	**2.3**	**1.3-4.0**	**0.004**
Presence of a quintuple mutation vs fewer mutations	**3.4**	**1.3-8.7**	**0.013**
Season 2004 vs. 2003	0.6	0.2-1.8	0.344
2005 vs. 2003	1.6	0.5-5.3	0.430
Site Catuane vs Boane	Unable to estimate due to no treatment failures at Catuane		
Magude vs Boane	1.1	0.3-3.8	0.858
Namaacha vs Boane	2.3	0.7-8.1	0.198
Temperature Day 0 (°C)	**1.9**	**1.3-2.7**	**0.002**
History of vomiting vs. no history of vomiting	1.4	0.3-6.3	0.626
History of vomiting combined with fever Day 0 vs. no such combination	2.0	0.3-15.1	0.500
Weight (kg)	**0.97**	**0.94-1.0**	**0.047**

Statistically significant variables in bold type

A statistically significant univariate association was found between the mg/kg dose of artesunate and the relative hazard of treatment failure (p=0.004) which is related to a particular influential outlying study subject who was an early treatment failure on Day 1 and only received a Day 0 dose. The mg/kg artesunate variable could not thereafter be brought into the model.

Using these univariate models as a starting point, several multivariate models were considered and compared to find the best subset of combined risk factors that are predictive of the relative hazard of treatment failure (Table 14).

Table 14: Time to treatment failure best model without treatment, PP dataset

Variable	Hazard ratio	95% CI	p
Age > 7 years vs. age ≤ 7 years	0.2	0.1-0.6	0.005
Presence of a quintuple mutation vs fewer mutations	4.0	1.5-10.6	0.005
Temperature Day 0 (°C)	1.7	1.2-2.6	0.008
Dose of pyrimethamine (mg/kg)	0.3	0.7-1.1	0.072

As found in the intention-to-treat dataset, the table indicates that those over 7 years have a decreased relative hazard of failure compared to younger children (by 80%) while those subjects with a quintuple mutation have an increased relative hazard of failure compared to those with fewer mutations (4-fold), and an increased hazard of failure as body temperature increases (by 70% for each °C). In addition there was trend towards a 70% decreased relative hazard of failure with higher mg/kg doses of pyrimethamine.

Table 15 presents the model with treatment group added to the selected subset of risk factors presented in Table 14. The variable "mg/kg dose of pyrimethamine" became non-significant and was removed from the model. Interaction terms between all the final variables selected were tested and no significant interactions were found, indicating that the treatment effect is the same for all age and mutations categories and for all temperatures. The association between treatment and failure, when modelled with the risk factors, was almost identical to that found when unadjusted.

Table 15: Time to treatment failure final model, PP dataset

Variable	Hazard ratio	95% CI	p
Treatment with AS plus SP	0.2	0.0-0.7	0.018
Age > 7 years vs. age ≤ 7 years	0.3	0.1-0.8	0.022
Presence of a quintuple mutation vs fewer mutations	3.4	1.3-9.3	0.014
Temperature Day 0 (°C)	1.6	1.0-2.4	0.033

Again, the final model was tested with parasite density replacing temperature. This new model fit was not quite as good as the final model chosen and parasite density itself was not statistically significant, although there was a trend towards significance (p=0.07).

The final model suggests that, in addition to treatment with AS plus SP which decreases the relative risk of treatment failure by 80% compared with SP monotherapy, other important risk factors include being aged over 7 years (which decreases the relative risk of failure by 70%), the body temperature on Day 0 (which increases the relative risk of failure by 60% for each additional °C) and having a quintuple mutation (which increases the relative risk of failure 3.4 fold compared to fewer mutations). These results confirmed those found in the ITT dataset.

6.4 Secondary outcome analysis: time to parasite clearance

6.4.1 Intention-to-treat (ITT) analysis

The ITT analysis of time to parasite clearance included the same dataset as the ITT analysis of the primary outcome. One additional subject was automatically excluded as he did not have 2 consecutive zero parasite densities due to a missed visit. Actual days were used therefore a few subjects did not clear parasites until Day 8 or 9. Kaplan Meier estimates showed that only 52.0% (95% CI 45.0%;59.3%) of those taking SP monotherapy had cleared parasites by 48 hours compared to 76.3% (95% CI 69.9%;82.3%) taking AS plus SP; demonstrating the superior effect of AS plus SP (log rank p<0.001) (Figure 10 and Table 16).

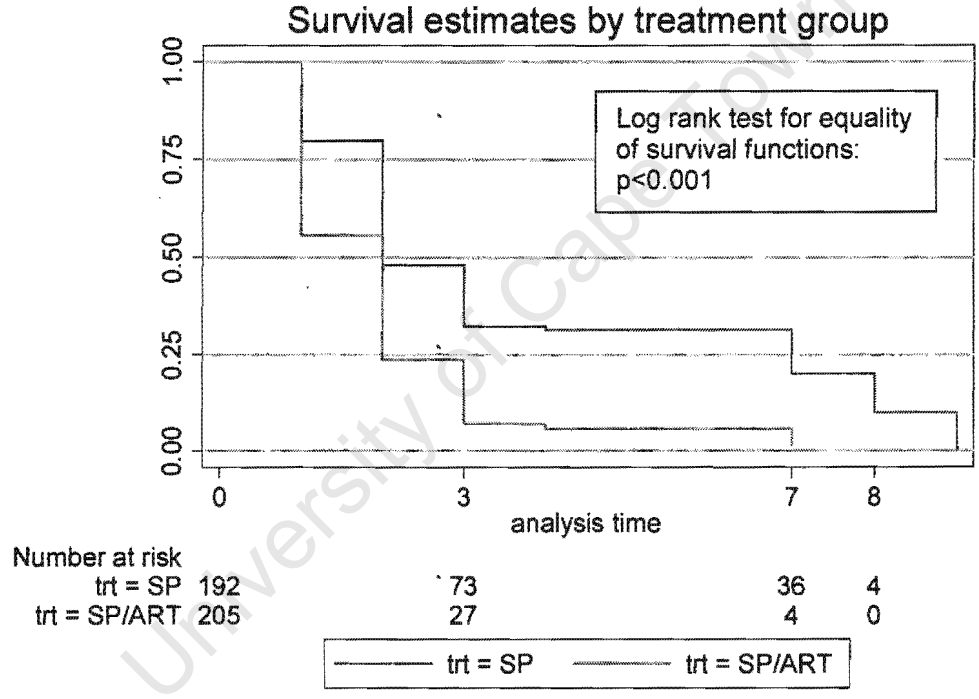


Figure 10: Parasite clearance time KM survival curve, ITT dataset

Table 16: Parasite clearance time KM survival estimates, ITT dataset

Time (days)	SP		AS plus SP	
	N	Survivor function (95% CI)	N	Survivor function (95% CI)
0	0	100	0	100
1	192	79.7 (73.3 – 84.7)	205	53.6 (48.5 – 62.0)
2	146	48.0 (40.7 – 55.0)	94	23.7 (17.7 – 30.1)
3	73	32.2 (25.3 – 39.4)	27	7.0 (3.4 – 12.4)
7	36	20.1 (13.8 – 27.1)	4	1.5 (0.1 – 6.5)
8	4	10.0 (2.7 – 23.1)		

A univariate Cox regression model estimating the unadjusted treatment effect showed that the relative hazard of parasite clearance increased by 90% on AS plus SP compared to SP alone (Hazard ratio 1.9, 1.5;2.3, p<0.001). The following univariate relationships were found for the other risk factors (Table 17):

Table 17: Parasite clearance time univariate analyses of potential risk factors, ITT dataset

Variable	Hazard ratio	95% CI	p
Age > 7 years vs. age ≤ 7 years	**1.3**	**1.0-1.7**	**0.023**
Anaemia, presence vs. absence	0.9	0.7-1.1	0.154
Severe anaemia, presence vs. absence	0.7	0.3-1.7	0.417
AS total dose (mg/kg)	1.0	1.0-1.1	0.515
Pyrimethamine total dose (mg/kg)	1.0	0.8-1.2	0.827
Diarrhoea in the first 24 hours post-dose, presence vs. absence during that time	1.0	0.6-1.7	0.897
Diarrhoea between days 2-7 presence vs. absence on these days	0.7	0.3-1.4	0.280
Duration of malaria symptoms before Day 0 (days)	1.0	0.9-1.0	0.669
Gender, male vs. female	0.9	0.8-1.2	0.572
Haemoglobin Day 0 (g/dL)	1.0	1.0-1.1	0.130
Log_{10} parasite density (per µl blood)	**0.7**	**0.7-0.8**	**0.000**
Presence of a quintuple mutation vs fewer mutations	0.8	0.6-1.0	0.062
Site Catuane vs Boane	**1.8**	**1.2-2.5**	**0.003**
Magude vs Boane	0.8	0.6-1.1	0.151
Namaacha vs Boane	**0.7**	**0.5-1.0**	**0.031**
Catuane (compared to others)	**2.1**	**1.5-2.9**	**<0.001**
Temperature Day 0 (°C)	1.0	1.0-1.0	0.681
History of vomiting vs. no history of vomiting	**0.7**	**0.4-1.0**	**0.043**
History of vomiting combined with fever Day 0 vs. no such combination	**0.5**	**0.3-1.0**	**0.043**
Repeat dose of study drug due to vomiting	1.0	0.6-1.6	0.958
Weight (kg)	1.0	0.99-1.0	0.107
Use of other drug with anti-malarial activity	0.9	0.6-1.3	0.479

Statistically significant variables in bold type

Several multivariate models were compared to find the best subset of combined risk factors that are predictive of the relative hazard of clearing parasites in the ITT dataset (Table 18). The variables relating to concomitant medication with another drug having inherent antimalarial activity and repeat dosing of study drugs due to vomiting, which were only tested in the ITT

analysis, were both not significantly associated with the hazard of clearing parasites.

