Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal

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Abstract

Background

Recent malaria epidemics in KwaZulu-Natal indicate that effective anti-malarial therapy is essential for malaria control. Although artemether-lumefantrine has been used as first-line treatment for uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal since 2001, its efficacy has not been assessed since 2002. The objectives of this study were to quantify the proportion of patients treated for uncomplicated *P. falciparum* malaria with artemether-lumefantrine who failed treatment after 28 days, and to determine the prevalence of molecular markers associated with artemether-lumefantrine and chloroquine resistance.

Methods

An observational cohort of 49 symptomatic patients, diagnosed with uncomplicated *P. falciparum* malaria by rapid diagnostic test, had blood taken for malaria blood films and *P. falciparum* DNA polymerase chain reaction (PCR). Following diagnosis, patients were treated with artemether-lumefantrine (Coartem®) and invited to return to the health facility after 28 days for repeat blood film and PCR. All PCR *P. falciparum* positive samples were analysed for molecular markers of lumefantrine and chloroquine resistance.

Results

Of 49 patients recruited on the basis of a positive rapid diagnostic test, only 16 were confirmed to have *P. falciparum* by PCR. At follow-up, 14 were PCR-negative for malaria, one was lost to follow-up and one blood specimen had insufficient blood for a PCR analysis. All 16 with PCR-confirmed malaria carried a single copy of the multi-drug resistant (*mdr1*) gene, and the wild type asparagine allele *mdr1* codon 86 (*mdr1* 86N). Ten of the 16 samples carried the wild type haplotype (CVMNK) at codons 72-76 of the chloroquine resistance transporter gene (*pfcrt*); three samples carried the resistant CVIET allele; one carried both the resistant and wild type, and in two samples the allele could not be analysed.

Conclusions

The absence of *mdr1* gene copy number variation detected in this study suggests lumefantrine resistance has yet to emerge in KwaZulu-Natal. In addition, data from this investigation implies the possible re-emergence of chloroquine-sensitive parasites. Results from this study must be viewed with caution, given the extremely small sample size.

Recommendations

A larger study is needed to accurately determine therapeutic efficacy of artemether-lumefantrine and resistance marker prevalence. The high proportion of rapid diagnostic test false-positive results requires further investigation.

Declaration

I .Charles Hervey Vaughan-Williams declare that

I. The research reported in this dissertation, except where otherwise indicated, is my original research.

II. This dissertation has not been submitted for any degree or examination at any other university.

III. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Manuscript in press

26 December 2012: 'Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal: an observational cohort study' accepted for publication in '*Malaria Journal*'.

Acronyms and Abbreviations

ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
crt gene	Chloroquine resistance transporter gene
DNA	Deoxyribonucleic acid
HRP	Histidine-rich protein
kg	Kilogram
mdr gene	Multi-drug resistance gene
P. falciparum	*Plasmodium falciparum*
pfcrt gene	*Plasmodium falciparum* chloroquine resistance transporter gene
PCR	Polymerase chain reaction
qPCR	Quantitative real-time polymerase chain reaction
RDT	Rapid diagnostic test
SP	Sulfadoxine-pyrimethamine
UKZN	University of KwaZulu-Natal
WHO	World Health Organization

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CHAPTER I: INTRODUCTION

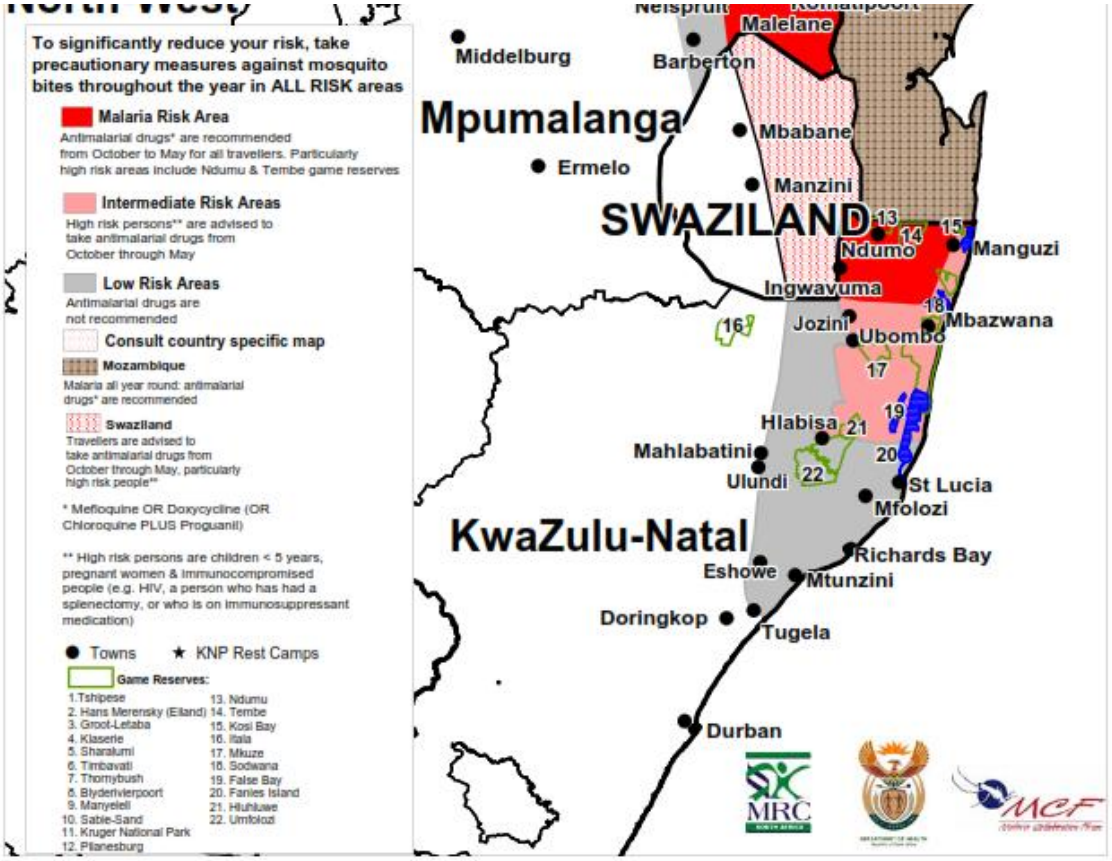
Background

Artemether-lumefantrine (AL) has been used as the first line treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal since 2001 [1, 2] and is recommended for this purpose by the South African Department of Health [3]. The recent history of malaria epidemics in KwaZulu-Natal indicates that the therapeutic effectiveness of the anti-malarial medication used to treat patients suffering malaria is vital for malaria control and must be regularly assessed. Failure to do so risks the undetected emergence of antimalarial drug resistance, contributing to a malaria epidemic, which has major public health and economic consequences for the area, province and even country. A study of the effectiveness of AL should either confirm the continuing efficacy of the medicine, or provide a warning as to the acquisition of resistance by malaria parasites and the need to seek an alternative therapy before an epidemic occurs.

Recent history of *P. falciparum* antimalarial drug resistance in KwaZulu-Natal

South Africa, along with neighbouring Namibia, Botswana, Zimbabwe and Swaziland, is classified by the World Health Organisation (WHO) as a low malaria transmission country [4]. Most of South Africa's population is not at risk from malaria, however malaria remains endemic mainly along the north-eastern border with Mozambique and Swaziland [4]. Mozambique is classified as a high malaria transmission country, with most of the population at high risk of malaria [4]. The Province of KwaZulu-Natal borders on both Mozambique and Swaziland, and malaria risk areas may be seen in figure 1 [5].

Figure 1: Malaria Risk Map of KwaZulu-Natal



Chloroquine resistance was first detected in South Africa in 1985 [6]. In 1988, due to the emergence and spread of chloroquine-resistant *P. falciparum* malaria, sulfadoxine-pyrimethamine (SP) replaced chloroquine as the first-line treatment for uncomplicated *P. falciparum* malaria in KwaZulu-Natal [1, 7, 8]. This drug remained effective until 1996 when malaria incidence increased dramatically. Between 1996 and 2000, northern KwaZulu-Natal suffered increasingly severe malaria epidemics with more than 40 000 malaria notifications in 2000 [9]. Only in 2000 did a clinical study show that *P. falciparum* parasites in the region had developed resistance to sulfadoxine-pyrimethamine, the then recommended first line treatment for malaria, rendering it largely ineffective in northern KwaZulu-Natal [7]. Subsequently the change of first-line medication to artemether-lumefantrine (AL), together with change of insecticide used for residual house spraying to DDT, dramatically reduced malaria incidence in northern KwaZulu-Natal [1, 2]. It has been estimated that the delay in changing first-line

treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to the size of the epidemic [10]. Artemisinin based combination therapy (ACT) is recommended for the treatment of uncomplicated *P. falciparum* malaria by the World Health Organization, and artemether-lumefantrine is one recommended combination [4, 8]. Artemether-lumefantrine is recommended for the treatment of uncomplicated *P. falciparum* by the South African Department of Health [3]. Studies of the therapeutic efficacy of artemether-lumefantrine in 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in northern KwaZulu-Natal [1, 11, 12].

Statement of problem

Since 2002 there have been no further studies in KwaZulu-Natal, or South Africa, of the continuing therapeutic effectiveness of AL in the treatment of uncomplicated malaria.

What needs to be known?

The WHO recommends routinely monitoring antimalarial resistance at least every three years, and a change in antimalarial medicine if the treatment failure proportion ≥ 10% after follow-up for at least 28 days [4, 8].

Purpose of research

For these reasons it was considered that an assessment of the efficacy of AL in KwaZulu-Natal was overdue and required in the interests of public health.

Specific objectives

The specific objectives of the study are: to quantify the proportion of patients treated with artemether-lumefantrine, who fail to clear *P. falciparum* from their blood 28 days after treatment; test for molecular markers of malarial drug resistance to AL and chloroquine; publish the results, and use the results to inform policy as to the best choice of drug for the treatment for uncomplicated malaria in Umkhanyakude Health District.

CHAPTER II: LITERATURE REVIEW

Purpose of literature review

The purpose of this literature review is to provide an understanding of the present situation with regards to malaria drug resistance in KwaZulu-Natal and the need for drug efficacy testing. For this the author felt that the history of antimalarial medication and malaria control should be included, as well as an outline of the biology of malaria which is necessary to understand some of the challenges faced in controlling the disease. Any discussion which involves malaria control must include reference to vector control as well as antimalarial medication.

Introduction

According to the WHO World Malaria Report, in 2010 there were 216 million episodes of malaria and 655 000 deaths from malaria worldwide [4]. Of these, 81% of the malaria cases and 91% of the deaths were in Africa [4]. The Institute for Health Metrics and Evaluation estimates a higher global mortality of 1,2 million of which 1,1 million were in Africa [13]. Resistance of *Plasmodium falciparum* malaria, the most virulent form of the disease, to antimalarial drugs has been one of the main obstacles to malaria control in the world and South Africa [6, 14].

Biology of malaria

Malaria is caused by a single-cell protozoan parasite called *Plasmodium* [15]. There are now five kinds of malaria described that infect humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi*, which was recently described in Borneo in 2009 [16, 17].

Most deaths are caused by *Plasmodium falciparum*, the other types rarely causing a fatal illness [15].

Malaria has a complicated life-cycle involving humans and the female *Anopheles* mosquito [18]. Malaria is transmitted to humans through the bite of an infected female *Anopheles* mosquito. The malaria parasites are first transported to the liver, where they multiply, and then to the blood stream where they multiply further and destroy blood cells. If an infected human is then bitten by an uninfected mosquito, the ingested parasites then develop in the mosquito, taking 10-18 days to mature and appear in the mosquito's salivary glands, and are then able to infect a new human subject bitten by the mosquito [18]. The lifespan of a mosquito is similar to the time taken for malaria parasites to mature in the mosquito. In colder weather the lifespan is shortened preventing maturation of the malaria parasites, hence malaria transmission occurs mainly in warm climates [19].

Brief history of malaria – World

Symptoms of malaria have been described for thousands of years [18]. There have been a number of important events contributing to the understanding of the biology of malaria. In 1880 Laveran discovered the malaria parasite. In 1886 Golgi discovered different forms of the disease, with different periodicity of fevers; in 1890 Grassi and Filetti named *Plasmodium vivax* and *Plasmodium malariae* parasites; in 1897 Welch named the *Plasmodium falciparum* parasite; and in 1897 Ross demonstrated that malaria is transmitted by mosquitoes [18].

Important steps in the treatment and prevention of malaria

Treatment

There have been a number of significant historical steps in the treatment and prevention of malaria. In China in 340 AD, the Qinghao plant or *Artemesia annua* was recognized as a treatment for fever [18]. In 1971 the antimalarial drug artemesinin was isolated by Chinese scientists, and today derivatives of artemesinin are used in many antimalarial medications throughout the world, often in combination with other drugs [18].

In Peru in 17th Century the bark of the Chinchona or quina tree was introduced to Jesuit missionaries as a treatment for fevers by the indigenous people [18]. In 1820 quinine was isolated from the bark by Pierre Pelletier and Joseph Caventou and became the standard treatment for malaria, until the development of chloroquine in 1946, which then became the preferred drug for treatment of malaria [18, 20, 21].

From 1946, chloroquine became increasingly used as a cheap effective antimalarial with few side effects. By the 1960s it was widely available over the counter throughout Africa [22].

Insecticide

Dichloro-diphenyl-trichloroethane (DDT) was first synthesised in 1874, however the insecticidal properties of the compound were only discovered in 1939. DDT was first used for malaria control during the Second World War by the Allies [18].

The Global Eradication Campaign was launched by the WHO in 1955 using insecticide house spraying, antimalarial treatment and surveillance [18, 23]. It was successful in eradicating malaria from the more temperate countries with seasonal malaria, but less successful in most of the tropical endemic malarious countries. Often, an initial reduction in malaria cases was followed by a rebound epidemic due to reduction of population immunity to malaria [22].

History of antimalarial drug resistance - World

Malaria parasite resistance to antimalarial drugs, as well as mosquito insecticide resistance, has been a major obstacle to malaria control worldwide, and remains so to this day [4, 6, 23].

Pyrimethamine parasite resistance was noted in Kenya in the 1950s following regular administration of the drug to villagers as part on an eradication programme, after which use of the drug was stopped [23]. Between 1960 and 1962, the first reports of chloroquine resistant malaria parasites came from South America and South East Asia [23]. By 1973 chloroquine resistance had been reported more widely. The first

chloroquine-resistant parasites from Africa were reported in Kenya and Tanzania in 1978 [21]. In subsequent years chloroquine resistance spread throughout Africa and the world, such that by 1989 almost all malarious countries had chloroquine-resistant *P. falciparum* [21, 23].

Sulfadoxine-pyrimethamine (SP) replaced chloroquine as first-line malaria treatment in a number of countries – starting with Thailand in 1973, and including KwaZulu-Natal, South Africa in 1988, Malawi in 1993, and Kenya and Botswana in 1997; however resistance to SP quickly emerged [21].

Since 1999, artemesinin combination therapy has been recommended as a means of reducing emergence of antimalarial resistance [24]. The mechanism of action of the combination is through a rapid reduction in parasite numbers by artemesinin, and clearance of the remaining parasites by a longer acting combination drug [24]. These combinations are now recommended by the WHO [8]. Artemether-lumefantrine is the recommended treatment for uncomplicated *P. falciparum* malaria by the South African Department of Health [3].