Table 18: Parasite clearance time best model without treatment, ITT dataset

Variable	Hazard ratio	95% CI	p
Log_{10} parasite density (per µl blood)	0.8	0.7 – 0.8	<0.001
Catuane (compared to other sites)	1.8	1.3 – 2.5	<0.001
Age > 7 years vs. age ≤ 7 years	1.2	1.0 – 1.6	0.091

This table shows a significant negative association between parasite density and the time to parasite clearance, indicating that those subjects with a higher baseline parasite density have a decreased relative hazard of clearing parasites compared to those having lower baseline parasite densities. Conversely, subjects enrolled at Catuane have an increased relative hazard of clearing parasites. There was a trend towards subjects over 7 years of age clearing parasites more rapidly.

Table 19 presents the model with treatment group added to the selected subset of risk factors presented in Table 18. The addition of treatment necessitated the removal of the age category variable as it became non-significant (p=0.133). Otherwise the model did not significantly change and the superior treatment effect of AS plus SP compared to SP monotherapy in terms of clearing parasites when modelled with risk factors was similar to that found when unadjusted.

Table 19: Parasite clearance time final model, ITT dataset

Variable	Hazard ratio	95% CI	p
Treatment with AS plus SP vs. SP	1.8	1.4 – 2.3	<0.001
Log_{10} parasite density (per µl blood)	0.8	0.7 – 0.8	<0.001
Catuane (compared to other sites)	1.8	1.3 – 2.5	<0.001

These results show that treatment with AS plus SP increases the relative hazard of parasite clearance by 80% compared with SP monotherapy. Other important factors that had an impact include being a subject enrolled at Catuane clinic which led to an 80% increase in the relative hazard of parasite clearance, while each 10 fold increase in parasite density decreased the relative hazard of parasite clearance by 20%.

When interaction terms between all the final variables were tested, a significant interaction was found between treatment group and log parasite density suggesting that the AS plus SP treatment effect was amplified when the parasite density was higher (Table 20). However, the relative hazard of clearing parasites was reasonably similar for both treatment groups when they were modeled separately (SP: Hazard ratio 0.7 [95% CI 0.6;0.8], AS plus SP: Hazard ratio 0.8 [95% CI 0.7;1.0]) indicating that the global results from the final model presented in Table 19 above holds true for all patients generally.

Table 20: Parasite clearance time final model including interaction, ITT dataset

Variable	Hazard ratio	95% CI	p
Treatment with AS plus SP vs. SP	0.9	0.4 – 1.8	0.693
Log_{10} parasite density (per µl blood)	0.7	0.6 – 0.8	<0.001
Catuane (compared to other sites)	1.8	1.3 – 2.5	<0.001
Treatment/log_{10} parasite density interaction	1.2	1.0 – 1.5	0.033

6.4.2 Per-protocol (PP) analysis

The PP analysis of time to parasite clearance included the same dataset of subjects as for the PP analysis of the primary outcome, time to treatment failure. One additional subject was automatically excluded as he did not have 2 consecutive zero parasite densities due to a missed visit. Actual days were used therefore a few subjects did not clear parasites until Day 8. AS plus SP was found to increase the relative risk of parasite clearance compared to SP monotherapy (log rank p<0.001) as shown by Kaplan-Meier curves (Figure 11) and survival estimates (Table 21):

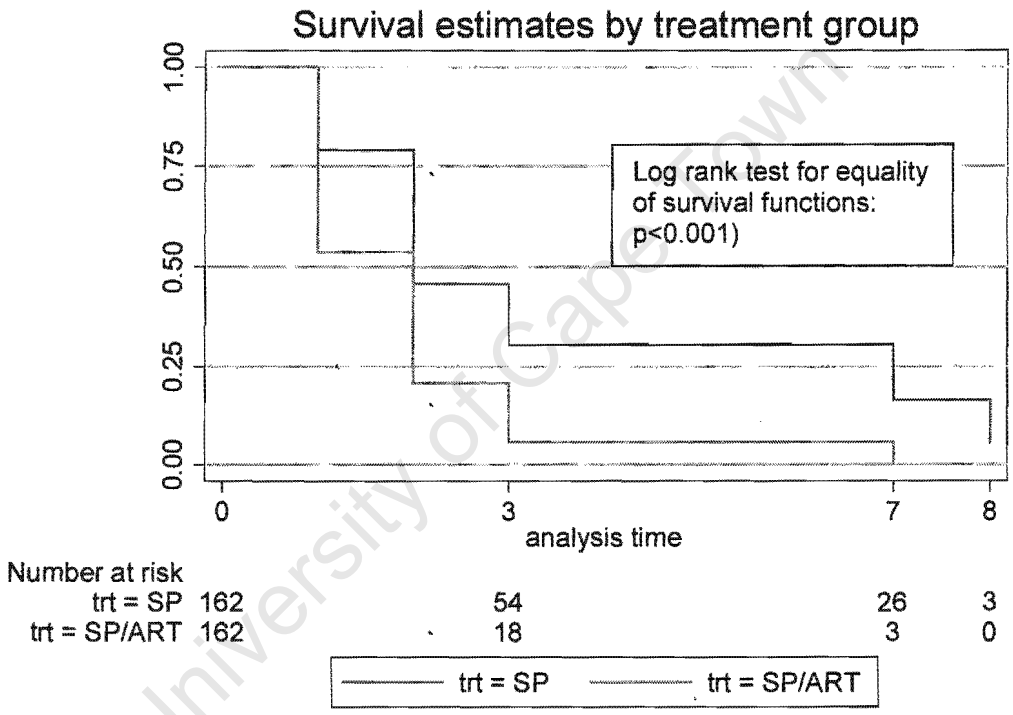


Figure 11: Parasite clearance time KM survival curve PP dataset

Table 21: Parasite clearance time KM survival estimates, PP dataset

Time (days)	SP		AS plus SP	
	N	Survivor function (% and 95% CI)	N	Survivor function (% and 95% CI)
0	0	100	0	100
1	162	79.10 (71.90 – 84.32)	162	53.70 (45.73 – 61.02)
2	121	45.71 (37.73 – 53.32)	70	20.71 (14.38 – 27.86)
3	54	30.47 (22.93 – 38.33)	18	57.5 (2.2 – 11.7)
7	26	16.41 (10.0 – 24.2)	3	0.0
8	3	5.5 (0.6 – 19.4)	3	

These data show that only 54.3% (95% CI 46.7%;62.3%) of those taking SP monotherapy had cleared parasites by 48 hours compared to 79.3% (95% CI 72.2%;85.6%) taking AS plus SP.

A univariate Cox regression model estimating the unadjusted treatment effect showed that the relative hazard of parasite clearance increased by 90% for AS plus SP compared to SP alone (Hazard ratio 1.9, 95% CI 1.4;2.4, p<0.001). The following univariate relationships were found for the other risk factors (Table 22):

Table 22: Parasite clearance time univariate analyses of potential risk factors, PP dataset

Variable	Hazard ratio	95% CI	p
Age > 7 years vs. age ≤ 7 years	**1.5**	**1.1 - 1.9**	**0.004**
Anaemia, presence vs. absence	0.9	0.7 - 1.1	0.285
Severe anaemia, presence vs. absence	0.5	0.2 - 1.5	0.194
AS total dose (mg/kg)	1.0	0.9 - 1.1	0.659
Pyrimethamine total dose (mg/kg)	1.0	0.7 - 1.3	0.734
Diarrhoea in the first 24 hours post-dose, presence vs. absence during that time	1.0	0.6 - 1.7	0.977
Diarrhoea between days 2-7 presence vs. absence on these days	0.7	0.3 - 1.3	0.226
Duration of malaria symptoms before Day 0 (days)	1.0	1.0 - 1.1	0.844
Gender, male vs. female	1.0	0.7 - 1.2	0.716
Haemoglobin Day 0 (g/dL)	**1.1**	**1.0 - 1.1**	**0.045**
Log_{10} parasite density (per µl blood)	**0.7**	**0.7 - 0.8**	**<0.001**
Presence of a quintuple mutation vs presence of fewer mutations	**0.7**	**0.5 – 1.0**	**0.042**
Site Catuane vs Boane	**1.8**	**1.2 - 2.6**	**0.006**
Magude vs Boane	0.8	0.6 - 1.1	0.175
Namaacha vs Boane	**0.7**	**0.5 - 1.0**	**0.056**
Catuane (compared to others)	**2.1**	**1.5 – 3.0**	**<0.001**
Temperature Day 0 (°C)	1.0	0.1 - 1.0	0.698
History of vomiting vs. no history of vomiting	**0.6**	**0.4 – 1.0**	**0.044**
History of vomiting combined with fever Day 0 vs. no such combination	0.5	0.2 - 1.0	0.051
Weight (kg)	**1.0**	**1.00 -1.01**	**0.032**

Statistically significant variables in bold type

Several multivariate models were compared to find the best subset of combined risk factors that are predictive of the relative hazard of clearing parasites in the PP dataset (Table 23):

Table 23: Parasite clearance time best model without treatment, PP dataset

Variable	Hazard ratio	95% CI	p
Log_{10} parasite density (per µl blood)	0.8	0.7 – 0.9	<0.001
Catuane (compared to other sites)	1.4	1.1 – 1.8	0.021
Age > 7 years vs. age ≤ 7 years	1.8	1.3 – 2.5	0.001
History of vomiting combined with fever Day 0 vs. no such combination	0.5	0.2 – 1.1	0.082

As found in the intention-to-treat dataset, the table indicates that those subjects with a higher baseline parasite density have a decreased relative hazard of clearing parasites compared to those having lower baseline parasite densities, while subjects enrolled at Catuane and those over 7 years of age have an increased relative hazard of clearing parasites. A difference found in the analysis of this PP dataset compared to the ITT dataset is that we also observed a trend for a negative association between subjects with a medical history of vomiting combined with a fever on Day 0 and the relative hazard of clearing parasites. The inclusion of this particular variable improved the model fit overall.

Table 24 presents the model with treatment group added to the selected subset of risk factors presented in Table 23. The addition of treatment decreased the statistical significance of the variable relating to the age categories. The variable relating to subjects with a medical history of vomiting combined with a fever on Day 0, although not significant itself, was retained as its inclusion improved the model overall. The statistically significant superior treatment effect of AS plus SP compared to SP monotherapy in terms of clearing parasites when modelled with the risk factors was similar to that found when unadjusted.

Table 24: Parasite clearance time final model, PP dataset

Variable	Hazard ratio	95% CI	p
Treatment with AS plus SP	1.8	1.4 – 2.3	<0.001
Log_{10} parasite density (per µl blood)	0.8	0.7 – 0.9	<0.001
Age > 7 years vs. age ≤ 7 years	1.3	1.0 – 1.7	0.046
Catuane (compared to other sites)	1.8	1.3 – 2.6	0.001
History of vomiting combined with fever Day 0 vs. no such combination	0.5	0.3 – 1.2	0.116

Interaction terms between all final variables selected were tested and no significant interactions were found (although trends towards significance were observed) indicating that the treatment effect is essentially the same regardless of age, site, parasite density and vomiting/fever categories.

The final results suggest that treatment with AS plus SP increased the relative hazard of parasite clearance by 80% compared with SP monotherapy, In addition, subjects aged over 7 years had a 30% increase, and those enrolled at Catuane clinic, had an 80% increase in the relative hazard of parasite clearance. Conversely, each 10-fold increase in parasite density lowered the relative risk of parasite clearance by 20%.

The only real difference found between the analyses of the two datasets was that age over 7 years compared to younger was a significant univariate risk factor for clearing parasites in the PP dataset and not in the ITT dataset.

6.5 Secondary outcome analysis: fever clearance

Any subject who had a fever on Day 0 was included in the subset for this analysis (n=187, 45.5%). Of these, 89.4% taking SP and 88.2% taking AS plus SP had cleared the fever by Day 1, with 97.9% and 98.9%, respectively, clearing parasites by Day 2. Only 1 subject (who was in the AS plus SP group) had not cleared fever by Day 3. There was no statistically significant difference for the fever clearance between treatment groups (chi^2 p=0.356).

6.6 Model validation

The models were built and validated according to the methods described in section 5.2 and a summary is given in Appendix 4. In all 4 survival analyses there were a small selection of Cox-Snell residuals (differences between predicted and actual observations) that suggested the models did not have an ideal fit. In addition, although the proportional hazard assumptions were satisfied overall in each model, they were not satisfied for the mutation category in the treatment failure models and for the parasite density category in the ITT parasite clearance model.

7 DISCUSSION

7.1 Summary of findings

The aim of this study was to provide evidence to Mozambique policy-makers regarding the efficacy and safety of sulfadoxine-pyrimethamine (SP) with or without artesunate (AS) for the treatment of uncomplicated malaria in Southern Mozambique. This MPH thesis was limited to the efficacy analysis.

In a survival analysis restricted to those subjects who complied fully with the protocol, both drug regimens were found to be efficacious (above 90%). However, when AS was combined with SP the outcome was significantly improved with regard to both the relative risks of achieving an ACPR and of shortening the time to clearing of peripheral parasites. When data were re-analysed in an intention-to-treat dataset, the results were almost identical for both outcomes.

The findings of the study are consistent with the literature that combining AS, an artemisinin-derivative, with SP improves efficacy should the efficacy of SP be sufficiently high, as the drugs have independent modes of action. The combined efficacy of AS plus SP was above 95%, the minimum level recommended by the WHO necessary for when adopting a new treatment policy[7]. The increased parasite clearance of the combination regimen corroborates data that show the artemisinins have a much higher parasite reduction ratio, in the order of 10^3-10^5 parasites per asexual life cycle, compared to 10-10^3 for sulfadoxine-pyrimethamine.[35]

A similar risk of re-infection with a new parasite during the 42-day follow up period was found for both treatments, which is as expected as AS is rapidly eliminated and both drug arms contained SP. Slowly-eliminated anti-malarials, such as SP, are responsible for preventing re-infection (post-treatment prophylaxis), as opposed to more rapidly eliminated artemisinins.[36] The positive impact of using drugs with delayed elimination leading to this prophylactic effect has public health importance - any malarial episode, whether caused by re-infection or recrudescence is to the detriment of the individual and the community, and re-infection rates have relatively greater consequence when drugs are highly efficacious (as the risk of true

recrudescence is then lower). However, the benefits of this prophylaxis needs to be weighed against the risk of increased selection of resistant parasites conferred by slowly-eliminated drugs.[37,38]

No significant difference was found between treatments with regard to fever clearance suggesting that the more rapid clearance of parasites by AS did not translate into a better clinical outcome relating specifically to fever. As our inclusion criteria allowed for a history of fever as well as current fever, the number of subjects enrolled with fever was limited. Previous studies have found the combination of AS plus SP to clear fever more quickly compared to SP monotherapy, but used different methodology to ours by counting those reporting a history of fever that remained afebrile as also clearing fever.[39]

7.2 Other significant associations with outcome

Several of the demographic and clinical characteristics modelled with the main treatment variables were found to be important independent risk factors for treatment failure and/or clearance of peripheral parasites in multivariate analyses. Possible associations between risk factors themselves make interpretations difficult and, despite numerous malaria efficacy studies conducted in many regions of the world, few investigate other risk factors with which to compare our results. Furthermore, there are known factors that influence this complicated disease which we did not assess, or analyse yet, such as the developmental stage of parasites at the time of treatment, pharmacokinetic parameters, concomitant medical conditions such as HIV/AIDS.

7.2.1 Age

Increased age was an important predictor of outcome; those aged over 7 years were 70% less at risk of treatment failure than those 7 and under and were 30% more likely to clear parasites more rapidly (the latter found only in the per-protocol analysis). These results are consistent with what is known about immunity to malaria infection increasing with age, and findings in other studies. Repeated exposure to *falciparum* malaria usually in early childhood, unless it causes severe disease and death, results in an acquired partial immunity (premunition).[40,41] This protective effect, however, is not understood well as there is no clear immunological marker with which to validate it, and

the physiological and environmental risk factors for malaria are entwined due to the vector (mosquito), host (human subject), and agent (parasite) relationships. Immunity is believed to be a combination of anti-disease and anti-parasite roles which impact on the severity of disease experienced by an individual and the chance of recovery with or without treatment.[42] Both roles are age-dependent; anti-disease immunity is generally associated with younger ages (less than 5 years) than anti-parasite immunity (adolescent/early adulthood).[43]

Another hypothesis for associations between lower age and poorer treatment outcome is that physiological concentrations of anti-malarials given at the labelled dose, particularly SP, may be sub-optimal in younger patients.[44,45] Dosing regimens derived from clinical studies that recruit adults may lead to inappropriate recommendations for children particularly if regimens are age- rather than weight-based, although this is more practical in resource-poor environments. Moreover, the disposition of drugs in children may be altered leading to concentrations that are below the therapeutic threshold.[46] Conversely, adults may be under-dosed if they weigh more than the average adult in age-based regimens. We found under-dosing of artesunate by mg/kg was not associated with younger age, probably because the dose regimens in our study were weight-based rather than according to the age-based SP package inserts. Ultimately, the pharmacokinetic data, which are not included in this report, may help explain the differential age-related failure risks.

7.2.2 Genetic mutations in the *falciparum* parasite

Sulfadoxine and pyrimethamine are anti-folate drugs that inhibit the enzymes dihydropteroate reductase (*dhps*) and dihydrofolate reductase (*dhfr*) respectively. Resistance to SP develops due to the accumulation of point mutations in the genes that encode these enzymes and has been found to be associated with treatment failure.[47,48] Data from prevalence surveys conducted in Southern Mozambique show that, as resistance to SP increased between 1999 and 2005, the number of mutations increased in parallel, reflecting increased drug pressure and an influx of resistant genes from neighbouring countries (Raman J, unpublished).[49]

In our study the presence of a quintuple mutation (*dhfr* S108N/N511/C59R and *dhps* A437G/K540E) was associated with a more than 3-fold increase in the risk of treatment failure compared to fewer mutations, a strong predictor of outcome and offering further evidence for the spread of resistance to SP in this area. Similar observations elsewhere have motivated proposals that molecular markers of resistance be actively used to predict future therapeutic response. Undoubtedly this is a useful tool, particularly at the population level due to the myriad factors playing a role in each individual.[50] However, other studies have shown no such associations, presumably due to differences in the populations or regions (such as host factors, parasite variations or transmission dynamics) or study methodology (relating to the follow-up time or the laboratory techniques), signalling the need for further work to standardise methodologies in a variety of settings.[51] To this end, there has recently been a call to action for a global database of both clinical and molecular resistance markers which will allow for the standardisation of data relating to the associated technology, their analysis and interpretation.[52,27]

7.2.3 Body temperature

The study showed that there was a significant association between a higher body temperature at baseline and the higher relative risk of treatment failure. The association between fever (both as a single measure at baseline and in terms of its persistence after treatment) and increased risk of failure has been found in other studies, but data are limited (or contradictory), and study methodologies are diverse.[41,53] Moreover, the biological mechanism for the association between higher temperature and an increased risk of failure has not been proposed by other authors.

The role of fever in malaria is not completely understood but it is known to be mediated by tumour necrosis factor (TNF) that is, paradoxically, associated with both severe infection and development of the immune response.[54] *In vitro* studies show that higher temperatures, analogous to fever in human hosts, may promote the growth of parasites, and high parasitaemias have been found to increase the risk of treatment failure.[55,56] There is, however, evidence that, *in vivo*, body temperature and parasitaemia fluctuate independently due to sequestration-release, which may be why we did not find them correlated in our study although this has been reported

elsewhere.[57] The temperature fluctuations inherent in malaria and the fact that temperature may be confounded by other undiagnosed causes of fever may indicate it is an inconsistent predictor of outcome, as appears to be reflected in the literature.[41]

7.2.4 Parasite density

While having a higher parasitaemia at baseline was not seen as an independent risk factor for treatment failure in our study, as opposed to what has been seen in other malaria efficacy studies, it was a significant factor in both datasets' univariate analyses, and was associated with a delayed clearance of parasites after adjustment by treatment, age, site and other indicators of more disease severity (vomiting and fever combined).[58] It makes intuitive sense that if there are more parasites present then the time to clear them will be longer, and thus a greater likelihood of parasites remaining after drug levels reach a minimum inhibitory concentration leading to their re-multiplication. There is a greater chance of a resistant strain being present if parasite densities are higher.[59] The impact of parasite density in our study may have been adjusted due to the inclusion of data relating to mutations. The interaction that we observed between treatment and parasite density in the intention-to-treat analysis of parasite clearance time suggested that the treatment effect was amplified at higher parasitaemias as would be expected given the specific indication for artemisinin derivatives in uncomplicated hyperparasitaemia.[60] However, this interaction did not translate into a clear difference between treatments.