Unfortunately resistance to artemisinin, characterised by slow clearing of the parasite has now been reported from South-East Asia [25], and suggested in Kenya [26].

History of malaria control and antimalarial drug resistance in South Africa

Vector control

In 1932, the first malaria control measures in South Africa were stimulated by the adverse effect of the disease on sugar production [6]. The first malaria control measures in South Africa were the use of Pyagra (liquid pyrethrum and kerosene) for indoor spraying, which proved effective [6]. Pyagra was replaced by DDT in 1946 [6]. DDT remained the insecticide used the house spraying in northern KwaZulu-Natal until 1996, when DDT was replaced by the pyrethroid insecticide deltamethrin [27]. This change in insecticide was followed by a dramatic increase in the area between 1996 and 2000 [1,

2, 27]. In 2000 deltamethrin resistant, but DDT sensitive *Anopheles funestus* mosquitoes were identified [27]. This discovery led to the reintroduction of DDT house-spraying in northern KwaZulu-Natal [1], which was followed by a dramatic increase in the incidence of malaria in the region [1].

Antimalarial drug resistance

Chloroquine resistance was first detected in KwaZulu-Natal in 1985 [6, 28]. This resistance was shown to have increased by 1988 [29] which led to sulfadoxine-pyrimethamine (SP) replacing chloroquine as the first line treatment for uncomplicated *P. falciparum* malaria in KwaZulu-Natal [1, 6]. This drug remained effective until 1996 when malaria incidence sharply increased. Between 1996 and 2000 northern KwaZulu-Natal suffered increasingly severe malaria epidemics with more than 40 000 malaria cases notified in 2000 [1, 2, 9]. Analysis of health institution statistics in northern KwaZulu-Natal indicated more than 60,000 malaria cases treated [1]. Only in 2000 did a clinical study show that *P. falciparum* parasites in the region had developed resistance to sulfadoxine-pyrimethamine, the then recommended first line treatment for malaria, rendering it largely ineffective in northern KwaZulu-Natal [7]. Subsequently the change of first-line medication to artemether-lumefantrine, together with change of insecticide used for residual house spraying to DDT, dramatically reduced malaria incidence in northern KwaZulu-Natal [1, 2]. It has been estimated that the delay in changing first-line treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to the size of the epidemic [10]. Artemesinin based combination therapy is recommended for the treatment of uncomplicated *P. falciparum* malaria by the World Health Organization [4], and artemether-lumefantrine (AL) is one of the recommended combinations [8]. Studies of the therapeutic efficacy of artemether-lumefantrine in 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in northern KwaZulu-Natal [1, 11, 12]. Since 2002, however, there have been no further studies in KwaZulu-Natal, or South Africa, of the continuing therapeutic effectiveness of AL in the treatment of uncomplicated malaria. The WHO recommends routinely monitoring antimalarial resistance at least every three years, and a change in antimalarial medicine if the

treatment failure proportion ≥ 10% by day 28, or the last day of follow-up if longer than 28 days [8, 30].

Pharmacology of artemether-lumefantrine

Artemether-lumefantrine is a combination of artemether and lumefantrine manufactured as Coartem® by Novartis: 20mg artemether and 120mg lumefantrine in tablet form [31]. The two drugs act in a complementary manner [32]. Artemether and its active metabolite, dihydroartemisin, rapidly kill most malaria parasites, while lumefantrine clears the remainder more slowly [32, 33]. As most parasites are killed by artemether, the likelihood of selecting resistant parasites to the partner drug, lumefantrine, is much reduced [32, 33]. Artemether is rapidly absorbed and metabolized, with a half-life of about two hours, whereas lumefantrine is absorbed more slowly and has a half-life of 3-4 days in malaria patients [32, 33].

Coartem® is taken as a six-dose oral regimen over 3 days [31]. The dosage depends upon the weight and age of the patient [31]. The adult dosage for persons aged 12 years or more, or children weighing 35kg and above, is four tablets as a single dose at the time of initial diagnosis, 4 tablets after 8 hours, and then 4 tablets twice daily on each of the following two days [31]. It is recommended that the tablets are taken with fatty food or milk to improve absorption [31-33].

Recommended protocol and follow-up period for antimalarial efficacy testing

The WHO protocol for assessment of clinical and parasitological response to antimalarial medication recommends patient assessment on days 0 (enrolment), 2, 3, 7, 14, 21, and 28. A 28 day follow-up is adequate for drugs with an elimination half-life of less than 7 days (such as lumefantrine) [30]. A longer follow-up is needed for drugs with a longer elimination half-life, such as mefloquine [34] and piperaquine [35] which require 42 days of follow-up [30, 36].

Inadequate responses to antimalarial treatment are classified as: ‘Early Treatment

Failure (ETF)' in which there are danger signs or failure to reduce parasitaemia levels by day 3; 'Late Clinical Failure (LCF)' in which there are danger signs or parasitaemia and fever between days 4 and 28, and 'Late Parasitological Failure (LPF)' in which there is parasitaemia without fever between days 4 and 28 [30]. 'Adequate Clinical and Parasitological Response (ACPR)' is defined as absence of parasitaemia on day 28 in patients who did not suffer any of the other inadequate responses to treatment [30]. It is noted that a longer follow-up period increases the losses, reducing a study's validity; however no similar comment is made about the number of return visits already required of patients with the present WHO protocols [30]. From these definitions it may be inferred that clinically assessing and testing a subject on day 28 should detect Late Clinical Failure and Late Parasitological Failure.

Molecular markers of malaria resistance

According to the 2002 WHO report, molecular markers may assist in clarifying the resistance situation [37]. Markers of resistance have been validated for a number of monotherapies including chloroquine [38] and lumefantrine [39]. The storing of blood samples on filter paper for future testing as new molecular markers become available is recommended [37]. Molecular markers may provide warning of developing resistance to an antimalarial drug in use in an area and give an indication of the persistence of resistance to a drug that has been withdrawn from an area [37].

At present the genetic basis for artemesinin tolerance or resistance has not been elucidated, leaving delayed parasite clearance times as the only clear determinant of artemesinin tolerance and resistance [40], and molecular markers of lumefantrine resistance as the only markers for resistance for AL.

Certain molecular markers are linked with resistance to lumefantrine, the partner drug in AL, specifically the *pfmdr1* copy number [39], and the *mdr1*86N allele at *pfmdr1* codon 86 [41, 42].

Use of molecular markers to distinguish between re-infection and recrudescence

A polymerase chain reaction (PCR) test is recommended as mandatory by the WHO to distinguish between *P. falciparum* recrudescence and re-infection 7 days or more after treatment in areas of both low to moderate, and high, transmission [30].

Genotyping of *P. falciparum* DNA extracted from dried blood spots based on variations in the genes coding for the glutamine-rich protein and merozoite surface protein 1 and 2 (msp-1, msp-2), may be used to distinguish between different strains of *P. falciparum* [43, 44]. The banding patterns of the three molecular markers may be compared before and after treatment, and if *P. falciparum* markers are still present, determine if treatment failure is due to a re-infection or recrudescence of the original infection.

CHAPTER III: PAPER ACCEPTED FOR PUBLICATION IN 'MALARIA JOURNAL'

The following research paper has been submitted to the journal: 'Malaria Journal' to be considered for publication.

Title

Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal: an observational cohort study

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Abstract

Background

Recent malaria epidemics in KwaZulu-Natal indicate that effective anti-malarial therapy is essential for malaria control. Although artemether-lumefantrine has been used as first-line treatment for uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal since 2001, its efficacy has not been assessed since 2002. The objectives of this study were to quantify the proportion of patients treated for uncomplicated *P. falciparum* malaria with artemether-lumefantrine who failed treatment after 28 days, and to determine the prevalence of molecular markers associated with artemether-lumefantrine and chloroquine resistance.

Methods

An observational cohort of 49 symptomatic patients, diagnosed with uncomplicated *P. falciparum* malaria by rapid diagnostic test, had blood taken for malaria blood films and *P. falciparum* DNA polymerase chain reaction (PCR). Following diagnosis, patients were treated with artemether-lumefantrine (Coartem®) and invited to return to the health facility after 28 days for repeat blood film and PCR. All PCR *P. falciparum* positive samples were analysed for molecular markers of lumefantrine and chloroquine resistance.

Results

Of 49 patients recruited on the basis of a positive rapid diagnostic test, only 16 were confirmed to have *P. falciparum* by PCR. At follow-up, 14 were PCR-negative for malaria, one was lost to follow-up and one blood specimen had insufficient blood for a PCR analysis. All 16 with PCR-confirmed malaria carried a single copy of the multi-drug resistant (*mdr1*) gene, and the wild type asparagine allele *mdr1* codon 86 (*mdr1* 86N). Ten of the 16 samples carried the wild type haplotype (CVMNK) at codons 72-76 of the chloroquine resistance transporter gene (*pfcrt*) ; three samples carried the resistant CVIET allele; one carried both the resistant and wild type, and in two samples the allele could not be analysed.

Conclusions

The absence of *mdr1* gene copy number variation detected in this study suggests lumefantrine resistance has yet to emerge in KwaZulu-Natal. In addition, data from this investigation implies the possible re-emergence of chloroquine-sensitive parasites. Results from this study must be viewed with caution, given the extremely small sample size. A larger study is needed to accurately determine therapeutic efficacy of artemether-lumefantrine and resistance marker prevalence. The high proportion of rapid diagnostic test false-positive results requires further investigation.

Key words: *Plasmodium falciparum* malaria, artemether, lumefantrine, therapeutic efficacy, resistance markers, KwaZulu-Natal

Background

The World Health Organization (WHO) has recommended that drug efficacy be regularly assessed [1, 2]. Failure to detect the emergence of anti-malarial drug resistance, could lead to a drug-resistant malaria epidemic, which would have major public health and economic consequences for an area, province and country. The most recent malaria epidemics in KwaZulu-Natal, one of three provinces in South Africa with endemic malaria, were partially attributed to unrecognized resistance to the anti-malarial therapy being used at the time [3]. Artemether-lumefantrine (AL) has been first-line treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal since it was introduced in response to these drug-resistant epidemics in 2001 [4, 5]. Studies should be performed to confirm the continued efficacy of AL, or provide a warning of emerging resistance, and the need to seek alternative therapy before a malaria epidemic occurs.

Recent history of *Plasmodium falciparum* anti-malarial drug resistance in KwaZulu-Natal

Chloroquine resistance was first detected in KwaZulu-Natal in 1985 [6], and had increased by 1988 [7], leading to sulfadoxine-pyrimethamine (SP) replacing chloroquine as the first-line treatment for uncomplicated *P. falciparum* malaria in KwaZulu-Natal [4, 8, 9]. SP remained effective until 1996 when malaria incidence increased sharply in KwaZulu-Natal. Between 1996 and 2000 northern KwaZulu-Natal suffered increasingly severe malaria epidemics, with more than 40,000 cases reported in 2000 [4, 5, 10].

Only in 2000, were *P. falciparum* parasites in the region shown to have developed at least 61% (and as high as 89%, excluding those lost to follow-up) resistance to SP in a clinical efficacy study, rendering the drug ineffective in northern KwaZulu-Natal [8]. The introduction of AL as the first-line medication for uncomplicated *P. falciparum* malaria, together with the reintroduction of DDT insecticide for indoor residual house spraying in 2001, dramatically reduced malaria incidence in the area [4, 5]. It has been estimated that the delay in changing first-line treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to

the size of the epidemic [3]. Malaria notifications in KwaZulu-Natal between 1991 and 2011 are shown (Figure 1).

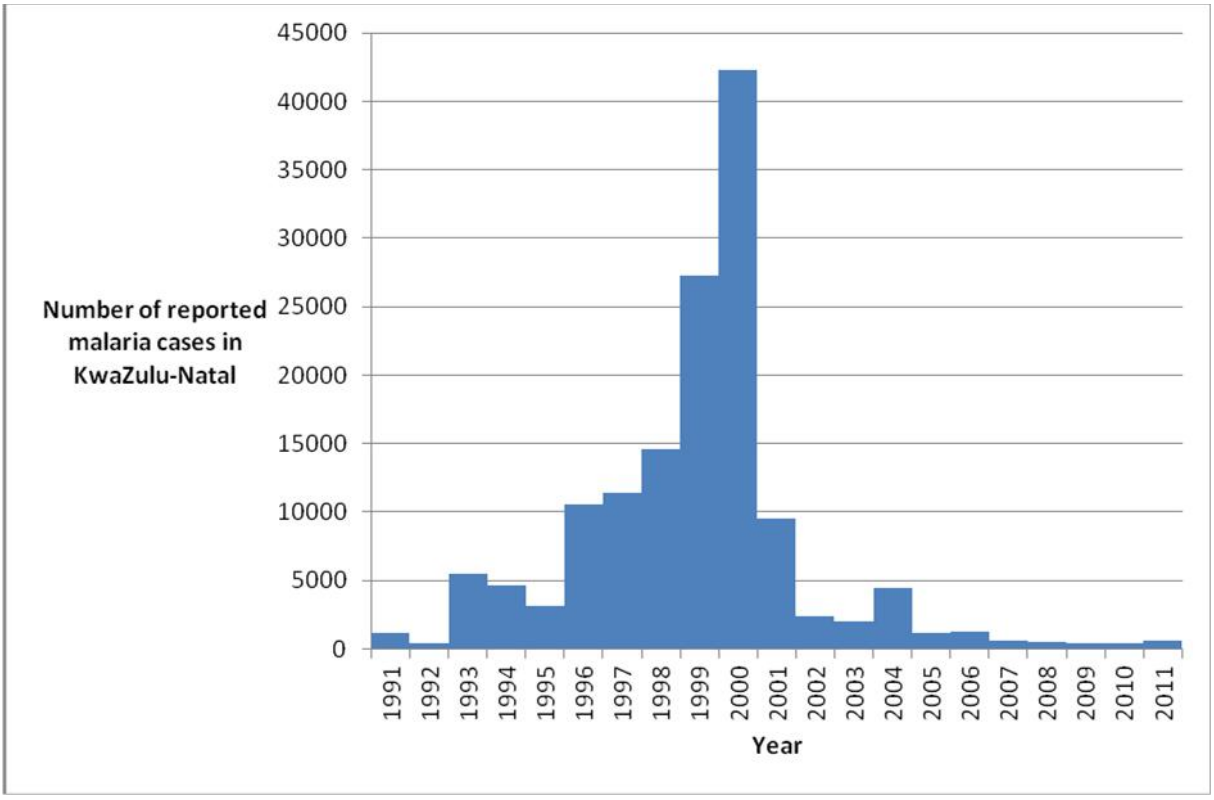

Figure 2: Malaria notifications in KwaZulu-Natal from 1991 to 2011.

Source: Data 1996-2011 - KwaZulu-Natal Department of Health Malaria Control Programme; Data 1991-1995 - Knight SE, Anyachebelu EJ, Geddes R, Maharaj R: Impact of delayed introduction of sulfadoxine-pyrimethamine and artemether-lumefantrine on malaria epidemiology in KwaZulu-Natal, South Africa. *Trop Med Int Health* 2009, 14:1086-1092

It has been estimated that in 2000, at the height of the epidemic, the malaria incidence amongst the exposed population in northern KwaZulu-Natal was 5,972 per 100,000 [3]. It should be noted that the malaria notification system became overloaded during these epidemics, and that the notifications were incomplete. For example during the year 2000 one clinic, Ndumo Clinic, in northern KwaZulu-Natal, saw 30,885 cases based on laboratory results, a 50-fold increase compared to 1995 [5], and equivalent to 73% of the total provincial notifications of 42,248 [10].