7.2.5 Study site

We found that subjects recruited at Catuane cleared parasites faster compared to the other sites. It was not unexpected to find a difference in an outcome between sites as, at the time the study was conducted, disparities in their recruitment strategies was noted. Despite information from clinic files that Catuane had a high prevalence of symptomatic malaria infections the numbers of cases confirmed by microscopy once the study started was low, and it is likely that some cases usually treated at that clinic were misdiagnosed as a result of their using clinical criteria only. To enhance recruitment the study team approached parents of children at a local school and prospectively screened those with fever or history of fever. The other

sites, meanwhile, continued to recruit patients who actively sought treatment due to their symptoms.

While the age of subjects at Catuane was not significantly lower compared to the other sites despite approaching the school, there was evidence of significantly lower parasite densities and shorter duration of symptoms in those enrolled at Catuane indicating they may be suffering from less severe malaria infection (although the site effect was still present after adjustment for fever combined with a recent history of vomiting, a possible marker of disease severity). Data from surveys in the region showed prevalence of malaria in the community was 92% at Catuane, the highest in Maputo Province indicating acquired immunity at a young age resulting in lower parasite densities and clinical symptoms.[61]

7.3 Efficacy versus effectiveness of treatment

This study was designed to compare the efficacy of two drug regimens, however clinical trial methodology, in general, does not offer a good representation of what happens during routine clinical practice; eligibility criteria are generally too strict, the follow-up of subjects too rigorous and all doses are planned to be observed. Effectiveness, conversely, refers to how interventions perform in routine clinical practice settings given the myriad factors that may impact.[62] One such factor is non-adherence whereby the drug is not taken according to the prescribed recommendations.[63] In addition there may be malabsorption due to relevant co-existing pathology or vomiting, or concomitant consumption of other drugs that could interfere with therapeutic efficacy (and its measurement). Generally effectiveness will be over-estimated by efficacy trials. As subjects taking part in this study were not always withdrawn for protocol violations relating to the factors mentioned above, their data could be excluded or censored at the time of the violation for the per-protocol analysis which could approximate "efficacy" of treatment, and then included fully in our intention-to-treat analysis, to offer a more pragmatic assessment of the "effectiveness", of treatment. The intention-to-treat analysis is still not truly representative of a clinical practice situation, however, as whatever doses were taken were observed, and the follow-up schedule was still rigorous.

7.3.1 Violations relating to dose

The protocol violations included in this study related mainly to the dose of the study drug(s) and the concomitant use of other drugs with some anti-malarial properties. The dose-related violations were that the wrong dose was chosen by the clinical team, or a subject did not adhere to the recommended visit schedule to ensure an AS course for 3 days. While the protocols allowed for repeat dosing of subjects who vomited, this could also be considered as a dose-related problem (as it is uncertain how much of the original and repeat dose were absorbed). As such, subjects who were re-dosed were also removed from the per-protocol analysis, and their impact tested in the intention-to-treat dataset.

Courses of AS shorter than 3 days are not recommended, as they have been found to have lower efficacy.[13] If shorter courses of AS are combined with SP, which is eliminated more slowly and is responsible for the complete eradication of the parasitaemia, resistance may be more likely to emerge.[10] The SP may, however, be sufficient to successfully treat malaria in individual subjects who take a substantially reduced dose of AS should its own efficacy be high enough (i.e. when parasites are fully susceptible to SP), as demonstrated in this study.

The impact of mg/kg doses of pyrimethamine and artesunate with regard to treatment response are difficult to interpret particularly in the intention-to-treat analysis where some subjects had missed doses while others were given a repeat dose if they vomited. Subjects who did not complete the full course of AS or who were under-dosed by the study team choosing an inappropriate dosing level comprised about 30% of the total protocol violations. Including these subjects, and those who were re-dosed, did not substantially change the overall treatment response outcomes in terms of risk of failure or parasite clearance. Associations between repeated dose and treatment outcome, specifically, were not found to be statistically significant. Ultimately, as discussed before, the study results in regard to the effect of dose will be strengthened when pharmacokinetic data are incorporated.

7.3.2 Violations relating to concomitant medications with anti-malarial properties

Our study prohibited concomitant treatment with other anti-malarials in order to show that treatment outcomes were due to the study drugs and not to their use in combination with other drugs that have some antimalarial effects. Erythromycin specifically, although not licensed or used as an anti-malarial, has been found to have weak *in vitro* and *in vivo* activity against *Plasmodium falciparum*.[64,65] The retrospective addition of erythromycin to our list of prohibited drugs meant that it was actually the predominant contributor to this subset of violations (36/43), however no association was found with treatment outcome indicating that it did not play a large role in curing uncomplicated malaria or the rate of parasite clearance.

Other concomitant medications that may impact on treatment response include those that have pharmacokinetic interactions although this was not addressed by our study.

7.3.3 Summary of differences between the per-protocol and intention-to-treat analyses

The results of our per-protocol and intention-to-treat analyses for the primary outcome, time to treatment failure, were remarkably similar. Our results were robust whether protocol violators were included or excluded. Including data from subjects who did not always adhere to dose within this clinical trial where the intention was for observed therapy, or who took prohibited concomitant medications (e.g. erythromycin), is useful for informing the effect of these common practices in treatment response.

For the outcome relating to the relative hazard of clearing parasites the independently significant variables were also very similar for the per-protocol and intention-to-treat datasets. Age above 7 years was of borderline significance as an independent risk factor for the increased relative hazard of clearing parasites in the per-protocol dataset and not the intention-to-treat dataset; this is not explained by different age proportions in the datasets.

7.4 Strengths and limitations

In general, the internal validity and reliability of this study was maximised as it was designed and managed such that the sample size was adequate for the primary outcome, the quality control and monitoring component was detailed and intensive, and the duration of planned follow-up was in line with recommendations. Efforts were made to enhance follow up and ascertainment of outcomes considering the study was set in poor communities with inherent economic migration risk factors. Possible areas for bias were identified and addressed. Overall the survival analysis methodology used, and the inclusion of the intention-to-treat analysis, allowed for the maximum amount of data to be included offering a more realistic result compared to logistic regression against a single end point, and a per-protocol analysis only.[19]

Non-adherence limited the number of observations within our per-protocol analysis although our results were robust as shown by their similarity to the intention-to-treat analysis. Non-adherence is a recognized concern for treatments that rely on multiple out-patient doses; it has been found that the number of daily doses in a drug regimen is a stronger risk factor for poor adherence than socio-economic status.[66] The reasons for non-adherence, and whether there is an association specifically between adherence and outcome, were not explored in this study but it may be possible to assess adherence in relation to tolerability when the safety data are analysed. Conversely, if subjects who took AS plus SP felt significantly better, faster, as it decreased parasitaemia more rapidly than SP monotherapy, they may have not felt the need to come back for further doses. Ideally, studies should incorporate method to measure adherence (such as questionnaires) to help understand these complex issues, as the reality is that studies conducted in resource-poor settings in particular will have to grapple with related loss of data. As mentioned previously, our intention-to-treat analysis is not truly representative of routine clinical practice (i.e. it could not assess the "effectiveness" of treatments).

7.4.1 Duration of follow-up

A 14 day follow-up for clinical malaria efficacy clinical trials in areas of intense-transmission was initially advised by the WHO as adequate. This

recognised the difficulty of longer follow-up in resource-poor settings and the rationale that initial clinical response was the primary objective to determine anti-malarial treatment policy, given the likelihood of frequent re-infection. Only 14 days, however, has been found to be unreliable in determining efficacy in any transmission dynamic, and a 28 day follow up is now suggested as the minimum due to its superior predictive power, regardless of intensity of transmission. Furthermore, evidence has been found that drugs which are eliminated slowly should be evaluated for a minimum of 42 days to be sure of detecting the majority of recrudescences.[67] The results from this study showed that recrudescence may occur beyond 42 days, albeit in small numbers, validating the minimum schedule and suggesting that, should resources allow, follow up be extended further.

7.4.2 Quality control and monitoring

The study was managed by a Principal Investigator and Study Manager (the MPH candidate) from UCT's Division of Clinical Pharmacology, both experienced in managing clinical trials. It was conducted on site by staff of the Mozambique Provincial Ministry of Health who were selected based on their experience and training. The teams included medical doctors, medical and laboratory technologists and operational management staff. Extensive training was given by UCT team members to site teams prior to study commencement through workshops addressing the principles of Good Clinical Practice and study methodology. Regular monitoring visits by the UCT team reinforced this training while case report form data were verified against source (original) documentation. Data quality of both the Access database and the Stata files were maximised by extensive cross-referencing between fields and the generation of queries almost all of which were able to be resolved.

7.4.3 Ascertainment of outcomes and end points

Attempts were made to contact any patient who did not return for a follow up visit and a reason for the loss to follow up noted, if possible, with particular attention to possible malaria and/or adverse events. Specific training and quality control processes for key study end points were used for parasitology, haematology, medical history, adverse event and concomitant medication, dried blood spots to determine parasite genotype and differentiate

recrudescence from re-infection and for SP mutational analysis. The standardised training, methodologies for outcomes' measurements and quality control measures taken, especially those relating to parasitological end points, minimised the possibility of measurement error.

It may be that the selection of body temperature in the models that investigated treatment failure could be less reliable compared to parasite density, as the latter involved at least double slide reading by members of the study team not involved in assessing clinical response. The use of temperature as an indicator for treatment failure would also be compromised by the numerous other causes of fever and use of pyretics.

Many other factors impact on parasite clearance as a measure for response to treatment. These include the immune response of the patient (although biological markers are unclear), sensitivity of the parasite to the drug, the stage of the parasite life-cycle at treatment, whether the drug being used acts at that particular stage, how often measurements are taken, the quality of microscopy, the variability of parasite density (at lower levels or because of sequestration) and what drug levels are attained.[68,69,35] Unfortunately, most of these factors are difficult, or sometimes impossible, to standardise or incorporate in clinical trials in order to assess their relationships in multivariate models.

7.4.4 Bias

In general, an RCT study offers advantages over other study designs as the random treatment allocation brings it closest to a counter-factual concept and there should be no association between the exposure and confounders (known or, more importantly, unknown). When subjects are excluded from analysis due to data loss (a "per-protocol" dataset), the effect of randomisation may be lost, leading to bias in the estimates obtained. Including all subjects regardless of lost data is often preferred but involves a decision as to how to deal with missing outcomes. Survival analysis, as used in this study, maximises available data which are included to the point of loss and preserves the randomisation. The results from the intention-to-treat dataset being so similar to the per-protocol dataset suggest that our per-protocol efficacy estimations were, in fact, valid.

Allocation concealment was considered sufficient to prevent pre-selection of treatment (selection bias) and comparison of baseline characteristics demonstrated exchangeability.

Some risk factors, such as the one which combined a history of vomiting within the 24 hours prior to enrolment with actual fever, were collated from medical history data and introduced to the models retrospectively after a subsequent review of the literature suggesting its importance. There is debate as to the validity of unplanned analyses, however, since these data were solicited in a systematic way we do not expect this to be a particular problem in our study.[70]

It was not possible to blind staff to treatment allocation for all measurements therefore detection bias could affect some assessments, especially clinical outcomes relating to malaria, adverse events and concomitant medication. However, the primary efficacy end point (treatment failure) was determined by a laboratory process relating to parasite density calculations which is less likely to be affected by detection bias as laboratory staff were not given access to information on treatment allocation.

7.4.5 Statistical methods used

While the advantages of survival methods to analyse malaria efficacy studies have been advocated in this report it is apparent that there were some problems achieving good model fit and in satisfying all of the assumptions of proportional hazards which underpin the Cox regression used. It has been suggested that hazard ratios are not appropriate for malaria studies but, despite this, numerous malaria efficacy studies are analysed and reported using the Cox regression.[17] The problems we found in this study are not considered serious enough to invalidate our results; however it must be considered that the data may benefit from a parametric approach such as an accelerated failure time model (AFT).[32]

7.4.6 External validity

While the standard approach taken to the methodology of evaluating efficacy of malaria treatments allows some comparison between studies, this study may not be fully generalisable to other regions due to inherent differences

relating to the malaria parasites (including mixed infections) and transmission dynamics (including seasonality), populations' immunity, pharmacogenetics, concomitant diseases and treatments, and parasite resistance patterns. However, a statistically significant difference between SP monotherapy and AS plus SP in this area is consistent with that found in other places with a similar SP monotherapy efficacy.[13,15]

Within the study area itself, eligibility criteria were quite wide and only excluded very specific subsets at special risk (those under 1 year of age, pregnant or seriously ill), so may represent a good proportion of the local target populations with uncomplicated malaria. Many malaria efficacy studies only include children less than 5 years of age as recommended by the WHO for areas of high intensity malaria transmission. It was felt relevant, however, in Southern Mozambique, despite a history of intense malaria transmission, to include adults in our study as transmission was rapidly declining, and our results show that older patients are at risk of treatment failure.

7.5 Public health implications of the study

The study has public health importance because Mozambique policy makers subsequently changed national treatment policy to AS plus SP for uncomplicated malaria (implemented in 2007). This decision was based on the ACT recommendation from the WHO due to their generally higher cure rates, good tolerability, potential to delay resistance and reduced gametocyte carriage, and preliminary results available from the studies reported in this dissertation.

It is recognized that any combination with SP may have a limited useful life, and acknowledged SP short-comings include inadequate dosing in young children.[45] It is possible that efficacy has subsequently declined since due to *dhfr* and *dhps* SP mutations increasing as a result of a new national implementation of SP as intermittent preventive treatment in pregnancy during 2007 which is expected to further increase drug pressure and the spread of SP resistance, and the presence of the *dhfr* 164 mutation, associated with high-level pyrimethamine resistance, found in neighbouring Malawi.[71] Therefore a comparison of possible replacement combinations was

communicated to Mozambique policy-makers by Prof Karen Barnes in 2007 noting further reasons to support a further policy change.

As mentioned in section 7.2.2 a global database has been proposed in which to store data relating to molecular markers of resistance. A similar database for efficacy outcomes is already ongoing with data from this study included as detailed in the study methodology (section 4.7).[27] That initiative, and the planned publication of these results which may allow for our data to be included in systematic reviews or meta-analyses, may contribute to a wider public health benefit.

7.6 Conclusions and recommendations

This study found that the efficacy of AS plus SP was superior to that of SP alone, which supports the implementation of this combination in Southern Mozambique. However, there is a need to consider an alternative artemisinin-containing combination due to evidence of drug pressure from neighbouring regions selecting SP resistant parasites, and this is likely to increase now that SP has been introduced nationally as intermittent preventive treatment in pregnancy.

Other important risk factors that impacted negatively on treatment failure included younger age, presence of quintuple *dhfr/dhps* mutations and more severe disease (with temperature as a proxy). Important risk factors impacting negatively on the clearance of parasites included, again, younger age, plus higher parasitaemia (an indicator of more severe disease). Age-related risk factors are thought to relate to lower immunity although age-related dose issues and SP drug levels may also be playing a role. These factors reflect what is known about the disease and should allow those involved in treating malaria to be extra vigilant in following up patients who fit these profiles as they may be more likely to need a second line treatment.

Survival analysis is a good method for assessing the efficacy of malaria treatments as it maximises the data that may be included compared to traditional approaches.

The inclusion of non-protocol adherent subjects in this study did not change the results overall. While it would never be appropriate to advocate that studies ignore protocol violators these results suggest that, should adherence be difficult to ensure, the results may still be valid. Intention-to-treat and per-protocol results should be routinely compared to identify factors that impact on treatment response.

8 APPENDICES

Appendix 1: Source document

SEACAT
EVALUATION
Ensuring Effective Malaria Treatment

Código do doente (exemplo: MOM00X/MOB00X):
Endereço do doente/contacto

Notas Médicas SEACAT 01a ASSP/SP (in vivo) Dia 0	Localidade	

Paciente (nome):_____Data de nascimento (minimo ano): _____Data de visita:_____

Sexo		Masculino ☐	Feminino ☐	
Estará a doente grávida/amamentando a peito?		Sim ☐	Não ☐	
Exame de resultado rápido		Positivo ☐	Negativo ☐	
História de febre?		Sim ☐	Não ☐	
Peso:	Kg	Temperatura axilar		°C

EXCLUIR O DOENTE SE: Idade < 12 meses, peso < 10kg
Temperatura axilar é < 37.5 sem antecedents febris
Grávida ou amamentando a peito

Medicação recente (condições maláricas e outras)				
O doente tomou algum remédio nos últimos 7 dias?	Sim	☐	Não	☐
O doente tomou algum remedio anti malaricos 42 dias?	Sim	☐	Não	☐

EXCLUIR O DOENTE SE: Cotrimoxazole/chloramphenicol/trimethoprim/tetracyclines (doxycycline)/folate/outros anti-maláricos nos últimos 7 dias
ADICIONAR TODOS OUTROS REMÉDIOS Á PÁGINA DOS MEDICAMENTOS (anti-malaricos 42 dias)

Resumo de história clínica incluindo alergias a remédios (maláricos e outros)		
Diagnose ou sintomas	Datas de princípio e fim	Presente agora? (sim/não)

EXCLUIR O DOENTE SE: Deficiencia de G6PD, anteriormente alérgico ao remédio.

Achados na examinação física(maláricos e outros)		
Diagnose ou sintomas	História prévia?	Presente agora? (sim/não)

EXCLUIR O DOENTE SE: Sériamente doente (critério da WHO)
OBTER CONSENTO INFORMADO

Densidade parasitária (resultado)	Densidade de Gametocyte (resultado)	Níveis de remédio no sangue estabelecido (marque)	Observação de sangue em papel de filtro feito (marque)	Hemoglobina (resultado)

EXCLUIR O DOENTE SE: Resultado densidade parasitária > 500,000

NÚMERO DO REMÉDIO NO ESTUDO DISPENSADO			
Quantidade dada ao doente (SP ou SP/AS ou placebo	Grupo número	Registar os detalhes se a dose for repetida	Assinatura/Data de dispensa

REGISTAR O NOME DO DOENTE NA CAIXA DE REMÉDIOS

Razão de exclusão: ☐ contagem parasitária ☐ severidade malárica ☐ gravidez/amamentando a peito
(se aplicável) ☐ idade/peso ☐ outros(especificar):_____

Código do doente

Dia 1			
Data da visita		Temperatura axilar	°C
Densidade parasitária	Nível de remédios no sangue	Observação de sangue em papel de filtro	
Progressão a moderadamente severa ou a malária severa? ☐ Sim(**descontinuar**) ☐ Não			
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐ Não			
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não			

OBSERVAÇÃO DA DISPENSA DE REMÉDIOS Dia 1			
Quantidade dada ao doente (SP ou SP/AS ou placebo)	Número do grupo	Registe os detalhes se a dose for repetida	Assinatura/data de dispensa

Dia 2			
Data da visita		Temperatura axilar	°C
Densidade parasitária	Nível de remédio no sangue	Observação de sangue em papel de filtro	
A densidade parasitária é≥que o resultado do dia o? ☐ Sim (**descontinuar**) ☐ Não			
Progressão a moderadamente severa ou a malária severa? ☐ Sim (**descontinuar**) ☐ Não			
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐ Não			
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não			

OBSERVAÇÃO DA DISPENSA DE REMÉDIOS Dia 2			
Quantidade dada ao doente (SP ou SP/AS ou placebo)	Número do grupo	Registe os detalhes se a dose for repetida	Assinatura/data de dispensa