Artemisinin-based combination therapy (ACT) is advocated for the treatment of uncomplicated *P. falciparum* malaria because of the rapid reduction in parasite load caused by artemisinin or its derivative; the consequent reduced likelihood of resistance

emerging to the partner drug; the reduction in gametocyte carriage, and rapid clinical response [11]. ACT is recommended by WHO for the treatment of *P. falciparum* malaria [12], and AL is one of the recommended combinations [2, 12]. Studies of AL therapeutic efficacy in northern KwaZulu-Natal during 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in the area [4, 13, 14]. Since 2002, there have been no further studies of the continuing therapeutic efficacy of AL in KwaZulu-Natal, or South Africa. The WHO recommends routinely monitoring anti-malarial resistance at least every three years, and a change in anti-malarial medicine if the treatment failure proportion is equal to or greater than 10% by day 28, or the last day of follow-up, if longer than 28 days [1, 12].

Pharmacology of artemether-lumefantrine

Artemether-lumefantrine is a combination of two drugs, artemether and lumefantrine, manufactured in tablet form as Coartem® by Novartis. Each tablet contains 20 mg artemether and 120 mg lumefantrine [15]. The two drugs act in an independent but complementary manner at different stages of the parasite life cycle [16]. Artemether and its active metabolite, dihydroartemisinin, rapidly kill most circulating malaria parasites, while lumefantrine clears the remainder more slowly [16, 17]. The probability of selecting parasites resistant to the partner drug, lumefantrine, is theoretically reduced due to the small parasite load remaining following activity of artemether [16, 17]. Artemether is rapidly absorbed and metabolized, with a half-life of about two hours, whereas lumefantrine is absorbed more slowly and has a half-life of 3-4 days in malaria patients [16, 17].

Coartem® is taken as a six-dose oral regimen over three days. The dosage depends mainly upon the weight of the patient. The dosage for persons aged 12 years or more, or younger children weighing 35 kg and above, is four tablets as a single dose at the time of initial diagnosis, four tablets after eight hours, and then four tablets twice daily on each of the following two days [15]. It is recommended that the tablets are taken with fatty food or milk to improve absorption [15, 17, 18].

Choice of follow-up period for anti-malarial efficacy testing

In the 2009, WHO anti-malarial drug efficacy testing guide [1], inadequate responses to anti-malarial treatment are classified as: 'early treatment failure' in which there are danger signs or failure to reduce parasitaemia levels by day 3; 'late clinical failure' in which there are danger signs or parasitaemia and fever occurring between days 4 and 28, and 'late parasitological failure' in which there is parasitaemia without fever between days 4 and 28. 'adequate clinical and parasitological response' is the absence of parasitaemia on day 28 (or day 42 for longer acting drugs), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure. For drugs with a half-life of less than seven days, such as artemisinin and lumefantrine, evaluation of clinical and parasitological response up to 28 days is recommended [1]. For those with longer half-lives such as mefloquine (three weeks [19]) and piperaquine (two to three weeks [20]), a follow-up of 42 days is recommended [1, 12, 21].

Although there has been no anecdotal evidence of resistance to AL in KwaZulu-Natal since its implementation, artemisinin resistance, characterised by slow clearing of parasite has been confirmed in South East Asia [22], and suggested in Kenya [23]. Previous research by Roper and colleagues demonstrated that SP resistance spread to southern Africa from East Africa [24]. In neighbouring Mozambique increase in prevalence of molecular markers associated with lumefantrine resistance since initial use of AL suggest the need for continued surveillance for the emergence of resistance to the drug [25]. The primary objective of this study was to screen for late AL clinical and parasitological failure, the first indication of emerging resistance to AL in South Africa.

Requiring patients to return a clinic six or seven times in one month for assessment requires considerable resources and the risk of drop-out from the study is high. A single follow-up assessment at 28 days was therefore chosen which required a patient to return only once. PCR is recommended by the WHO to distinguish between *P. falciparum* recrudescence and re-infection seven days or more after treatment in areas of both low to moderate, and high, transmission [1].

Molecular markers of malaria resistance

According to the 2002 WHO report [26], molecular markers may assist in determining resistance and provide an early warning of developing drug resistance before it becomes clinically apparent. Markers of resistance have been validated for a number of monotherapies, including chloroquine [27] and lumefantrine [28]. As the genetic basis for artemisinin resistance is not known, efficacy of AL is assessed by determining the molecular markers of resistance for the partner drug, lumefantrine. Certain molecular markers have been linked with resistance to lumefantrine, the partner drug in AL, namely the *P. falciparum* multidrug resistant (*mdr)1* gene copy number [28], and the *mdr1*86N allele [29, 30]. Multiple copies of the *mdr1* gene has been linked with lumefantrine resistance in Southeast Asia [28], while mutations at the codon 86 of the *mdr1* gene modulate lumefantrine efficacy [31].

Storage of blood samples on filter paper for future testing as new molecular markers become available is recommended [26].

Ethical issues

The study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee, and by the Health Research Committee of the KwaZulu-Natal Department of Health.

Methods

The study population included symptomatic persons presenting to health facilities, diagnosed with uncomplicated *P. falciparum* malaria in Umkhanyakude Health District, northern KwaZulu-Natal, using the *P. falciparum* malaria rapid diagnostic test (First Response, malaria antigen *P. falciparum* (HRP2) detection rapid card test manufactured by Premier Medical Corporation Limited, Kachigam, Daman (UT) 396215, India).

Inclusion criteria

Symptomatic patients aged from five years to 69 years, self presenting to health facilities, diagnosed with uncomplicated malaria in Umkhanyakude Health District between January and May 2012, were invited to participate in the study.

Exclusion criteria

Patients with the following danger signs or symptoms of severe malaria: unable to drink; vomiting everything; a convulsion during previous seven days; lethargic or decreased level of consciousness; unable to stand or sit [32], were excluded. Pregnant women, patients aged less than five years and more than 69 years, and patients treated for malaria during the previous two weeks were also excluded.

Information provided

At recruitment patients were provided with an information sheet in English and *isiZulu* detailing the purpose of the study, which was also explained verbally. Patient queries were answered after which they were invited to provide written consent.

Investigations

Finger-prick blood spots blotted on to Guthrie 903 filter paper cards (Munktell GmbH, Barenstien, Germany), and blood samples were collected from all participants for molecular analysis and malaria microscopy. The patient was then asked to return in four weeks for repeat malaria film microscopy and blood spot collection, with the offer of ZAR50 (US$5.79) in travelling expenses upon return. RDT was not performed at follow-up due to persistence of histidine-rich protein, HRP-2, in patients for as long as 28 days after parasite clearance [14, 33]. Blood spots taken by nurses were sent to the investigator at the local hospital. These were then collected by the Principal Investigator, usually twice per month, and posted to the researcher performing the molecular analysis more than 400 km away.

Thick and thin blood films were prepared according to the National Health Laboratory Service standard operating procedure for processing specimens for malaria parasites [34]. Slides were stained using the rapid modified Wright-Giemsa stain (Rapidiff); thin films being fixed with methanol before staining. Parasitaemia was calculated from the percentage of red cells containing malaria parasites observed in 10 microscope fields using the 100x lens.

Parasite DNA was extracted from all blood spots using the QIAamp DNA Mini Kit (QIAGEN, Whitehead Scientific). The extracted DNA was then subjected to

quantitative real-time PCR (qPCR) and nested PCR analysis to confirm the presence of *P. falciparum* parasites [35, 36].

All samples confirmed as *P. falciparum* positive by PCR, were subjected to mutational analysis to detect the prevalence of molecular markers linked with resistance to lumefantrine, (*mdr1* gene copy number amplification) and chloroquine (mutations at *mdr1* codon 86, [27] and codons 72 to 76 of the chloroquine resistance transporter (*crt*) gene) [37]. At the follow-up visit, the patient was clinically assessed, and a further finger prick blood sample taken for *P. falciparum* PCR and blood film.

Results

A total of 49 patients with a diagnosis of malaria based on a rapid diagnostic test were enrolled in the study. Two patients did not have their age recorded**.** The age range of the remaining 47 patients was 2 – 69 years; median 15 years, and mean 21.1 years. The largest group comprised those less than 10 years of age (Figure 2). Four patients were less than minimum age of five years stipulated in the study protocol. Their treatment, however, was identical to that in the National Treatment Guidelines [38], and they were included in the analysis.

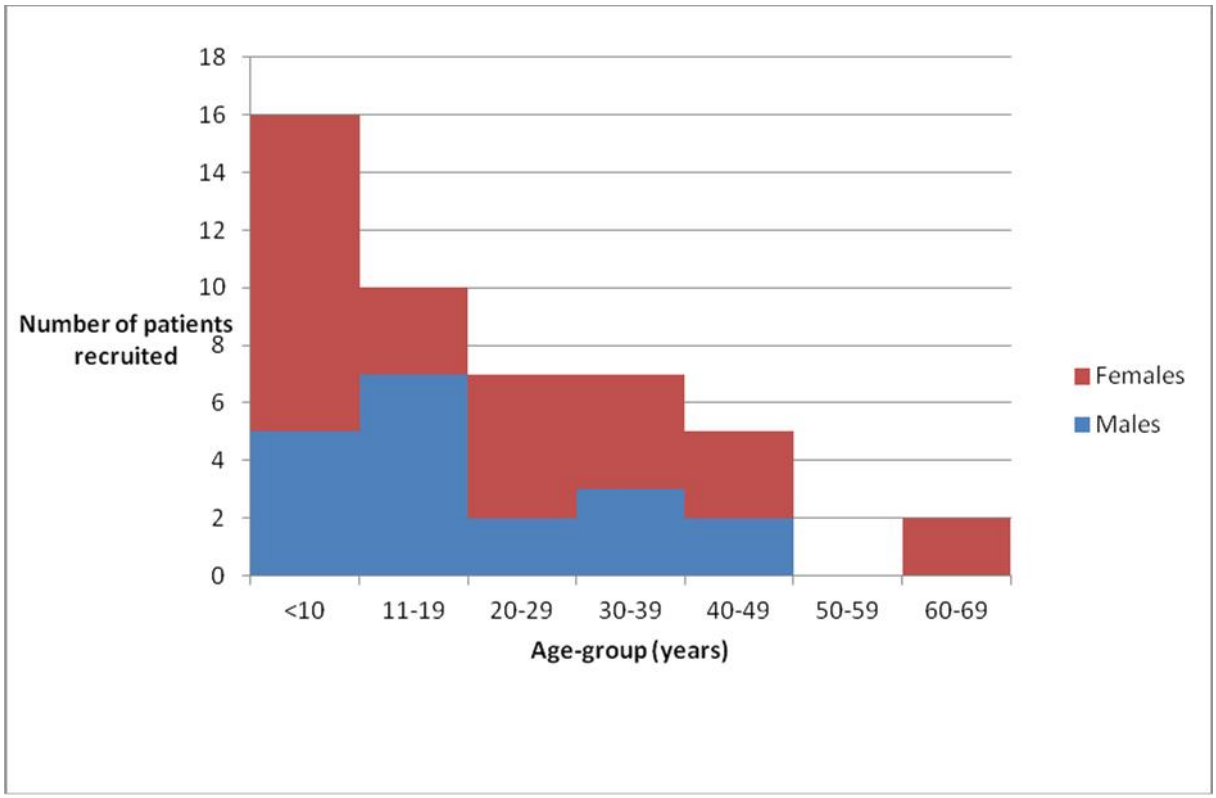

Figure 3: Age and gender of patients with malaria RDT-positive recruited from Umkhanyakude Health District, January to May 2012 (N=47)

Confirmation of *Plasmodium falciparum* malaria by PCR

Only 33% (16/49) patients were confirmed to have *P. falciparum* malaria by PCR (Figure 3).

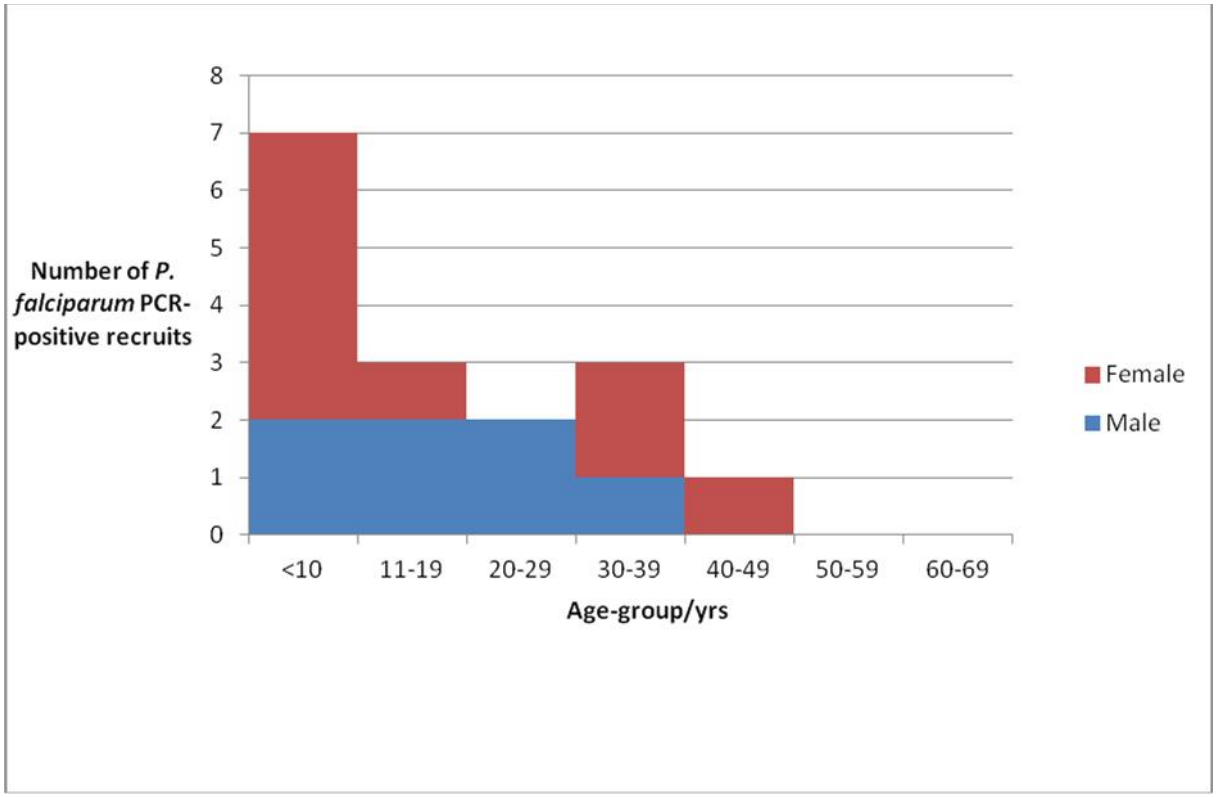

Figure 4: Age and gender of patients with *P. falciparum* malaria confirmed by PCR recruited from Umkhanyakude Health District, January to May 2012 (N=16)

Closer inspection of the RDTs revealed that frequently too much blood had been used, rendering the test virtually impossible to interpret. The age range of those confirmed with *P. falciparum* was 2 - 40 years with median age 14.5 years and mean 16.8 years. Nearly half (7/16) of those with PCR-confirmed malaria were aged less than 10 years. Results for the *P. falciparum* PCR positive patients are summarized in Table 1.

Table 1: Results for patients with PCR confirmed *Plasmodium falciparum* malaria at enrolment

Age	Gender	Travel 60 days prior to illness	Temp	*mdr1* copy number (lumefantrine sensitivity)	*mdr1*N86Y gene (chloroquine sensitivity)	Chloroquine resistance transporter gene *crt* K76T codons 72-76	Follow-up day	Follow-up *P. falciparum* malaria PCR
40	Female	Mozambique	36.5	1(sensitive)	Asparagine (*mdr1* 86N - sensitive)	CVMNK(sensitive)	28	Negative
7	Male	Not recorded	39.4	1	asparagine	CVMNK and CVIET(resistant)	191	Negative
32	Female	None	36.4	1	asparagine	CVIET	168	Insufficient sample
24	Male	None	39.3	1	asparagine	CVMNK	49	Negative
9	Female	Not recorded	Not recorded	1	asparagine	CVMNK	29	Negative
31	Male	Mozambique	38.3	1	asparagine	CVMNK	32	Negative
8	Female	None	36.7	1	asparagine	CVIET	29	Negative
15	Male	Mozambique	38.0	1	asparagine	CVIET	106	Negative
20	Male	Mozambique	41.0	1	asparagine	CVMNK	127	Negative
37	Female	Not recorded	38.7	1	asparagine	CVMNK	86	Negative
6	Female	None	39.0	1	asparagine	CVMNK	28	Negative
2	Female	Mozambique	Not recorded	1	asparagine	CVMNK	NA	Lost to follow-up; returned to Mozambique
17	Male	None	38.2	1	asparagine	CVMNK	69	Negative
14	Female	None	38.0	1	asparagine	Not obtained	92	Negative
4	Female	Mozambique	38.0	1	asparagine	CVMNK	59	Negative
3	Male	Not recorded	38.0	1	asparagine	Not obtained	57	Negative

Presence of fever

Fever (auxiliary temperature ≥37.5°C) was recorded in 64% (27/42) of all patients who were initially diagnosed with malaria and 77% (11/14) of the PCR malaria confirmed cases, while 57% (16/28) of those PCR negative had a fever. Temperature was not recorded for two malaria PCR positive patients.

Recent travel

Six of the 16 PCR malaria-confirmed cases reported having travelled to Mozambique within the previous month.

Blood films

Due to liaison difficulties with a local laboratory, blood films were only obtained in 15/49 recruited patients, examined by the hospital laboratory technicians. Of those, only four were PCR-confirmed malaria samples, and of those four, one was *P. falciparum* positive by microscopy with a parasitaemia of 0.25%. All other 14 blood films were microscopy-negative for *P. falciparum*.

Molecular markers

All 16 PCR *P. falciparum* positive samples collected at enrolment had a single copy of the *mdr*1 gene and carried the wild type asparagine allele at codon 86 of the *mdr1* gene (*mdr1* 86N) (Table 1). Results for 14 of the 16 samples were *crt* 72-76 genotyped. Ten samples carried the wild chloroquine sensitive haplotype (CVMNK), three the pure mutant haplotype (CVIET) associated with chloroquine treatment failure, while one carried both wild and mutant alleles. Two samples proved inadequate for *crt* genotyping.

Follow-up

Only 14% (7/49) of the patients returned for follow-up, of which six were *P. falciparum* PCR positive at enrolment. Malaria control personnel tracked down and obtained blood specimens for follow-up PCR from a further nine non-returning PCR-confirmed patients, at varying intervals from four weeks following recruitment. Of the initial PCR positive cohort, 14 were found to be negative at follow-up, one sample contained insufficient blood for testing and one patient was lost to follow-up.

Discussion

The primary aim of this study was to assess the therapeutic efficacy of AL, the current first-line treatment of uncomplicated *P. falciparum* malaria in northern KwaZulu-Natal. The study was hampered by a scarcity of diagnosed malaria cases. Of 49 patients enrolled in the study on the basis of a positive *P. falciparum* rapid diagnostic test, only 16 were subsequently confirmed to have *P. falciparum* malaria by PCR analysis.

Amplification of *mdr1* gene copy number associated with lumefantrine resistance in South East Asia was not detected in this study [39]. This result confirms the research findings in neighbouring Mozambique [25], where no variation in *mdr1* copy number was observed following two years of AL deployment, and supports the hypothesis that *mdr1* amplification is rare in Africa [31].

The *mdr1*N86Y mutation associated with chloroquine resistance [27] was completely absent in this study. This finding together with the high prevalence of the *crt* 72-76 wild type haplotype (CVMNK) in the study area suggests AL deployment removed chloroquine drug pressure, allowing chloroquine sensitive parasites to re-emerge as seen in Malawi [40] and Mozambique [25]. This return of parasite sensitivity to chloroquine could result in the re-introduction of chloroquine in combination with a partner drug as an anti-malarial.

All follow-up samples were PCR negative for *P. falciparum*, implying sustained AL efficacy, 11 years after it was initially rolled out in KwaZulu-Natal. On a cautionary note, the high *mdr1*86N allele prevalence is a cause for some concern. It has been suggested that increases in *mdr1*86N prevalence is the first step towards lumefantrine tolerance [30]. Sustained lumefantrine drug pressure is probably driving the selection of the *mdr1*86N allele in KwaZulu-Natal. In contrast, the removal of chloroquine drug pressure probably selected for this allele in Mozambique [25]. Given the wide use of AL in southern Africa and the high prevalence of resistance markers associated with lumefantrine resistance, close monitoring of AL efficacy and lumefantrine resistance markers is recommended to ensure effective first-line treatments are available.

Positive study outcomes

Despite the extremely small sample, some valuable data was produced by this study.

Blood spots for molecular analysis

In a low malaria-incidence setting, blood spot samples proved to be a good source for molecular analysis. Collection of the filter paper samples was relatively easy and inexpensive. Once collected and correctly stored the samples were resilient to delays in collection and transport. PCR is capable of detecting malaria parasites at a density of five parasites per microlitre, whereas thick film microscopy is only reliable at a density of 50 parasites per microlitre, meaning PCR is more than ten times more sensitive than microscopy for the diagnosis of malaria parasitaemia [41, 42]. PCR also has a specificity of nearly 100% for both *P. falciparum* and *P. vivax* [43].

Monitoring molecular markers of drug resistance, while a less rigorous method of assessing drug efficacy than *in vivo* sensitivity studies, is much less expensive and time consuming, and is a reasonable method of surveillance for emerging drug resistance [25]. The WHO recommends that in countries with very low levels of transmission, such as South Africa, studies of molecular markers of resistance should be conducted every year [1]. Molecular markers in 10 of the 14 available blood specimens indicated sensitivity to chloroquine, suggesting that chloroquine resistance may have decreased following removal of the selection pressure from using chloroquine as first-line therapy. Similar findings have been demonstrated in neighbouring Mozambique and been attributed to the withdrawal of chloroquine [25].

Study limitations

Enrolment procedure and administration

The incidence of malaria in the study area remained low during the study period and cases were geographically scattered, presenting to several different clinics and hospitals in Umkhanyakude District. Management and control of the patient records and specimens was difficult. The cooperation of healthcare workers from several health facilities was required and consistency of the enrolment procedure was difficult to

achieve. Blood was sent by clinic nurses to the local hospital laboratory which declined to perform blood films on many patients.

Of the 49 recruited patients only 16 were PCR confirmed malaria. The high proportion of false positive RDT results was probably mainly due to incorrect use of the test, indicating a lack of familiarity, and the need for more training. It is possible that false positive RDT results have erroneously inflated the notified malaria cases in the district for some time, and is deserving of further investigation.

Sample size

The study aimed to obtain a sample size of 50, which is the minimum recommended by the WHO regardless of rates of failure anticipated, in order to be representative [1]. However, this study could only include the malaria cases available. In an area which suffered severe malaria epidemics within the past 12 years, partly attributable to a lack of parasite resistance data required for upgrading antimalarial treatment policy [3], it is important to undertake regular drug resistance monitoring, or risk repeating the mistakes of the past. The last published malaria resistance studies in KwaZulu-Natal took place in 2002 [4, 14], and the data in this study could be used to inform a larger study.

Follow-up

Despite the financial incentive offered to recompense for travelling expenses, most patients had to be tracked down by malaria control personnel at varying time intervals after treatment.

Use of single follow-up visit

Use of a single follow-up visit on day 28, rather than follow-up visits on days 1,2,3,7,14 and 28, as recommended by the WHO [1, 26, 32], meant that in the event of persistence of *P. falciparum* parasitaemia by day 28, it would not be possible to distinguish between early treatment failure, late clinical failure, and late parasitological failure. The finding of persistence of parasitaemia by day 28 would provide a motivation for a further study following the WHO protocol [1] to distinguish the degree of resistance. However, as already mentioned, persuading patients to return for six follow-up visits is not easy, evidenced by the difficulty faced in this study of obtaining even a single follow-up from

patients. The single 28 day follow-up should detect most clinical and treatment failures, and seems particularly suitable for screening for late clinical failure and late parasitological failure.

Conclusions

Determining drug efficacy, particularly as malaria transmission approaches zero, is critical, as the last remaining parasites are most likely the most resistant [44]. Since therapeutic efficacy of AL in KwaZulu-Natal had not been assessed recently, this study attempted to address the issue. Unfortunately the extremely low incidence of malaria in northern KwaZulu-Natal impacted negatively on patient recruitment. As drug efficacy data is essential to inform policy, particularly as South Africa embarks on an elimination agenda [45], every attempt to obtain robust valid resistance data must be made. Future options include larger studies across multiple sites, and the follow-up of all malaria cases at 28 days with annual molecular marker studies [1].

Although 49 patients were recruited into the study based on RDT results, only 16 were confirmed *P. falciparum* positive by PCR. Preliminary investigations appear to indicate that incorrect use of RDT was the principal reason for the high proportion of false-positive results. Since definitive diagnosis is a fundamental tenet of the elimination agenda, further investigation into the cause of the false-positive RDT results is indicated, and corrective measures put in place to prevent misdiagnosis.

Despite the small sample size, all samples were malaria negative at Day 28, or longer, suggesting sustained AL efficacy in KwaZulu-Natal. Support for this is provided by the absence of *mdr1* copy number amplification found in this study. However rigorous regular lumefantrine resistance monitoring is recommended given the high prevalence of the *mdr1*86N allele associated with lumefantrine tolerance and widespread use of AL in southern Africa.

Abbreviations

ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
crt gene	Chloroquine resistance transporter gene
DNA	Deoxyribonucleic acid
HRP	Histidine rich protein
mdr gene	multidrug resistance gene
pfcrt gene	*Plasmodium falciparum* chloroquine resistance transporter gene
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
SP	Sulfadoxine-pyrimethamine
WHO	World Health Organization

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors have reviewed the final draft and agreed to its submission. CHVW initiated the research project, wrote the protocol, co-ordinated the research, collated the data, and composed most of the research report. JR contributed to study design, performed the PCR and molecular analysis, and assisted with editing of the manuscript. ER participated in the study design and supervised Malaria Control Personnel for the follow-up of non-returning patients. EI co-ordinated the recruitment of patients at local clinics, the collection of samples, and was responsible for overseeing clinical care of recruited patients. HR co-ordinated local recruitment of patients, collection of samples, and was responsible for overseeing clinical care of recruited patients. KG reviewed the

study protocol, co-ordinated local recruitment of patients, and was responsible for overseeing clinical care of recruited patients. SK assisted with the development of the protocol and the write-up of the report of the research.

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CHAPTER IV: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Introduction

In this chapter the lessons learned from the research will be discussed. Some issues have already been mentioned in the paper submitted for publication in the previous chapter; however in addition the practical challenges and lessons learned will be expanded upon.

Therapeutic efficacy of artemether-lumefantrine

The study found no definite evidence, either clinically or from molecular marker analysis, of resistance to artemether-lumefantrine, providing some reassurance that the drug is still efficacious, and that there is, as yet, no evidence of a need to change first line therapy for the treatment of uncomplicated malaria in northern KwaZulu-Natal. None of the 14 confirmed *P. falciparum* patients followed up were *P. falciparum* PCR positive at a follow-up. Nor did any of the 16 confirmed *P. falciparum* PCR-positive patients show an increase in the *pfmdr1* copy number associated with lumefantrine resistance [45]. A slight concern is that all 16 samples carried the *mdr1*86N (asparagine) allele. Although this means that there was no *mdr1*86Y allele associated with chloroquine resistance [38], an increase in the frequency of the *mdr1*86N allele has been suggested as a step towards lumefantrine tolerance [42] . As mentioned already, the final sample was very small, meaning that the results must be treated with caution.

Use of blood spots for PCR analysis

The study demonstrated the robustness of PCR analysis of blood spots as an investigative tool, in a rural area with staff unfamiliar with research. Blood spots for PCR are routinely used for the detection of HIV in exposed infants [46]. The study blood spots were easy to obtain, and resilient to delay in transport to the laboratory more than 400km away. PCR is capable of detecting malaria parasites at a density of five parasites per microlitre or less [47, 48], whereas thick film microscopy is only reliable at a density of 50 parasites per micro litre or more [48]. PCR has also been shown to

have a specificity approaching 100% for both *P. falciparum* and *P. vivax* [49]. Ideally blood spots should be obtained from all *P. falciparum* cases in KwaZulu-Natal, while the present low malaria incidence persists so that a continuous molecular marker surveillance may be carried out [30].

Challenges

The study encountered a number of challenges:

Malaria incidence

The incidence of malaria in the study area remained low during the study period, which severely hampered the research project. Too low a malaria incidence to research was a gamble taken when the study was embarked upon. However, in 2011 there were circumstances which suggested that malaria incidence in 2012 could be sufficient to undertake the research.

District Hospitals in northern Umkhanyakude District had reported a surge in cases in early 2011. Malaria Control Programme statistics showed 84 ‘passive’ (reported by health institutions) cases for Jozini and Umhlabuyalingana Districts in northern KwaZulu-Natal for the period January to June 2011. Total active (cases actively sought out by the Malaria Control Programme) and passive cases in the two sub-districts for 2011 were 153, compared to 72 in 2010.

In addition to this apparent increase in malaria cases in 2011 compared to 2010, it appeared that malaria control measures in neighbouring southern Mozambique were deteriorating. Insecticide spraying in southern Mozambique has been one of the highly successful malaria control measures undertaken by the Lubombo Spatial Development Initiative implemented since 2000 [50] . However, it was reported at a meeting on 28 September 2011 between a Mozambiquan delegation from Maputo Province and a delegation from the KwaZulu-Natal Department of Health, that there was no funding to continue the insecticide spraying. A resulting resurgence of malaria in southern Mozambique would be likely to spill over into northern KwaZulu-Natal.