Dia 3				
Data da visita		Temperatura axilar	°C	
Densidade parasitária	Densidade de Gametocyte	Nível de remédio no sangue	Observação de sangue em papel de filtro	Hemoglobina
Qual é a densidade parasitária como % do Dia 0 ?	Densidade parasitária≥ 25% do Dia 0? ☐ Sim(**descontinuar**) ☐ Não			
Progressão a moderadamente severa ou a malária severa? ☐ Sim (**descontinuar**) ☐ Não				
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐ Não				
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não				

Código do doente:

Dia 7				
Data da visita		Temperatura axilar		°C
Densidade parasitária	Densidade de Gametocyte	Nível de remédio no sangue	Observação de sangue em papel de filtro	Hemoglobina
Existencia parasitária?			☐ Sim (**descontinuar**)	☐ Não
Progressão a moderadamente severa ou a malária severa?			☐ Sim (**descontinuar**)	☐ Não
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐Não				
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não				

Dia 14				
Data da visita		Temperatura axilar		°C
Densidade parasitária	Densidade de Gametocyte	Nível de remédio no sangue	Observação de sangue em papel de filtro	Hemoglobina
Existencia parasitária?			☐ Sim (**descontinuar**)	☐ Não
Progressão a moderadamente severa ou a malária severa?			☐ Sim (**descontinuar**)	☐ Não
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐Não				
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não				

Dia 21				
Data da visita		Temperatura axilar		°C
Densidade parasitária	Densidade de Gametocyte	Nível de remédio no sangue	Observação de sangue em papel de filtro	Hemoglobina
Existencia parasitária?			☐ Sim (**descontinuar**)	☐ Não
Progressão a moderadamente severa ou a malária severa?			☐ Sim (**descontinuar**)	☐ Não
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐Não				
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não				

Dia 28				
Data da visita		Temperatura axilar		°C
Densidade parasitária	Densidade de Gametocyte	Nível de remédio no sangue	Observação de sangue em papel de filtro	Hemoglobina
Existencia parasitária?			☐ Sim (**descontinuar**)	☐ Não
Progressão a moderadamente severa ou a malária severa?			☐ Sim (**descontinuar**)	☐ Não
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐ Não				
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não				

Código do doente:

Day 42/visita de descontinuação				
Data da visita		Temperatura axilar		°C
Densidade parasitária	Densidade de Gametocyte	Nível de remédio no sangue	Observação de sangue em papel de filtro	Hemoglobina
Outros remédios tomados ou receitados por si desde da última visita (para condições maláricas e outras). ☐ Sim (**adicionar á página de medicação**) ☐ Não				
Acontecimentos adversos (condições maláricas e outras). ☐ Sim (**adicionar á página de acontecimentos adversos**) ☐ Não				
Se o doente for descontinuado dê a razão aqui	☐ falha parasitológica ☐ deterioração clínica ☐ acontecimento adverso/acontecimento adverso sério ☐ doente/pedido do guardião do doente ☐ perdido para seguimento ☐ falha investigativa ☐ outro: _____			
É favor tentar contactar o doente se descontinuado e notar a sua condição:				

Código do doente:

Acontecimentos Adversos (desde do dia 0)				
Descrição	Sério S/N	Datas de começo e fim	Em progresso no fim do estudo (marque)	Medicação (S/N) (junte á tábua seguinte)

*categories sérias: Morte do doente
Doente vitima de acontecimento adverso com perigo de vida
Doente tornou-se bastante incapacitado
Doente foi hospitalisado(ou hospitalisação foi prolongada)durante o estudo
Doente engravidou durante o estudo

Informação de contacto para acontecimentos adversos sérios:
Tunisio Camba (Gerente Local): 082 456 497
Dr Karen Barnes (Investigadora Principal): + 27 21 406 6758/+ 27 82 440 2542
Liz Nettleship (Gerente SA): + 27 21 406 6758/+ 27 83 679 4915
Lauren Hill (Gerente SA): + 27 21 4066758/+ 27 82 785 2053
Tracey Fourie (Assistente da Dra Barnes): + 27 21 406 6758/+ 27 83 400 1922
Dr Ushma Mehta (Gerente de Segurança): + 27 82 773 1440

Medicação(de 7 dias antes do dia 0 e durante o estudo)				
Nome do medicamento	Datas de início e fim	Em progresso no fim do estudo (marque)	Dose	Razão de uso

Study site:	Patient initials:	Patient study code:	Date of birth:	Sex M/F:	Weight (kg) D0:	History of fever Y/N:

Drug Accountability

Study drug number:	Dose given		Dose repeated (Y/N)		Total number of tablets dispensed	
	SP	Artesunate	SP	Artesunate	SP	Artesunate
Day 0						
Day 1						
Day 2						

Study assessments

	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Day 42
Date Ddmmyy									
Axillary temp ^{0}C									
Parasite density									
% day 0 parasite density	N/A								
Gametocyte density		N/A	N/A						
Haemoglobin (g/dL)		N/A	N/A						

Reason for patient withdrawal if applicable:
- ☐ parasitological failure
- ☐ clinical deterioration
- ☐ adverse event/serious adverse event
- ☐ allergic reaction
- ☐ lost to follow up/ patient or patient's guardian request
- ☐ protocol deviation/violation
- ☐: other _____

Study outcome:
- ☐ adequate clinical parasitological response
- ☐ early treatment failure
- ☐ late treatment failure – late clinical failure
- ☐ late treatment failure – late parasitological failure
- ☐ Comment:_____

Polymerase chain reaction result: (UCT/MRC use only)
- ☐ recrudescence
- ☐ re-infection
- ☐ indeterminate

Comment: _____

Name/signature of Site Manager or designee: _____ Date:_____

Patient study code:	SEACAT in vivo 01a ASSP SP CRF Moz 141003

MH no.	Medical history clinical diagnosis or symptom	Started ddmmyy	Resolved ddmmyy	Ongoing (tick)	Medication taken*?	
					Yes	No

* Please enter any medication in the medication boxes of the AE CRF

Patient study code:

AE no.	Adverse Event clinical diagnosis or symptom	Started ddmmyy	Resolved ddmmyy	Ongoing (tick)	Serious?			Medication given?		Relationship to study drug (UCT use only)
					Yes	No	Code	Yes	No	

Medication name	Form	Started ddmmyy	Stopped ddmmyy	Ongoing (tick)	Dose			AE Ref.
					Strength/quantity		Frequency	
						Mg G Mls		
						Mg G Mls		
						Mg G Mls		
						Mg G Mls		

SAE codes

A Fatal

B Life-threatening

C Persistent/significant disability/incapacity

D In-patient hospitalisation

E Prolonged in-patient hospitalisation

F Congenital anomaly/birth defect

G Important medical event

Use additional form if insufficient space

Forms:
Tab = tablet
Cap = capsule
Syrup
Susp = suspension
Inj = injection
ND = Nose drops/spray
ED = Eye drops
Lot = lotion
Cream
Oint = ointment

Frequency:
QDS = four times a day
TDS = three times a day
BD = twice a day
OD = once a day
ON = at night
PRN = when required
STAT = immediately (once only)

Appendix 3: Ethical approvals

Research Ethics Committee
Faculty of Health Science
E46-26 Old Main Building, Groote Schuur Hospital, Observatory, 7925
Queries : Xolile Fula
Tel : (021) 406-6492 Fax: 406-6411
E-mail : Xfula@curie.uct.ac.za

21 January 2003

REC REF: 299/99

Dr K Barnes
Pharmacology

Dear Dr Barnes

THE SOUTH EAST AFRICAN COMBINATION ANTI-MALARIAL THERAPY (SEACAT) EVALUATION

Thank you very much for your letter to the Research Ethics Committee dated 07 January 2003.

It is a pleasure to inform you that the Research Ethics Committee has ***approved*** *Protocol Amendment no.04 dated 20 December 2002 for the above mentioned study.*

Please quote the above Rec. reference number in all correspondence

Yours sincerely

PROF CR SWANEPOEL
CHAIRPERSON

UNIVERSITY OF CAPE TOWN

Research Ethics Committee
Faculty of Health Sciences
OMB E53 Room 44.1, GSH
Queries : Xolile Fula
Tel : (021) 406-6492 Fax: 406-6411
E-mail : Xfula@curie.uct.ac.za

07 November 2003

REC REF: 336/2003

Dr K Barnes
Pharmacology

Dear Dr Barnes

AN OPEN- LABEL, RANDOMIZED, PARALLEL GROUP IN VIVO DRUG STUDY TO EVALUATE A COMBINATION ANTI- MALARIAL THERAPY (CAT), ARTESUNATE AND SULFADOXINE-PYRIMETHAMINE (ASSP) VERSUS SULFADOXINE- PYRIMETHAMINE (SP) ALONE, IN TERMS OF THERAPEUTIC EFFICACY, PREVALENCE OF GAMETOCYTE CARRIAGE AND PREVALENCE OF MOLECULAR MARKERS ASSOCIATED WITH SP RESISTANCE IN UNCOMPLICATED PLASMODIUM FALCIPARUM INFECTIONS

Thank you for submitting your study to the Research Ethics Committee for review.

Date Considered: 31 October 2003

Decision: Approved subject to correction of grammatical and typographical errors.

The following documentation has been approved:

- Protocol: SEACAT in vivo 01a ASSP/SP, 8th October 2002.
- Information and consent form: SEACAT 01a ASSP/SP, 8th October 2002.
- Investigator's Brochure: SEACAT (Oral Artesunate) , 1 July 2001.
- Prescribing information for Fansidar plus guidelines for the use of Artesunate plus SP for Mpumalanga as supporting information.
- Mcc Approval.

Please find attached the list of members who attended the meeting.
Please quote the above Reference number in all correspondence.

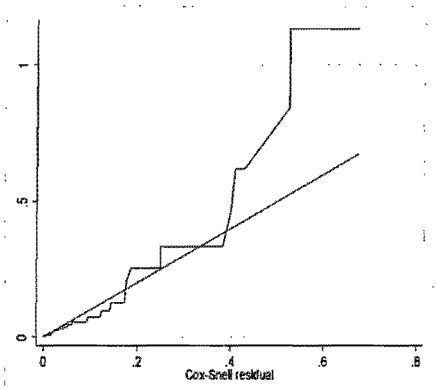
Yours sincerely

PROF T. ZABOW
CHAIRPERSON

Appendix 4: Model validation diagnostics

1 Primary outcome: time to treatment failure

1.1 ITT dataset



Cox-Snell residuals suggest model fit problems

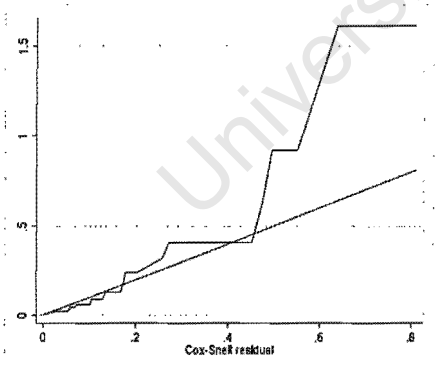
Potential influential observations were identified as:

- MOB2004_012, MON2003_013 (temperature)
- MOB2004_053, MOM2004_080, MOM2004_191 (age)
- MOM2004_190 (age and temperature)
- MON2003_048, MOM2004_167, MOM2004_193 (age and treatment)
- MON2003_051 (temperature and treatment)
- MOB2004_049 (age, temperature and treatment)

Subject MON2003_048 was dropped as he/she had a clinical deterioration on Day 1 despite the profile suggesting treatment success.

Overall the proportional hazard assumption was satisfied (Schoenfeld p=0.1075) but not for the mutation category (Schoenfeld p=0.0368)

1.2 PP dataset



Cox-Snell residuals suggest model fit problems

Potential influential observations were identified as:

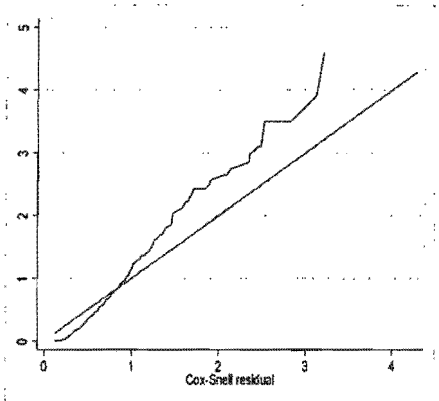
- MOB2004_012, MON2003_013 (temperature)
- MOB2004_053 (age)
- MON2003_048, MOM2004_190, MOM2004_193 (treatment and age)
- MOB2004_049 (temperature and age)

Subject MON2003_048 was dropped as had a clinical deterioration on Day 1 despite his profile suggesting treatment success.

Overall the proportional hazard assumption was satisfied (Schoenfeld p=0.6572) but not for the mutation category (Schoenfeld p=0.0323).

2 Secondary outcome: time to parasite clearance

2.1.1 ITT dataset



Cox-Snell residuals suggest model fit problems

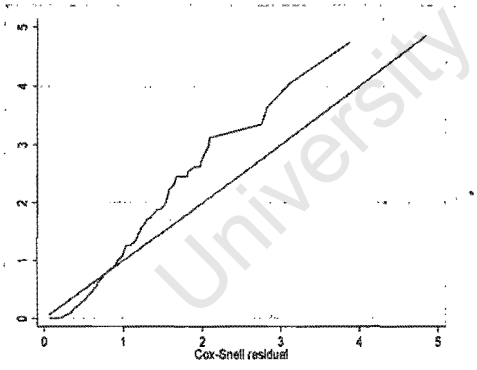
Potential influential observations were identified as:

- MON2003_051 (parasite density)
- MOC2003_011 (Site and treatment)

Both were dropped. MON2003_051 was an ETF Day 3 however parasite density was 32% of a very low Day 0 result (47 parasites per µl blood). MOC2003_011 took longer to clear parasites compared to what would be expected taken the profile.

Overall the proportional hazard assumption was satisfied (Schoenfeld p=0.0677) but not for the parasite density category (Schoenfeld p=0.0123)

2.1.2 PP dataset



Cox-Snell residuals suggest model fit problems

Potential influential observations were identified as:

- MON019 (age)
- MOC011, (site)
- MOC032 (parasite density, age and site)
- MOB060 (History of vomiting combined with fever)

No subjects were removed from the dataset.

The proportional hazard assumption was satisfied both overall and for all variables individually (Schoenfeld p=0.7707).

9 REFERENCES

[1]Greenwood BM, Bojang K, Whitty CJM, Targett GAT. Malaria. Lancet 2005;365:1487–98.

[2]World Health Organisation. The Africa Malaria Report 2006 [cited 2008, Apr 4]. Available from: http://www.afro.who.int/malaria/publications/annual_reports/africa_malaria_report_2006.pdf

[3]Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A. Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. Lancet 1997;349 (9066):1650-54.

[4]Centers for Disease Control. Malaria. [cited 2008, Arp 4] . Available from: http://www.cdc.gov/malaria/index.htm

[5]Chilundo B, Sundby J, Aanestad M. Analysing the quality of routine malaria data in Mozambique. Malar J. 2004;3:3.

[6]Sharp BL, Kleinschmidt I, Streat E, Maharaj R, Barnes KI, Durrheim DN. Seven years of regional malaria control collaboration-Mozambique, South Africa and Swaziland. Am J Trop Med Hyg. 2007;76(1):42-47.

[7]World Health Organisation. Guidelines for the treatment of malaria 2006. WHO/HTM/MAL/2006.1108.

[8]Drakeley CJ, Secka I, Correa S, Greenwood BM, Targett GA. Host hematological factors influencing the transmission of Plasmodium falciparum gametocytes to Anopheles gambiae s.s mosquitoes. Trop Med Int Health. 1999;4:131-8.

[9]Wellems TE, Plowe CV. Chloroquine-resistant malaria. J Infect Dis. 2001;184:770–76.

[10]Nosten F White NJ. Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg. 2007; 77(6):181-92

[11]Price RN, Nosten F, Luxemburger C, ter Kuile FO, Paiphun L, Chongsuphajaisiddhi T et al. Effects of artemisinin derivatives on malaria transmissibility. Lancet 1996; 347:1654 - 8.

[12]Price RN, Nosten F, Simpson JA, Luxemburger C, Phaipun L, ter Kuile F et al. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. Am J Trop Med Hyg. 1999; 60:1019 - 23

[13]von Seidlein L, Milligan P, Pinder M, Bojang K, Anyalebech C, Gosling R. Efficacy of artesunate plus pyrimethamine-sulphadoxine for uncomplicated malaria in Gambian children: a double-blind, randomised, controlled trial. Lancet. 2000; 355: 352–57.

[14]Rapid responses to Plowe CV, Kublin JG Dzinjalamala FK, Kamwendo DS, Mukadam RA, Chimpeni P et al. Sustained clinical efficacy of sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Malawi after 10 years as first

line treatment: five year prospective study. BMJ. 2004;328(7439):545-8. Epub 2004 Feb 2.

[15] Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N: International Artemisinin Study Group. Artesunate combinations for treatment of malaria: meta-analysis. Lancet. 2004;363(9402):9-17.

[16] Taylor W, White NJ. Antimalarial drug toxicity: a review. Drug Safety 2004;27(1):25-61.

[17] Stepniewska K, White NJ. Some considerations in the design and interpretation of antimalarial drug trials in uncomplicated falciparum malaria. Malar J. 2006;5:127.

[18] World Health Organisation. Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. 2003. WHO/HTM/RBM/2003.50.

[19] Guthmann JP, Pinoges L, Checchi F, Cousens S, Balkan S, van Herp M et al. Methodological issues in the assessment of antimalarial drug treatment: analysis of 13 studies in eight African countries from 2001 to 2004. Antimicrob Agents Chemother. 2006;50(11):3734-9. Epub 2006 Sep 5.

[20] Barnes K. An open label In Vivo drug study (with both single and parallel group components) to evaluate combination anti-malarial therapy (CAT), in terms of therapeutic efficacy, prevalence of gametocyte carriage and prevalence of molecular markers associated with SP resistance in uncomplicated Plasmodium falciparum infections. Including Amendment 4 (Dec 20 2002). karen.barnes@uct.ac.za.

[21] Barnes K. An open-label, randomized, parallel group in vivo drug study to evaluate a combination anti-malarial therapy (CAT), artesunate and sulfadoxine-pyrimethamine (ASSP) versus sulfadoxine-pyrimethamine (SP) alone, in terms of therapeutic efficacy, prevalence of gametocyte carriage and prevalence of molecular markers associated with SP resistance in uncomplicated Plasmodium falciparum infections. (Oct 8 2003). karen.barnes@uct.ac.za.

[22] World Health Organisation. Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission 1996. WHO/MAU96.1077.

[23] Carter JY, Loolpapit MP, Lema OE, Tome JL, Nagelkerke NJD, Watkins WM Reduction of the efficacy of antifolate antimalarial therapy by folic acid supplementation. Am J Trop Med Hyg. 2005;73:166-70.

[24] World Health Organisation. Severe falciparum malaria. Trans R Soc Trop Med Hyg 2000; 94(1):62.

[25]World Health Organisation. Basic malaria microscopy. Part 1. Learner's Guide.1991.

[26]Snounou G, Beck HP. The Use of PCR Genotyping in the Assessment of Recrudescence or Reinfection after Antimalarial Drug Treatment. Parasitology Today. 1998; 14 (11): 462-7.

[27]Price RN, Dorsey G, Ashley EA, Barnes KI, Baird JK, d'Alessandro U et al. World Antimalarial Resistance Network I: Clinical efficacy of antimalarial drugs . Malar J. 2007; 6:119.

[28]World Health Organisation. International Statistical Classification of Diseases and Related Health Problems 10th Revision Version for 2007. Available from: http://www.who.int/classifications/apps/icd/icd10online.

[29]Division of Pharmacology, Faculty of Health Sciences, University of Cape Town, editors. South African Medicines Formulary Sixth Edition. Health and Medical Publishing Group of the South African Medical Association.