In the event, and revealed partly as a result of the research project, it appears that many of the reported 'passive cases' may have been incorrectly diagnosed. Active cases are diagnosed by microscopy and blood films obtained by Malaria Control Programme personnel. The field workers in Northern KwaZulu-Natal (Jozini and Umhlabuyalingana Sub-districts) obtain approximately 2000 blood films per month in the high risk Mozambiquan border area, targeting symptomatic persons and contacts of confirmed cases on which malaria microscopy is performed. For the period January to May in 2011, four positive *P. falciparum* cases were identified between the two sub districts, and in 2012, nine cases were diagnosed. In contrast, 'passive cases' are those cases presenting to health facilities and diagnosed by the health facility, usually by rapid diagnostic test (RDT). For the period January to May 2011, 87 passive cases were reported, and for the same period in 2012, 96 passive cases were reported. These statistics show many more 'passive' cases are reported, based on RDT diagnosis, than 'active' cases diagnosed by microscopy. From the large number of false positive rapid test results found during the study, it would appear that RDT- diagnosed passive cases may contain many false positive results, supported by the persistently low number of cases diagnosed by active surveillance. From the Malaria Control Programme statistics it seems unlikely that there were a substantial number of cases missed by the study, and that the incidence of malaria really was low.

False positive rapid diagnostic test results

The finding that 33/49 patients were diagnosed falsely positive by RDT was a major hindrance and disappointment in the conduct of the study. At one stage of the study a particular clinic was enrolling a surprisingly large number of patients, which prompted Malaria Control Personnel to visit the clinic to investigate a possible outbreak. It turned out that one nurse at the clinic was putting far too much blood onto the rapid tests such as to make them uninterpretable. Some of these tests are shown in figure 4, lined up below correctly used tests for comparison. It should be noted that not all false positive RDTs came from the one clinic, which indicates that they were not all due to misuse by one nurse.

The product leaflet of the RDT used claims 100% sensitivity and specificity compared to an in house group of samples [51]. The WHO rates the First Response® RDT as

100% sensitive at 200 parasites per µl, with only 3% false positive results, equivalent to 97% specificity [52]. With low malaria incidence a poor predictive value of a positive RDT is quite possible, even with a specificity as high as 97%. It would never-the-less appear that there is a need for further education and training regarding the use of malaria RDTs in the District.

Figure 5: Rapid Diagnostic Tests from Study

Enrolment and administration

This low malaria incidence resulted in cases occurring in a scattered distribution and patients presenting to several different clinics and hospitals within the district. Neither the Principal Investigator, nor even any of his co-authors were able to personally enrol patients and prepare blood films for review by Malaria Control Laboratory staff, as originally envisaged. To assist with patient enrolment, nurses from several health clinics were recruited to assist the study; however consistency of the enrolment procedure was difficult to achieve, with several different staff involved, each enrolling only a small number of cases.

Most blood samples for malaria film and blood counts were sent by clinic nurses to one local hospital laboratory with whom co-operation was not fully achieved, meaning that blood counts and blood films on many patients were not performed. One reason for the difficulty with the laboratory seems to have been that instead of simply filling in the laboratory request form in the usual way, the nurses were writing headings such as 'Malaria Research' on the forms, which seems to have contributed to the samples being disowned by the laboratory staff. This was a difficulty not anticipated, as the Head of District National Health Laboratory Services had been consulted and given approval, and the tests requested were nothing more than usual for a case of malaria. Also, the onsite Medical Manager was a co-investigator. It had been hoped that it would be possible for one of the investigators to personally make the blood films and bring them back for microscopy by the Malaria Research Programme, however the scarcity and scattering of cases prevented this. In hindsight greater consultation should have taken place with the individual hospital laboratory managers, especially when it became apparent that cases would be scattered and sporadic, and that blood films could only be done on samples sent from clinics or hospitals. This unforeseen difficulty also illustrates that once incidence of malaria incidence decreases to a low level, interest in malaria amongst healthcare workers and public may also decrease. Laboratories may become less likely to have the reagents necessary to prepare malaria films, and instead rely entirely on rapid diagnostic tests. It should be noted that the National Guidelines for the Treatment of Malaria [3], recommend either a rapid diagnostic test or microscopy of blood smear.

A number of other administrative difficulties occurred. Initially clinic nurses were advised to keep completed recruitment forms at the clinic, sending only the blood spots, and submitting all forms together upon the return of the patient for follow up. This system was quickly found to be ineffective, as most patients did not return for follow-up. After the first few patients, nurses were requested to submit the enrolment form with the enrolment blood spots taken for PCR and molecular markers. This lessened the likelihood of the enrolment form being lost, and through the information on place of residence, patients who did not return for follow-up could be tracked down by Malaria Control personnel.

Four children were recruited into the study below the stipulated minimum age of 5 years. The author had decided to set this age limit for a combination of reasons, including possible difficulty in swallowing tablets with younger children, which might affect the efficacy results. Malaria is an unpredictable, potentially lethal disease, even if treated correctly at an early stage, and although the study involved no change to the standard treatment, the author was reluctant to include very young children in the study. However the WHO specifically recommends efficacy trials to include children younger than 5 years [37, 53]. In the event younger children were included due to misunderstanding of the recruiting nurses. Once recruited, and treated, and with the small number of proven cases, there appeared to be no disadvantage to the children involved, not to include their results, and their data was valuable. The follow-up, which would be less likely to occur outside the study, could be to their advantage.

The study could only include the malaria cases available, and it should also be noted that had a sample of even this small size been used to audit therapeutic efficacy of sulfadoxine-pyrimethamine in 1998 or 1999 in KwaZulu-Natal, it would probably have demonstrated the need to change first line medication earlier than in 2000.

Monitoring molecular markers of drug resistance, while a less rigorous method of assessing drug efficacy than *in vivo* sensitivity studies, is much less expensive and time consuming, and is a reasonable method of surveillance for emerging drug resistance [54]. The WHO recommends that in countries with very low transmission, such as South Africa, studies of molecular markers of resistance should be conducted every year. Most of the specimens (10/14) from molecular markers appeared to be sensitive to chloroquine. This suggests that chloroquine resistance may have decreased following removal of selection pressure from chloroquine, as has been demonstrated in neighbouring Mozambique [54] .

Single follow-up visit

The standard WHO protocol for assessment of antimalarial drug efficacy demands at least 6 follow-up visits [30]. Use of one follow-up at 28 days, or longer for drugs with longer half-lives, means that it is not possible to distinguish between the different classifications of failed response to treatment (early treatment failure, late clinical

failure, and late parasitological failure [30]). However none-the-less it would be expected that all the types of treatment failure would show parasites at the final follow-up, so a 28 day follow-up should be an effective tool for screening for parasite resistance, especially in the early stages of development of resistance. The difficulty of achieving multiple follow-up visits without a large drop-out rate seems to be underplayed in the protocol literature, although the large amounts of time, labour and money required have been noted [54]. In this study only 7/49 subjects returned for follow-up. They were mostly recruited from a rural area covered by a District Hospital more than 100km from the District Office where the author and Malaria Control Programme were based. Blood spots had to be first sent to the local hospital, and then usually collected by the author every two weeks, who would post them to Durban for analysis. This meant that with delay in samples being sent to the local hospital, combined with the collection interval from the hospital, it could take a month before it could be definitely ascertained that a patient had not returned. Malaria Control Programme personnel were then asked to trace the subject, which could also take time, as the subject was not always at home. It had not originally been envisaged that it would be practical to conduct any study with only the few scattered cases that finally presented.

In the opinion of the author the arduous protocol for the surveillance of the therapeutic efficacy of antimalarial medicines [30] is one factor in the lack of resistance data available in South Africa, and probably many other malarious areas of the world. There appears to be a need for a much simpler WHO-approved antimalarial resistance screening tool, such as the method used in this study.

Conclusions

This study found no definite evidence, either from follow-up after treatment, or molecular markers, of acquired *P. falciparum* resistance to artemether-lumefantrine in northern KwaZulu-Natal. Molecular markers also suggested that most cases of *P. falciparum* malaria would be sensitive to chloroquine.

Recommendations

South Africa is committed to the goal of malaria elimination [55]. With very low malaria incidence, as is presently the situation in northern KwaZulu-Natal, with an average of only one microscopically confirmed active surveillance case per month, according to KZN Malaria Control Programme statistics, drug resistance marker studies should be undertaken annually, and all cases of *P. falciparum* malaria should be followed up at 28 days, as recommended by the WHO [30]. Another option is to pool data from several sites to obtain a sample large enough to be statistically significant, although this method could be at the expense of being able to determine resistance patterns in specific localities. Use of PCR to confirm diagnosis and for follow-up monitoring provides an effective alternative to microscopy which may be easier to use in a rural setting, but requires adequate technical expertise and resources.

Further research

As well as continuing ongoing surveillance of antimalarial drug efficacy, further research appears to be needed into the reliability of ‘passive’ malaria cases reported by health institutions in northern KwaZulu-Natal, mainly on the basis of rapid tests. The study found a high false-positive proportion of cases reported by clinics, at least partly due to incorrect use of rapid tests. The difference in magnitude of ‘passive’ cases diagnosed by rapid tests and ‘active’ cases diagnosed by microscopy since at least 2010 in northern KwaZulu-Natal, suggests persistent over-diagnosis by health institutions for some time, and a possible chronic over-estimate of malaria incidence.

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ADDENDA

Research Project Protocol

Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal

STUDENT

Name: Dr Charles Hervey Vaughan-Williams

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Tel.: 035 5721327 e-mail: herveyvw@gmail.com Cell: 072 584 3472

Student's signature:

SUPERVISOR:

Dr Stephen Knight

Department of Public Health Medicine, University of KwaZulu-Natal

Tel.: 031 260 4226 e-mail: knights@ukzn.ac.za Cell: 0837623123

Signature:

CO-INVESTIGATORS

1. Dr Jaishree Raman

   Malaria Research Unit,

   South African Medical Research Council,

   Durban 4067

   Role: Molecular Biologist responsible for genetic analysis of blood samples using malaria polymerase chain reaction (PCR)

   Signature……

2. Mr Eric Raswiswi

   Co-ordinator

   Malaria Control Programme

   Umkhanyakude Health District Office

   Role: Supervise blood film microscopy, and assist with specimen and data collection

   Signature……

3. Dr Etienne Immelman

   Medical Manager

   Manguzi Hospital

   KwaNgwanase

   Role: Recruitment and care of subjects and data collection

   Signature…….

4. Dr Holger Reichel

   Medical Officer

   Mosvold Hospital

   Ingwavuma

   Role: Recruitment and care of subjects and data collection

   Signature……

5. Dr Kelly Gate

   Medical Manager

   Bethesda Hospital

   Ubombo

   Role: Recruitment and care of subjects and data collection

   Signature……

Purpose of Protocol:

Protocol for research dissertation contributing towards Master of Public Health degree with the University of KwaZulu-Natal

Summary

Background

The recent history of malaria epidemics in northern KwaZulu-Natal shows that resistance to the standard malaria treatment may arise quickly and contribute to epidemics. Artemether-lumefantrine has now been used for 10 years as the first line treatment for uncomplicated *P. falciparum* malaria, but has not been assessed for therapeutic efficacy for 9 years.

Purpose

The purpose of the study is to assess the therapeutic efficacy of artemether-lumefantrine for patients with uncomplicated malaria in Umkhanyakude Health District in 2011-12, and test for molecular markers of antimalarial drug resistance.

Objectives

The main objective is to quantify the failure proportion after 28 days of a sample of patients treated for uncomplicated malaria with artemether-lumefantrine. Molecular analysis of blood samples will search for molecular markers of resistance to artemether-lumefantrine and chloroquine.

Study design

The study will be an observational cohort.

Settings

The study will take place in 3 hospitals, and 28 attached clinics in Umkhanyakude Health District.

Study population

The study population will be patients with uncomplicated *P. falciparum* malaria presenting to 3 hospitals and their 28 attached clinics in Umkhanyakude Health District.

Study sample

A sample of between 50 and 100 patients aged between 5 and 69 years with uncomplicated *P.*

falciparum malaria will be recruited between September 2011 and May 2012.

Data collection

Patients will be tested for malaria using blood films and blood spots for molecular analysis at diagnosis and after 28 days. Molecular analysis will also be conducted for markers of chloroquine and artemether-lumefantrine resistance.

Statistical methods

The failure proportion will be calculated, with 95% confidence intervals. In the event of a substantial number of failures, association will be sought between failure and parasitaemia count, age and gender. The proportion of patients with markers for malarial drug resistance will be calculated with 95% confidence intervals.

Introduction /background

Artemether-lumefantrine (AL) has been used as the first line treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal since 2001 [1, 2].

The recent history of malaria epidemics in KwaZulu-Natal indicates that the therapeutic effectiveness of the anti-malarial medication used to treat patients suffering malaria, together with the effectiveness at killing mosquitoes of the insecticide used for residual house-spraying, are vital for malaria control and must be regularly assessed. Failure to do so risks a recurrence of malaria epidemics, which has major public health and economic consequences for the area and province as a whole.

A study of the effectiveness of AL should either confirm its' continuing efficacy or provide a warning as to the acquisition of resistance by malaria parasites and the need to seek an alternative therapy before an epidemic occurs.

Literature Review

In 1988, due to the emergence and spread of chloroquine – resistant *P. falciparum* malaria, sulfadoxine-pyrimethamine (SP) replaced chloroquine as the first line treatment for uncomplicated *P. falciparum* malaria in KwaZulu-Natal [1, 3]. This drug remained effective until 1996 when malaria incidence began to increase. Between 1996 and 2000 northern KwaZulu-Natal suffered increasingly severe malaria epidemics with more than 60,000 cases reported in 2000 [1, 2]. Only in 2000 did a clinical trial, in conjunction with genetic studies, show that *P. falciparum* parasites in the region had developed resistance to sulfadoxine-pyrimethamine, the then recommended first line treatment for malaria, rendering it largely ineffective in northern KwaZulu-Natal [3]. Subsequently the change of first-line medication to artemether-lumefantrine, together with change of insecticide used for residual house spraying to DDT, dramatically reduced malaria incidence in northern KwaZulu-Natal [1, 2]. It has been estimated that the delay in changing first-line treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to the size of the epidemic [4]. Artemether combined with lumefantrine is one of the artemesinin-based combination therapies (ACT) recommended for the treatment of uncomplicated *P. falciparum* malaria by the World Health Organization (WHO) [5]. Studies of the therapeutic efficacy of artemether-lumefantrine in 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in northern KwaZulu-Natal [1, 6, 7]. Since 2002, however, there have

been no further studies in KwaZulu-Natal, or South Africa, of the continuing therapeutic effectiveness of AL in the treatment of uncomplicated malaria. The WHO recommends routinely monitoring antimalarial resistance [8], and a change in antimalarial medicine if the treatment failure proportion ≥ 10% [5].

Pharmacology

Artemether-lumefantrine is a combination of artemether and lumefantrine manufactured as Coartem® by Novartis: 20mg artemether and 120mg lumefantrine in tablet form [9]. The two drugs act synergistically. Artemether and its active metabolite, dihydroartemesin, rapidly kill most malaria parasites, while lumefantrine mops up the remainder more slowly [10]. Artemether is rapidly absorbed and metabolized, with a half-life of about two hours, whereas lumefantrine is absorbed more slowly and has a half-life of 3-4 days in malaria patients [10].

Coartem® is taken as a 6 dose oral regimen over 3 days. The dosage depends upon the weight and age of the patient. The adult dosage for persons aged 12 years or more, or children weighing 35kg and above is four tablets as a single dose at the time of initial diagnosis, 4 tablets after 8 hours, and then 4 tablets twice daily on each of the following two days [9]. It is recommended that the tablets are taken with fatty food or milk to improve absorption [9].

Choice of follow-up period

A WHO protocol for the assessment of therapeutic efficacy of antimalarial drugs recommends assessment of patients on days 0, 1, 2, 3, 7 and 14, and defines three categories of therapeutic response:

- Adequate clinical response
- Early treatment failure – occurring up to day 3
- Late treatment failure, defined as: 'Development of danger signs or severe malaria in the presence of parasitaemia on any day from day 4 to 14 without previously meeting any of the criteria for early treatment failure' [11].

A more recent WHO report recommends a change in the definition of late treatment failure in areas of low transmission to:

> Presence of parasitaemia on any day from day 4 to day 28 and a measured axillary temperature of ≥ 37.5°C, without previously meeting any of the criteria of early treatment failure [12].

Professor NJ White points out that for rapidly eliminated drugs a 28 day follow-up is needed, and longer for more slowly eliminated drugs [13]. The 2010 WHO Malaria Treatment Guidelines recommend at least 28 days follow-up to assess parasitological cure, and 42 days for regimes containing mefloquine and piperaquine [5].

As yet there has been no anecdotal evidence of resistance to AL in KwaZulu-Natal. Follow-up malaria films of patients with malaria treated with AL performed by the Malaria Control Program in 2011 in KwaZulu-Natal have been predominantly negative. The primary objective of the study is to seek evidence of late clinical failure, which would be the first sign of resistance to AL. Requiring patients to return for assessment several times requires considerable resources, and the risk of drop-outs is high.

Considering:

- The 2002 WHO report on monitoring antimalarial drug resistance [12],
- KwaZulu-Natal is presently an area of low malaria transmission;
- The analysis of Professor White [13],
- Lumefantrine, the more slowly eliminated drug in AL has a half-life of a few days, and not weeks like mefloquine [10], and
- The fact that requiring a patient to return once four weeks later is a fairly simple request,

a single follow-up assessment at 28 days is chosen.

Purpose of the study

- The purpose of the study is to assess the therapeutic efficacy of artemether-lumefantrine (AL) for the treatment of uncomplicated *P. falciparum* malaria in Umkhanyakude District, northern KwaZulu-Natal in 2011, and to search for molecular markers of drug resistance.

Specific objectives

The specific objectives are:

- To quantify the proportion of patients treated with artemether-lumefantrine, who fail to clear *P. falciparum* from their blood 28 days after treatment
- Test for molecular markers of malarial drug resistance to AL and chloroquine

- Publish the results
- Use the results to inform policy as to the best choice of drug for the treatment for uncomplicated malaria in Umkhanyakude District.

Type of research

The research project will be a clinical epidemiological study

Definitions

Early Treatment Failure (ETF) [11]

Development of danger signs or severe malaria on Day 1, Day 2, or Day 3 in the presence of parasitaemia;

Axillary temperature ≥ 37.5°C on Day 2 with parasitaemia > Day 0 counts;

Axillary temperature ≥ 37.5°C on Day 3 in the presence of parasitaemia, and

Parasitaemia on day 3 ≥ 25% of count on Day 0.

Late Treatment Failure (LTF) [12]

Presence of parasitaemia on any day from day 4 to day 28 and a measured axillary temperature of ≥ 37.5°C, without previously meeting any of the criteria of early treatment failure

Late Parasitological Failure [12]

Presence of parasitaemia on any day from Day 7 to Day 28 and axillary temperature <37.5°C without previously meeting any of the criteria of early treatment failure or late clinical failure

Danger Signs include

- Not able to drink or breastfeed;
- Vomiting everything;
- Recent history of convulsion;
- Lethargic or unconscious state; and

- Unable to sit or stand up [11].

Uncomplicated malaria:

Symptomatic infection with malaria parasitaemia without signs of severity and/or evidence of vital organ dysfunction [5].

Research Methods

Study setting

The study will be set in 3 hospitals and 28 attached clinics in Umkhanyakude District.

Study design

An observational descriptive cohort study design will be used.

Target population

The target population will be persons diagnosed as suffering from uncomplicated *P. falciparum* malaria treated with artemether-lumefantrine in KwaZulu-Natal. As malaria drug resistance can vary considerably between countries and even provinces, the results will only be generalisable to KwaZulu-Natal.

Study population

The study population will include persons, aged between 5 and 69 years, diagnosed with uncomplicated *P. falciparum* malaria in Umkhanyakude Health District, presenting to 3 hospitals or their 28 attached clinics in northern KwaZulu-Natal.

Inclusion / Exclusion criteria

Inclusion criteria:

Patients diagnosed with malaria rapid diagnostic test (RDT) as suffering *P. falciparum* malaria, with diagnosis subsequently confirmed by blood film.

Exclusions

The following categories of patients will not be included in the study:

- Patients with severe or complicated malaria;
- Pregnant women;
- Patients aged less than 5 years and more than 69 years; and
- Patients treated for malaria during the previous two weeks.

Study Sample

Method of selecting sample

As far as possible, all patients, aged 5 years to 69 years, diagnosed with uncomplicated malaria in Umkhanyakude District between September 2011 and May 2012, will be invited to participate in the study. Recruitment will cease if 100 patients are recruited. Patients will be recruited who present to 3 hospitals or the 28 attached clinics. Patients will be attended by either a doctor or a primary healthcare trained nurse.

Size of sample

An attempt will be made to recruit 100 patients; however the feasibility of recruiting this number will depend upon the incidence of malaria in Umkhanyakude District. Taking

A 10% failure rate or higher as unacceptable;

Setting the probability of α, a type I error, at 0.05;

Setting the probability of β, a type II error at 0.2;

A minimum of 49 patients will be needed.

Data sources

Measurement instruments / data collection techniques

Patient data will be collected according to a proforma at recruitment.

Blood will be taken at recruitment and follow-up for

- malaria thick and
- thin films, and

- molecular analysis.

Genotyping of *P. falciparum* DNA extracted from dried blood spots based on variations in the genes coding for the glutamine-rich protein and meroziote surface protein 1 and 2, will be used to determine if treatment failure is due to a re-infection or recrudescence of the original infection [14, 15]. Infections will be classified as recrudescent if PCR products for all three markers from Day 0 and Day of failure parasites are identical. If the banding patterns for any marker differ between Day 0 and Day of failure parasites, then the infection will be classed as a re-infection.

This study will assess the prevalence of molecular markers linked with resistance in the partner drug lumefantrine, specifically the *mdr1* copy number [16], and mutations at *mdr1* codon 86 [17], since no verified marker for artemisinin resistance exists.

Measures to ensure validity

Internal

All patients will have a blood film made which will be analysed by an experienced micrcoscopist, and confirmed by another microscopist. Blood spots will also be taken and sent for malaria polymerase chain reaction (PCR).

A patient will be considered to be infected with *P. falciparum* malaria if:

- The blood film is definitely positive for *P. falciparum*, or
- The PCR test is positive.

A borderline blood film with negative PCR will be considered negative.

In the unexpected event of the blood film being clearly positive, but the PCR being negative, the result will be investigated.

Reduction of bias

Selection bias

Attrition bias

Effort will be made to reduce the number of follow-up drop outs. Patients will be offered R50 to assist with travelling expenses when they return for follow-up.

Selection bias

All eligible persons diagnosed with malaria will be invited to participate in the study

Information bias

Reporting bias:

There will be no discussion of results between the microscopists reporting on blood films and the technician performing the PCR test.

Detection bias:

Molecular analysis, combined with microscopy, should minimise the number of false negative results.

External Validity / Generalisability

Pilot study

There will be no pilot study, due to the low number of patients with malaria in the district.

List of Variables

- Age
- Gender
- Weight
- Duration of illness
- Other medications
- Travel history within past 60 days
- HIV status (if already known)
- Home address
- Artemether-lumefantrine dosage given
- The following at both enrolment and 28-day follow-up:
    - Temperature

- Pulse rate
- Blood pressure
- *P. falciparum* malaria rapid diagnostic test result
- Thick blood film for malaria diagnosis
- Thin blood film for malaria diagnosis
- Polymerase chain reaction analysis for *P falciparum*
- Reinfection/recrudescence assessment using nested PCR
- Assessment of mdr1 copy number (as an indicator of AL resistance)
- Assessment of Chloroquine sensitivity

Plan for Data collection

- A data collection sheet will be completed at enrolment and samples taken for
- 28-day follow-up data collection sheet
- Data to be collated in excel spreadsheet

Plan for Data handling/processing

Data to be collated in a Microsoft excel spreadsheet

Statistical methods

Descriptive statistics

The proportion of patients with confirmed uncomplicated *P. falciparum* malaria, treated with artemether-lumefantrine, and cleared of parasites by 28 days will be calculated.

The proportion of patients demonstrating molecular markers of resistance to antimalarial drugs will also be calculated.

Analytic statistics

The 95% confidence interval for failure ratio will be calculated and compared the proposed maximum 10% acceptable failures.

The 95% confidence interval for the proportion of patients testing positive for markers of

resistance to antimalarial drugs will also be calculated.

List of possible confounders

The study is not an analytical study with a control group

List of associations to be measured

In the event of sufficient failures to compare with other variables, failure to clear parasites would be compared to parasitaemia count, age, and gender.

Ethical considerations

Institutional Ethical Review Board

The study will be submitted to the University of KwaZulu-Natal Biomedical Research Ethics Committee for approval.

Permissions

Permission will be obtained from the District Manager, Umkhanyakude Health District Office, Jozini, and approval obtained from the KwaZulu-Natal Department of Health Provincial Health Research Committee.

Informed consent and participant information

All patients aged 18 years or over will be asked to sign a consent form written in English and Zulu. For children aged less than 18 years consent will be obtained from a parent or legal guardian.

Work plan

Budget

Travelling expenses for subjects	R50
Molecular cost per sample with test run in duplicate:	
Smear/RDT confirmation using qPCR:	R35

Reinfection/recrudescence assessment using nested PCR	R30
Assessment of mdr1 copy number	R40
Assessment of Chloroquine sensitivity	R35
Sample transport costs to Medical Research Council laboratory:	R60
Total cost per subject:	R250
Total budget if 100 patients recruited:	R25,000

Study period / Time lines

It is hoped that recruitment of patients may begin in September 2011. The study will continue until 100 patients are recruited or until May 2012, whichever is sooner.

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Acronyms & abbreviations

- AL: artemether-lumefantrine
- ACT: artemesinin-based combination therapy
- ETF: early treatment failure
- LFT: late treatment failure
- PCR: polymerase chain reaction
- RDT: rapid diagnostic test

Addenda

Enrolment Data collection sheet

Today's date	
Patient information sheet given?	Yes / No
Patient consent form signed?	Yes / No
Method of positive malaria diagnosis	Rapid Test (RDT) / Blood film
Active surveillance case?	Yes / No
Hospital or Clinic name	
Hospital/Clinic card number:	
Name	
Date of birth	
Age (Must be ≥ 5 yrs and < 70 yrs)	
Gender	Male / Female
Date became ill	
Other medication	
Travel 60 days prior to illness	
HIV status, if known	
Symptoms of complicated malaria: If any answer: 'Yes', do not enroll in study, but refer to hospital.	
Unable to Drink	Yes / No
Vomiting everything	Yes / No
Convulsion during past 7 days	Yes / No
Lethargic or decreased consciousness	Yes / No
Unable to stand or sit	Yes / No
Pregnant	Yes / No
If presenting to Clinic or Hospital:	
Temperature	
Pulse rate	
Blood Pressure	
Weight (kg)	
Dose Coartem (tablets bd)	

Address	
Area	
Chief / Induna	
Homestead Head	
Nearest adjacent homestead	
River	
School	
Store	
Magisterial District	
Country of origin	
Cellphone number/telephone number	
Study samples taken:	SA / Moz / Swaz / Other
Thick film	Yes / No
Thin film	Yes / No
Blood spot for molecular analysis	Yes / No
Return date (28 days)	Yes / No
Compiled by	

28 Day follow-up data collection sheet

28 Day follow-up data collection sheet	
Today's date	
Malaria Rapid Test Result today	Pos / Neg
Hospital or Clinic name	
Hospital/Clinic card number:	
Name	
Date of birth	
Age	
Gender	Male / Female
Date enrolled in study	
Symptoms of complicated malaria: If any answer 'Yes', please refer to hospital.	
Unable to Drink	Yes / No
Vomiting everything	Yes / No
Convulsion during past 7 days	Yes / No
Lethargic or decreased consciousness	Yes / No
Unable to stand or sit	Yes / No
Pregnant	Yes / No
Observations	
Temperature	
Pulse rate	
Blood Pressure	
Weight (kg)	
Dose Coartem (tablets bd)	
Were all tablets taken as instructed?	Yes / No
If not, which tablets were taken?	
Study samples taken:	
Thick film	Yes / No
Thin film	Yes / No
Sample for Malaria PCR	Yes / No
R50 given for travelling expenses, and signed for?	Yes / No
Payment of R50 received for travelling expenses	Patient signature
Compiled by	

Patient information sheet (Adults - English)

Dear Sir/Madam,

I am Dr CHV Williams, and work at

Umkhanyakude Health District Office
Jozini
3969

Telephone: 035 5721357
Cellphone: 072 584 3472
Email: hervey.williams@kznhealth.gov.za

A blood test shows that you have malaria which can be a serious illness.

Since the year 2001 coartem™ has been used to treat uncomplicated (less severe) malaria in KwaZulu-Natal. It seems to work well, but we want to check that it is still curing malaria. We wish to follow-up 100 patients with uncomplicated malaria in Umkhanyakude District.

We are inviting you help us by letting us take an extra drop of your blood today, to study the malaria, and to come back for a check-up after 4 weeks, when we will ask for another drop of blood to confirm the malaria has disappeared.

Your blood will only be used to test for malaria. In this study your treatment will be the same as for anyone else with malaria. We will just observing you more closely than usual.

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (approved number:…….)

In the event of any problems or concerns you may contact Dr CHV Williams at the above address, or the
Biomedical Research Ethics Committee at

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus Govan Mbeki Building
Private Bag X 54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

You are not obliged to participate in this study, and are free to change your mind and withdraw at any time. You will still receive the usual treatment for malaria.
If you return after 4 weeks, we will offer you R50 for traveling expenses.

It is intended to publish the results of the study, however no patient identities will be revealed. No personal information obtained in the study will be shared with anyone not involved in the research.
An anonymous dried frozen spot of blood will be kept at the Malaria Research Programme laboratory of the Medical Research Council in Durban for future malaria research.

Patient information sheet adults - Zulu translation

Iphepha lesaziso seziguli ngokuhlolwa kwezinsuku eziwu-28 emva kokuthatha amaphilisi i-coartemTM eyelapha umalaleveva

Mnumzane/Nkosikazi
Igama lami ngingu Dr CHV Williams, ngisebenza e-Mkhanyakude emnyangweni wezempilo. Imininingwane yekheli ikanje:

Umkhanyakude Health District Office
Jozini 3969
Izinombolo zocingo: 035 572 1357; 072 584 3472
I-Email: hervey.williams@kznhealth.gov.za

Ukuhlolwa kwegazi lakho kukhombise ukuthi unomalaleveva okungaba isifo esinobungozi kakhulu.