[30]Department of Health 2000. Guidelines for good practice in the conduct of clinical trails in South Africa. Department of Health, Pretoria, South Africa.

[31]Medical Research Council. Guidelines on ethics for medical research: General principles. (4th edition). Medical Research Council of South Africa, Tygerberg, South Africa.

[32]Collett D. Modelling survival data in medical research. 2nd ed. Chapman and Hall/CRC.Press LLC; 2003.

[33]Nosten F, ter Kuile F, Chongsuphajaisiddhi T, Luxemburger C, Webster HK, Edstein M et al. Mefloquine-resistant falciparum malaria on the Thai-Burmese border. Lancet. 1991; 337(8750):1140-3.

[34]Sowunmi A Fateye, BA. Adedeji A Gbotosho Happi TC Bamgboye AE et al. Predictors of the failure of treatment with pyrimethamine–sulfadoxine in children with uncomplicated falciparum malaria. Acta Tropica 2006; 98(1):6-14.

[35]White NJ. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. Minireview. Antimicrob Agents Chemother. 1997;41(7): 1413–22.

[36]White NJ. How antimalarial drug resistance affects post-treatment prophylaxis Malar J. 2008;7:9.

[37]Watkins WM, Mosobo M. Treatment of Plasmodium falciparum malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life. Trans R Soc Trop Med Hyg. 1993;87(1):75-8.

[38]Checchi F Piola P, Fogg C, Bajunirwe F, Biraro S, Grandesso F et al. Supervised versus unsupervised antimalarial treatment with six-dose artemether-lumefantrine: pharmacokinetic and dosage-related findings from a clinical trial in Uganda. Malar J. 2006;19;5:59.

[39]Tjitra E, Suprianto S, Currie BJ, Morris PS, Saunders JR, Anstey NM. Therapy of uncomplicated falciparum malaria: a randomized trial comparing artesunate plus

sulfadoxine-pyrimethamine versus sulfadoxine-pyrimethamine alone in Irian Jaya, Indonesia. Am J Trop Med Hyg. 2001;65(4):309-17.

[40]Aponte JJ, Menendez C, Schellenberg D, Kahigwa E, Mshinda H, Vountasou P et al. Age interactions in the development of naturally acquired immunity to Plasmodium falciparum and its clinical presentation PLoS Med. 2007;4(7) Erratum in: PLoS Med. 2007;4(12):e359.

[41]Dorsey G, Kamya MR, Ndeezi G, Babirye JN, Phares CR, Olson JE et al. Predictors of chloroquine treatment failure in children and adults with falciparum malaria in Kampala, Uganda. Am J Trop Med Hyg. 2000; 62(6):686-92.

[42]Enevold A, Nkya WM, Theisen M, Vestergaard LS, Jensen AT, Staalsoe T, et al. Potential impact of host immunity on malaria treatment outcome in Tanzanian children infected with Plasmodium falciparum. Malar J. 2007;16;6:153.

[43]Filipe JA, Riley EM, Drakeley CJ, Sutherland CJ, Ghani AC. Determination of the processes driving the acquisition of immunity to malaria using a mathematical transmission model. PLoS Comput Biol 2007;3(12): e255.

[44]Terlouw DJ, Courval JM, Kolczak MS, Rosenberg OS, Oloo AJ, Kager PA et al. Treatment history and treatment dose are important determinants of sulfadoxine-pyrimethamine efficacy in children with uncomplicated malaria in Western Kenya. J Infect Dis. 2003;187,467-76.

[45]Barnes KI, Little F, Smith PJ, Evans A, Watkins WM, White NJ. Sulfadoxine-pyrimethamine pharmacokinetics in malaria: pediatric dosing implications. Clin Pharmacol Ther. 2006; 80(6): 582-96.

[46]Barnes KI, Watkins WM, White NJ. Antimalarial dosing regimens and drug resistance. Trends Parasitol. 2008 Mar;24(3):127-34.

[47]Plowe CV, Cortese JF, Djimde A, Nwanyanwu OC, Watkins WM, Winstanley PA et al. Mutations in Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. J Infect Dis. 1997;176(6):1590-6.

[48]Kublin JG, Dzinjalamala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM et al. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of Plasmodium falciparum malaria. J Infect Dis. 2002;185(3):380-8.

[49]Raman J, Sharp B, Kleinschmidt I, Roper C, Streat E, Kelly V et al. Differential effect of regional drug pressure on dihydrofolate reductase and dihydropteroate synthetase mutations in southern Mozambique. Am J Trop Med Hyg. 2008;78(2):256-61.

[50]Talisuna AO, Nalunkuma-Kazibwe A, Langi P, Mutabingwa TK, Watkins WW, Van Marck E et al. Two mutations in dihydrofolate reductase combined with one in the

dihydropteroate synthase gene predict sulphadoxine–pyrimethamine parasitological failure in Ugandan children with uncomplicated falciparum malaria infection. Infect Genet Evol. 2004;4(4):321-7.

[51]J. Eriksen S, Mwankusye S, Mduma MI, Veiga A, Kitua G, Tomson MG et al. Antimalarial resistance and DHFR/DHPS genotypes of Plasmodium falciparum three years after introduction of sulfadoxine–pyrimethamine and amodiaquine in rural Tanzania Trans. R Soc Trop Med Hyg. 2008;102:137–42.

[52]Plowe CV, Roper C, Barnwell JW, Happi CT, Joshi HH, Mbacham W et al. World Antimalarial Resistance Network (WARN) III: molecular markers for drug resistant malaria. Malar J 2007;6:121.

[53]Hamer DH, MacLeod WB, Addo-Yobo E, Duggan CP, Estrella B, Fawzi WW et al. Age, temperature, and parasitaemia predict chloroquine treatment failure and anaemia in children with uncomplicated Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg. 2003;97(4):422-8.

[54]Kwiatkowski D, Bate CA, Scragg IG, Beattie P, Udalova I, Knight JC. The malarial fever response--pathogenesis, polymorphism and prospects for intervention. Ann Trop Med Parasitol. 1997;91(5):533-42.

[55]Pavithra SR, Banumathy G, Joy O, Singh V, Tatu U. Recurrent fever promotes Plasmodium falciparum development in human erythrocytes. J Biol Chem. 2004;279(45):46692-9.

[56]Sowunmi A, Adedeji AA, Fateye BA, Babalola CP. Plasmodium falciparum hyperparasitaemia in children. Risk factors, treatment outcomes, and gametocytaemia following treatment. Parasite. 2004;11(3):317-23.

[57]Delley V, Bouvier P, Breslow N, Doumbo O, Sagara I, Diakite M et al. What does a single determination of malaria parasite density mean? A longitudinal survey in Mali Tropical Medicine & International Health. 2000;5(6):404–12.

[58]Fontanet AL, Walker AM. Predictors of treatment failure in multiple drug resistant falciparum malaria: results from a 42-day follow-up of 224 patients in Eastern Thailand. Am J Trop Med Hyg. 1993;49(4)465-72.

[59]Ter Kuile FO Luxemburger C, Nosten F, Thwai KL, Chongsuphajaisiddhi T, White NJ. Predictors of mefloquine treatment failure: a prospective study of 1590 patients with uncomplicated falciparum malaria. Trans R Soc Trop Med Hyg. 1995; 89: 660-664.

[60]Luxemburger C, Nosten F, Raimond SD, Chongsuphajaisiddhi T, White NJ. Oral artesunate in the treatment of uncomplicated hyperparasitemic falciparum malaria. Am J Trop Med Hyg. 1995;53(5):522-5.

[61]Lubombo Spatial Development Initiative (Unplublished report).

[62]Gartlehner G, Hansen RA, Nissman D, Lohr KN, Carey TS. Criteria for distinguishing effectiveness from efficacy trials in systematic reviews. Technical Review 12 (Prepared by the RTI-International–University of North Carolina Evidence-based Practice Center under Contract No. 290-02-0016.) 2006. AHRQ Publication No. 06-0046. Rockville, MD: Agency for Healthcare Research and Quality.

[63]Smithuis F, Kyaw MK, Phe O, Aye KZ, Htet L, Barends M et al. Efficacy and effectiveness of dihydroartemisinin-piperaquine versus artesunate-mefloquine in falciparum malaria: an open-label randomised comparison. Lancet .2006;367(9528):2075-85.

[64]Nakornchai S, Konthiang P. Activity of azithromycin or erythromycin in combination with antimalarial drugs against multidrug-resistant Plasmodium falciparum in vitro. Acta Trop. 2006;100(3):185-91.

[65]Hamel MJ, Holtz T, Mkandala C, Kaimila N, Chizani N, Bloland P et al. Efficacy of trimethoprim-sulfamethoxazole compared with sulfadoxine-pyrimethamine plus erythromycin for the treatment of uncomplicated malaria in children with integrated management of childhood illness dual classifications of malaria and pneumonia. Am J Trop Med Hyg. 2005;73(3):609-15.

[66]Orrell C, Bangsberg D, Badri M, Wood R. Adherence is not a barrier to successful antiretroviral therapy in South Africa. AIDS. 2003;17(9):1369-75.

[67]Stepniewska K, Taylor WR, Mayxay M, Price R, Smithuis F, Guthmann JP et al. In vivo assessment of drug efficacy against plasmodium falciparum malaria: duration of follow-up. Antimicrob Agents Chemother. 2004;48(11):4271-80.

[68]Hugosson E, Montgomery SM, Premji Z, Troye-Blomberg M, Björkman A. Higher IL-10 levels are associated with less effective clearance of Plasmodium falciparum parasites. Parasite Immunol. 2004;26(3):111–117.

[69]Gregson A, PLowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev. 2005;57:117-45.

[70]Vandenbroucke JP. Observational research, randomized trials and two views of medical science. PLoS Med. 2008 11;5(3):e67.

[71]Alker AP Mwapasa V, Purfield A, Rogerson SJ, Molyneux ME, Kamwendo DD et al. Mutations associated with sulfadoxine-pyrimethamine and chlorproguanil resistance in Plasmodium falciparum isolates from Blantyre, Malawi. Antimicrob Agents Chemother. 2005;49(9): 3919-21.