Kusukela ngonyaka ka-2001 i-CoaratemTM yasetshenziswa ukwelapha umaleveva KwaZulu Natal. Ibonakale isebenza kahle kakhulu, kodwa sisafuna ukuhlola ukhuthi isaqhubeka nokusebenza kahle ekwelapheni kwalesisifo.

Ebesikucela kuwena ukuba usinikeze imvumo yokuthi sithathe elinye futhi iconsi legazi lakho namuhla ukuze sizokwazi ukuqhuba lolucwaningo lukamalaleveva egazini lakho. Uyacelwa futhi ukuba uphinde ubuye emva kwamasonto amane ukuzoqhubeka nokuhlolelwa lesisifo, lapho-ke sizophinde sikucele futhi ukuba usinikeze enye imvumo yokuthatha elinye iconsi legazi ukuze siqinisekise ukuthi welapheke ngempela kumalaleveva.

Igazi lakho lizosetshenziswa ukhuhlolelwa umalaleveva nje kuphela, hhayi okunye. Kulokhukuhlolwa amaphilisi owatholayo azoqhubeka afane nabobonke abanye abaphethwe umalaleveva umehluko ukuthi nje wena sizobe sikubheke eduze kunabanye ngokulokhu sikucela ukuba ubuye njalo emtholampilo uma kudingekile.

Lolu cwaningo luvunyelwe labhekwa ngokwesisekelo sezinkolelo yiUKZN Biomedical research ethics committee (inombolo esemtethweni…….)

Uma uneminye imibuzo/izinkinga ungangaxhumana noDr CHV Williams kulekheli elilandelayo:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research office , Westville campus Govan Mbeki Building
Private Bag X54001, Durban 4000
KwaZulu-Natal, South Africa
Tel: 27 31 2604769-Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

Sifisa ukukwazisa ukuthi awuphoqwanga ukuba yingxenye yalolucwaningo lokuhlolelwa umalaleveva nokuthi ulokhu ubuya njalo emva kwamasonto amane ukuzohlolwa emtholampilo . Uma ungathandi ukuba yingxenye yalolucwaningo uvumelekile ukwenqaba. Noma wenqabile uzoqhubeka ukunikwa amaphilisi okwelapha umalaleveva njengokwejwayelekile. Uma kumele ubuye emva kwamasonto amane uzonikezwa imali yokugibela engango R50.

Imiphumela yalolu cwaningo izoshicilelwa, kodwa ayikho imininigwane yomuntu

ezovezwa. Imininigwane yakho etholakale kulolucwaningo iyimfihlo, angeke ivezelwe abantu abangangekho emphathini walolucwaningo.
I consi legazi eliqandisiwe lanomawubani lizo bekwa eMalaria Research Programme laboratory ye Medical research council eThekwini lapho izosetshenziselwa eminye imicwaningo esikhathini esiphambilini.

- I-coartem okungamaphilisi kamalaleveva kufanele uthathe amaphilisi amane, kabili ngosuku izinsuku ezintathu. Kumele uyithathe nobisi noma emvakokudla uze uyiqede. Uma ungayithathanga njengoba uyaliwe maningi amathuba okuthi uphinde uphathwe umalaleveva futhi.

Uma uzizwa ungabingcono, noma ukugula kuqhubekela phambili emva kwezinsuku ezimbili, kumele ubuye masinyane emtholampilo noma esibhedlela.

Abakhulelwe, izingane ezingaphansi kweminyaka emihlanu (5) nabadala abangaphezu kweminyaka engamashumi ayisikhombisa (70) abavumelekile ukuba yingxenye yalolucwaningo.

Uyacelwa ukuba ubuye ngomhlaka……

Patient information sheet (Children - English)

Dear Sir/Madam,

I am Dr CHV Williams, and work at

Umkhanyakude Health District Office
Jozini
3969

Telephone: 035 5721357
Cellphone: 072 584 3472
Email: hervey.williams@kznhealth.gov.za

A blood test shows that your child has malaria which can be a serious illness.

Since the year 2001 coartem™ has been used to treat uncomplicated (less severe) malaria in KwaZulu-Natal. It seems to work well, but we want to check that it is still curing malaria. We wish to follow-up 100 patients with uncomplicated malaria in Umkhanyakude District.

We are inviting you to help us by letting us take an extra drop of your child's blood today, to study the malaria, and to come back for a check-up after 4 weeks, when we will ask for another drop of blood to confirm the malaria has disappeared.

Your child's blood will only be used to test for malaria. In this study your child's treatment will be the same as for anyone else with malaria. We will just observing your child more closely than usual.

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (approved number:……..)

In the event of any problems or concerns you may contact Dr CHV Williams at the above address, or the
Biomedical Research Ethics Committee at

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville CampusGovan Mbeki Building
Private Bag X 54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

You are not obliged to have your child participate in this study, and are free to change your mind and withdraw at any time. Your child will still receive the usual treatment for malaria.
If you return after 4 weeks, we will offer you R50 for traveling expenses.

It is intended to publish the results of the study, however no patient identities will be revealed. No personal information obtained in the study will be shared with anyone not involved in the research.

An anonymous dried frozen spot of blood will be kept at the Malaria Research Programme laboratory of the Medical Research Council in Durban for future malaria research.
Your child should take coartem™ …..tablets, 2 times per day for 3 days, with milk or a meal, until the pills are finished. Otherwise the malaria is more likely to come back.

If you feel that your child is not getting better within two days, or are getting worse, you must come back to the clinic or hospital at once.

We do not want to include in this study: pregnant women, children who are less than 5 years, or people who are more than 70 years.

Please Return on……(Date)

Patient information sheet - children - Zulu translation

Iphepha lesaziso seziguli ngokuhlolwa kwezinsuku eziwu-28 emva kokuthatha amaphilisi i-coartemTM eyelapha umalaleveva

Mnumzane/Nkosikazi

Igama lami ngingu Dr CHV Williams, ngisebenza e-Mkhanyakude emnyangweni wezempilo. Imininingwane yekheli ikanje:

Umkhanyakude Health District Office
Jozini 3969
Izinombolo zocingo: 035 572 1357; 072 584 3472
I-Email: hervey.williams@kznhealth.gov.za

Ukuhlolwa kwegazi lengane yakho kukhombise ukuthi inomalaleveva okungaba isifo esinobungozi kakhulu.

Kusukela ngonyaka ka-2001 i-CoaratemTM yasetshenziswa ukwelapha umaleveva KwaZulu-Natal. Ibonakale isebenza kahle kakhulu, kodwa sisafuna ukuhlola ukhuthi isaqhubeka nokusebenza kahle ekwelapheni kwalesisifo.

Ebesikucela kuwena ukuba usinikeze imvumo yokuthi sithathe elinye futhi iconsi legazi lengane yakho namuhla ukuze sizokwazi ukuqhuba lolucwaningo lukamalaleveva egazini lakho. Uyacelwa futhi ukuba uphinde ubuye emva kwamasonto amane ukuzoqhubeka nokuhlolelwa lesisifo, lapho-ke sizophinde sikucele futhi ukuba usinikeze enye imvumo yokuthatha elinye iconsi legazi lengane ukuze siqinisekise ukuthi welapheke ngempela kumalaleveva.

Igazi lengane yakho lizosetshenziswa ukhuhlolelwa umalaleveva nje kuphela, hhayi okunye. Kulokhukuhlolwa amaphilisi owatholayo azoqhubeka afane nabobonke abanye abaphethwe umalaleveva umehluko ukuthi nje sizobe simbheke umntwana eduze kunabanye ngokulokhu sikucela ukuba ubuye njalo emtholampilo uma kudingekile.

Lolu cwaningo luvnyelwe labhekwa ngokwesisekelo sezinkolelo yiUKZN Biomedical research ethics committee (inombolo esemtethweni…….)

Uma uneminye imibuzo/izinkinga ungangaxhumana noDr CHV Williams kulekheli elilandelayo:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research office , Westville campus Govan Mbeki Building
Private Bag X54001, Durban 4000
KwaZulu-Natal, South Africa
Tel: 27 31 2604769-Fax:27 31 2604609; Email: BREC@ukzn.ac.za

Sifisa ukukwazisa ukuthi awuphoqwanga ukuba yingxenye yalolucwaningo lokuhlolelwa umalaleveva nokuthi ulokhu ubuya njalo emva kwamasonto amane ukuzohlolwa emtholampilo . Uma ungathandi ukuba yingxenye yalolucwaningo uvumelekile ukwenqaba. Noma wenqabile uzoqhubeka ukunikwa amaphilisi okwelapha umalaleveva njengokwejwayelekile.

Uma kumele ubuye emva kwamasonto amane uzonikezwa imali yokugibela engango R50.

Imiphumela yalolu cwaningo izoshicilelwa, kodwa ayikho imininigwane yomuntu ezovezwa. Imininigwane yakho etholakale kulolucwaningo iyimfihlo, angeke ivezelwe abantu abangangekho emphathini walolucwaningo.

I consi legazi eliqandisiwe lanomawubani lizo bekwa eMalaria Research Programme laboratory ye Medical research council eThekwini lapho izosetshenziselwa eminye imicwaningo esikhathini esiphambilini.

- Ingane yakho kumele ithathe amaphilisi awu___ eCoartemTM, kabili ngosuku izinsuku ezintathu. Lamaphilisi kumele iwathathe nobisi noma emvakokudla aze aphele. Uma ingawathathanga njengoba uyaliwe maningi amathuba okuthi iphinde iphathwe umalaleveva.

Uma uzizwa ungabingcono, noma ukugula kuqhubekela phambili emva kwezinsuku ezimbili, kumele ubuye masinyane emtholampilo noma esibhedlela.

Abakhulelwe, izingane ezingaphansi kweminyaka emihlanu (5) nabadala abangaphezu kweminyaka engamashumi ayisikhombisa (70) abavumelekile ukuba yingxenye yalolucwaningo.

Uyacelwa ukuba ubuye ngomhlaka……

Consent Form (English-Adults)

Consent form for 28 day follow-up after treatment with coartem™ to treat *P. falciparum* malaria

I agree to return on……..for a blood test see if the malaria has gone away.

I,……., have been informed about the study entitled:
'**Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *plasmodium falciparum* malaria in northern KwaZulu-Natal'**
by (…….).

- I understand the purpose and procedures of the study.
- I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.
- I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher,

Dr CHV Williams,
Umkhanyakude Health District Office, Jozini, 3969
Telephone: 035 5721357; Cellphone: 072 584 3472,
Email: hervey.williams@kznhealth.gov.za

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville CampusGovan Mbeki Building
Private Bag X 54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

_____ _____
Signature of Participant **Date**

_____ _____
Signature of Witness (Where applicable) **Date**

_____ _____
Signature of Translator (Where applicable) **Date**

Consent form adults – Zulu translation

Imvume yokuhlolwa emva kwezinsuku eziwu-28 uthathe amaphilisi i-coartem™ eyelapha umalaleveva

Mina (igama nesibongo) _____Ngiyavuma ukubuya ngomhlaka (usuku)_____ngizohlolwa igazi, ukuze kubhekwe ukuthi welapheke ngempela umalaleveva.

Ungazisile u –(igama lomuntu okwazise ngalolucwaningo) _____ ngalolucwaningo lukamalaleveva lokubheka ukusebenza kwamaphilisi i-coartem asentshenziswa ekwelapheni lesisifo lapha KwaZulu Natal

- Ngiyasizwa isizathu nendlela ezosentshenziswa kulolucwaningo.
- Nginikiwe ithuba lokubuza imibuzo ngalolucwaningo futhi izimpendulo engizinikeziwe zingenelisile.
- Ngiyaqinisekisa ukuthi angiphoqwanga ukuba yingxenye yalolucwaningo nokuthi ngazisiwe ukuthi ngingaphuma noma yinini uma ngingasathandi ukuba yingxenye ngaphandle kokuphazamiseka kokwelashelwa kwami lesisifo.
- Ngiyazi futhi ukuthi uma ngineminye imibuzo/izinkinga namganoma yini engingayiqondi kahle mayelana nalolucwaningo ngingaxhumana nomhloli omkhulu walolucwaningo okungu -Dr CHV Williams, kulelikheli elilandelayo:

Umkhanyakude Health District office, Jozini, 3969
Izinombolo zocingo: 0355721357; 0725843472, I-Email: hervey.williams@kznhealth.gov.za

Ngiyazi futhi ukuthi uma ngineminye imibuzo ngamalungelo ami okuba yingxenye yalolucwaningo, nanoma ngabe yini engingayiqondi kahle ngalolucwaningo nangomcwaningi ngingaxhumana namahhovisi ezocwaningo kulelikheli elilandelayo:
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION,
Research Office, Westville Campus Govan Mbeki Building
Private Bag X54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Inombolo yocingo: 27312604769- Fax: 27312604609; Email: BREC@ukzn.ac.za

_____ _____
Isiginesha yami usuku

_____ _____
Isiginesha kafakazi (lapho edingekile) usuku

_____ _____
Isiginesha yomhumushi (lapho edingekile) usuku

Consent Form (English-Children)

Consent form for 28 day follow-up after treatment with coartem™ to treat *P. falciparum* malaria

I ……agree to return with my child…….
on……..for a blood test see if the malaria has gone away.

I,…….. have been informed about the study entitled:
'**Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *plasmodium falciparum* malaria in northern KwaZulu-Natal'**
by (……..).

- I understand the purpose and procedures of the study.
- I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.
- I declare that the participation of my child in this study is entirely voluntary and that I may withdraw my child at any time without affecting any treatment or care that my child would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher,

Dr CHV Williams,
Umkhanyakude Health District Office, Jozini, 3969
Telephone: 035 5721357; Cell phone: 072 584 3472,
Email: hervey.williams@kznhealth.gov.za

If I have any questions or concerns about the rights of my child as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus Govan Mbeki Building
Private Bag X 54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

_____ _____
Signature of Parent or Guardian **Date**

_____ _____
Signature of Witness **Date**
(Where applicable)

_____ _____
Signature of Translator **Date**
(Where applicable)

Consent form children – Zulu translation

Imvume yokuhlolwa emva kwezinsuku eziwu-28 uthathe amaphilisi i-coartem™ eyelapha umalaleveva

Mina _____(igama nesibongo) ngiyavuma ukuletha ingane yami ongu (igama nesibongo somntwana)_____ ngomhlaka (usuku)_____ ukuze ahlolwe igazi kubhekwe ukuthi welapheke ngempela kumalaleveva.

Mina ngazisiwe u–(igama lomuntu okwazise ngalolucwaningo) _____ ngalolucwaningo lukamalaleveva lokubheka ukusebenza kwamaphilisi i-coartem asentshenziswa ekwelapheni lesisifo lapha KwaZulu Natal

- Ngiyasizwa isizathu nendlela ezosentshenziswa kulolucwaningo.
- Nginikiwe ithuba lokubuza imibuzo ngalolucwaningo futhi izimpendulo engizinikeziwe zingenelisile.
- Ngiyaqinisekisa ukuthi angiphoqwanga ukuba yingxenye yalolucwaningo nokuthi ngazisiwe ukuthi ngingaphuma noma yinini uma ngingasathandi ukuba yingxenye ngaphandle kokuphazamiseka kokwelashelwa kwami lesisifo.
- Ngiyazi futhi ukuthi uma ngineminye imibuzo/izinkinga namganoma yini engingayiqondi kahle mayelana nalolucwaningo ngingaxhumana nomhloli omkhulu walolucwaningo okungu -Dr CHV Williams, kulelikheli elilandelayo:

Umkhanyakude Health District office, Jozini, 3969
Izinombolo zocingo: 0355721357; 0725843472, I-Email: hervey.williams@kznhealth.gov.za

Ngiyazi futhi ukuthi uma ngineminye imibuzo ngamalungelo ami okuba yingxenye yalolucwaningo, nanoma ngabe yini engingayiqondi kahle ngalolucwaningo nangomcwaningi ngingaxhumana namahhovisi ezocwaningo kulelikheli elilandelayo:
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION,
Research Office, Westville Campus Govan Mbeki Building
Private Bag X54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Inombolo yocingo: 27312604769- Fax: 27312604609; Email: BREC@ukzn.ac.za

_____	_____
Isiginesha yami	usuku
_____	_____
Isiginesha kafakazi (lapho edingekile)	usuku
_____	_____
Isiginesha yomhumushi (lapho edingekile)	usuku

Letter of permission from District Office

health

Department:
Health
PROVINCE OF KWAZULU-NATAL

Umkhanyakude Health District Office
Private Bag X 026, Jozini 3967
Tel: 035 5721327, Fax: 035 5721251
Email:makho.themba@kznhealth.gov.za

Reference :
Enquiries : Ms MP Themba
Telephone : 035-5721327

28 April 2011

To Whom It May Concern:

Re: Proposed research project entitled: 'Assessment of the therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated *plasmodium falciparum* malaria in northern KwaZulu-Natal'

This office supports the above-titled research project, with Dr CH Vaughan-Williams as principal investigator, being conducted in Umkhanyakude Health District in 2011 and 2012, subject to the necessary ethical approval being granted by the UKZN Biomedical Research Ethics Committee.

Signed,

APR 2011

Ms MP Themba,
District Manager
Umkhanyakude Health District Office

Data Analysis Sheet (part A)

Recruitment Date	Name	Hospital or Clinic name	Hospital/Clinic card number:	Date of birth	Age	Gender	Weight	Date became ill	Other medication	Travel 60 days prior to illness	HIV status, if known	Area	Country of origin

Data Analysis Sheet (Part B)

Enrolment										Follow-up					
Temperature	Pulse rate	Blood Pressure	Thick film	Thin film (+count)	Malaria PCR	Recrudescence PCR	mdr1 present (AL resistance)	Chloroquine resistance marker present	Dose Coartem (tablets bd)	Temperature	Pulse rate	Blood Pressure	Thin film (+count)	Thin film	Malaria PCR

Malaria Journal Instructions for authors

Malaria Journal is aimed at the scientific community interested in malaria in its broadest sense. It is the only journal that publishes exclusively articles on malaria and, as such, it aims to bring together knowledge from the different specialties involved in this very broad discipline, from the bench to the bedside and to the field. *Malaria Journal* offers a fast publication schedule while maintaining rigorous peer-review; this is achieved by managing the whole of the publication process electronically, from submission to peer-review.

Each article type published by *Malaria Journal* follows a specific format, as detailed in the corresponding instructions for authors; please choose an article type from the list on the left to view the instructions for authors.

The instructions for authors includes information about preparing a manuscript for submission to *Malaria Journal*, criteria for publication and the online submission process. Other relevant information about the journal's policies, the refereeing process and so on can be found in 'About this journal'.

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Research Articles

Submission process | Preparing main manuscript text | Preparing illustrations and figures | Preparing tables | Preparing additional files | Style and language

See 'About this journal' for descriptions of different article types and information about policies and the refereeing process.

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Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The submitting author takes responsibility for the article during submission and peer review.

Please note that *Malaria Journal* levies an article-processing charge on all accepted Research Articles; if the submitting author's institution is a BioMed Central member the cost of the article-processing charge may be covered by the membership (see About page for detail). Please note that the membership is only automatically recognised on submission if the submitting author is based at the member institution.

To facilitate rapid publication and to minimize administrative costs, *Malaria Journal* accepts only online submission.

Files can be submitted as a batch, or one by one. The submission process can be interrupted at any time; when users return to the site, they can carry on where they left off.

See below for examples of word processor and graphics file formats that can be accepted for the main manuscript document by the online submission system. Additional files of any type, such as movies, animations, or original data files, can also be submitted as part of the manuscript.

During submission you will be asked to provide a cover letter. Use this to explain why your manuscript should be published in the journal, to elaborate on any issues relating to our editorial policies in the 'About *Malaria Journal*' page, and to declare any potential competing interests. You will be also asked to provide the contact details (including email addresses) of potential peer reviewers for your manuscript. These should be experts in their field, who will be able to provide an objective assessment of the manuscript. Any suggested peer reviewers should not have published with any of the authors of the manuscript within the past five years, should not be current collaborators, and should not be members of the same research institution. Suggested reviewers will be considered alongside potential reviewers recommended by Editorial Board members or other advisers.

Assistance with the process of manuscript preparation and submission is available from BioMed Central customer support team.

We also provide a collection of links to useful tools and resources for scientific authors on our Useful Tools page.

File formats

The following word processor file formats are acceptable for the main manuscript document:

Microsoft word (DOC, DOCX)

Rich text format (RTF)

Portable document format (PDF)

TeX/LaTeX (use BioMed Central's TeX template)

DeVice Independent format (DVI)

Users of other word processing packages should save or convert their files to RTF before uploading. Many free tools are available which ease this process.

TeX/LaTeX users: We recommend using BioMed Central's TeX template and BibTeX stylefile. If you use this standard format, you can submit your manuscript in TeX format. If you have used another template for your manuscript, or if you do not wish to use BibTeX, then please submit your manuscript as a DVI file. We do not recommend converting to RTF.

Note that figures must be submitted as separate image files, not as part of the submitted manuscript file.

Preparing main manuscript text

General guidelines of the journal's style and language are given below.

Overview of manuscript sections for Research Articles

Manuscripts for Research Articles submitted to *Malaria Journal* should be divided into the following sections (in this order):

Title page
Abstract
Keywords
Background
Methods
Results and discussion
Conclusions
List of abbreviations used (if any)
Competing interests
Authors' contributions
Authors' information
Acknowledgements
Endnotes
References
Illustrations and figures (if any)
Tables and captions
Preparing additional files

The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database (EMBL), DNA Data Bank of Japan (DDBJ), GenBank at the NCBI (GenBank), Protein Data Bank (PDB), Protein Information Resource (PIR) and the Swiss-Prot Protein Database (Swiss-Prot).

You can download a template (Mac and Windows compatible; Microsoft Word 98/2000) for your article.

For reporting standards please see the information in the About section.

Title page

The title page should:

provide the title of the article

list the full names, institutional addresses and email addresses for all authors

indicate the corresponding author

Please note:

the title should include the study design, for example "A versus B in the treatment of C: a randomized controlled trial X is a risk factor for Y: a case control study"

abbreviations within the title should be avoided

Abstract

The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background**, the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract. **Trial registration**, if your research reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number (e.g. **Trial registration**: Current Controlled Trials ISRCTN73824458). Please note that there should be no space between the letters and numbers of your

trial registration number. We recommend manuscripts that report randomized controlled trials follow the CONSORT extension for abstracts.

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field. The section should end with a brief statement of what is being reported in the article.

Methods

The methods section should include the design of the study, the setting, the type of participants or materials involved, a clear description of all interventions and comparisons, and the type of analysis used, including a power calculation if appropriate. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses in the Methods section.

For studies involving human participants a statement detailing ethical approval and consent should be included in the methods section. For further details of the journal's editorial policies and ethical guidelines see 'About this journal'.

For further details of the journal's data-release policy, see the policy section in 'About this journal'.

Results and discussion

The Results and discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The Results and discussion sections may also be broken into subsections with short, informative headings.

Conclusions

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

Competing interests

A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organizations. Authors must disclose any financial competing interests; they should also reveal any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript.

Authors are required to complete a declaration of competing interests. All competing interests that are declared will be listed at the end of published articles. Where an author gives no competing interests, the listing will read 'The author(s) declare that they have no competing interests'.

When completing your declaration, please consider the following questions:

Financial competing interests

In the past five years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify.

Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.

Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.

Do you have any other financial competing interests? If so, please specify.

Non-financial competing interests

Are there any non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.

If you are unsure as to whether you, or one your co-authors, has a competing interest please discuss it with the editorial office.

Authors' contributions

In order to give appropriate credit to each author of a paper, the individual contributions of authors to the manuscript should be specified in this section.

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We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

Please acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for each author, and for the manuscript preparation. Authors must describe the role of the funding body, if any, in design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. Please also acknowledge anyone who contributed materials essential for the study. If a language editor has made significant revision of the manuscript, we recommend that you acknowledge the editor by name, where possible.

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Koonin EV, Altschul SF, Bork P: **BRCA1 protein products: functional motifs.** *Nat Genet* 1996, **13:**266-267.

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In press article
Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract
Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42:**s250.

Article within conference proceedings
Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

Book chapter, or article within a book
Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2.* 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

Whole issue of journal
Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings
Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book
Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series
Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.* Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology*, vol 1.]

Book with institutional author
Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis
Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL
The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]

Link / URL with author(s)
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Dataset with persistent identifier
Zheng, L-Y; Guo, X-S; He, B; Sun, L-J; Peng, Y; Dong, S-S; Liu, T-F; Jiang, S; Ramachandran, S; Liu, C-M; Jing, H-C (2011): Genome data from sweet and grain sorghum (Sorghum bicolor). *GigaScience*. http://dx.doi.org/10.5524/100012.

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Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine.

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UKZN Biomedical Research Ethics Committee Approval

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Website: http://research.ukzn.ac.za/Researchethics/BiomedicalResearchEthics.aspx

30 October 2011

Dr. V Vaughan- Williams
Department of Public Health
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Williams

PROTOCOL: Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal.
REF: BE099/11

PROVISIONAL APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your response dated 14 September 2011 to queries raised on 29 August 2011.

The study is given **PROVISIONAL APPROVAL** pending receipt of:

1. Permission from Umkhanyakude Health District and Hospital Managers and Clinics
2. Postgraduate Education Committee Approval

Only when full ethical approval is given, may the study begin. **Full ethics approval has not been given at this stage.**

PLEASE NOTE: Provisional approval is valid for 6 months only - should we not hear from you during this time - the study will be closed and reapplication will need to be made.

Your acceptance of this provisional approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC· 290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

KwaZulu-Natal Health Research Committee Approval

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

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Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : **HRKM169/11**
Enquiries : **Mrs G Khumalo**
Telephone : 033 – 3953189

12 December 2011

Dear Dr C H Vaughan-Williams

Subject: Approval of a Research Proposal

1. The research proposal titled '**Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal**' was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby **approved** for research to be undertaken at Umkhanyakude.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mrs G Khumalo on 033-3953189.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
KwaZulu-Natal Department of Health
Date: 13/12/2011.

UKZN Postgraduate Education Committee Approval

-----Original Message-----

From: Anna Voce [mailto:voceas@ukzn.ac.za]

Sent: 23 November 2011 04:19 PM

To: 983215818 Charles Hervey Vaughan-Williams

Cc: Stephen Knight; Mbokazi@ukzn.ac.za; oreilly@ukzn.ac.za

Subject: [NRMSM] Provisional Approval

Dear Dr Knight

RE: Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal. CH Vaughan-Williams.

The Postgraduate Education Committee considered the abovementioned application and raised various queries. These have been addressed and the protocol is given provisional approval for the Master of Public Health degree.

This decision will be ratified at a full sitting of the Committee scheduled for 6th December 2011.

Please note that the study may not begin without ethics approval.

Yours sincerely

Dr A Voce

Dean's Assistant: Coursework Programmes

Postgraduate Education Committee

University of KwaZulu-Natal

http://www.ukzn-nrmsm.co.za

Study Data Table, including data not published

Recruitment Date	Study Number	Age	Gender	Enrolment PCR sample received	Enrolment form received	Follow-up PCR sample received	Follow-up form received	Weight	Date became ill	Other medication	Travel 60 days prior to illness	HIV status, if known	Area	Country of origin	Temperature
2012/01/11	**1**	**40**	**f**	**Yes**	**Yes**	**Yes**	**Yes**	**65**	**2012/01/11**		**Moz**	**Neg**	**Thukuze**	**Moz**	**36.5**
2012/01/12	2	21	f	Yes	No	No	No								
2012/01/12	3			Yes	No	No	No								
2012/01/16	**4**	**7**	**m**	**Yes**	**Yes**	**No**	**No**	**19**	**2012/01/15**	**Paracetamol**	**Yes**	**Non-reactive**	**Lulwane**	**South Africa**	**39.4**
2012/01/19	**5**	**32**	**f**	**Yes**	**No**	**No**	**No**	**58**	**2012/01/28**	**Arvs**	**None**	**Pos**	**Thengani**	**South Africa**	**36.4**
2012/01/23	**6**	**24**	**m**	**Yes**	**Yes**	**Yes**	**Yes**	**57**	**2012/01/21**	**Nil**	**None**	**NK**	**Moz**	**Moz**	**39.3**
2012/01/31	**8**	**9**	**f**	**Yes**	**No**	**Yes**	**Yes**	**25**							
2012/01/31	7			Yes	No	No	No								
2012/02/10	9	30	f	Yes	Yes	Yes	No	61	2012/02/18	Paracetamol	None	NK	Skhemelele	South Africa	36.5
2012/02/26	**10**	**31**	**m**	**Yes**	**Yes**	**Yes**	**No**	**56**	**2012/02/23**	**Nil**	**Moz**	**NK**	**Manhlali**	**South Africa**	**38.3**
2012/02/27	11	6	m	Yes	No	Yes	Yes	20.9							
2012/02/29	**12**	**8**	**f**	**Yes**	**Yes**	**Yes**	**Yes**	**20.6**	**2012/02/23**	**antibiotics**	**None**	**NK**	**KwaMakhanya**	**South Africa**	**36.7**
2012/03/01	13	28	f	Yes	Yes	No	No	51.5	2012/02/25	Nil	Moz	NK	Mozambique	Mozambique	37.1
2012/03/09	14	28	f	Yes	Yes	No	No	71.7	2012/03/08	Paracetamol	None	Non-reactive	Embangweni	South Africa	36
2012/03/09	15	69	f	Yes	Yes	No	No	61	2012/03/08	Nil	Moz	NK	Makhabeleni	South Africa	38.7
2012/03/12	17	5	f	Yes	Yes	No	No	23.8	2012/03/06	Paracetamol	None	Non-reactive	Embangweni	South Africa	40
2012/03/12	16	6	f	Yes	Yes	No	No	20.7	2012/03/06	Paracetamol	None	Non-reactive	Embangweni	South Africa	38.1
2012/03/12	22	43	m	Yes	Yes	No	No	66	2012/03/10	Nil	Moz	nk	Mbangweni	South Africa	38.4
2012/03/13	**19**	**15**	**m**	**Yes**	**Yes**	**No**	**No**	**42**	**2012/03/09**	**Paracetamol**	**Moz**	**NK**	**Embangweni**	**South Africa**	**38**

2012/03/13	18	45	f	Yes	Yes	No	No		2012/03/10	Paracetamol	None	Non-reactive	Embangweni	South Africa	
2012/03/19	21	34	m	Yes	Yes	No	No	34						South Africa	35.8
2012/03/19	23	48	m	Yes	Yes			65	2012/03/01	Paracetamol	None	NK	Mbangweni	South Africa	37.1
2012/03/26	24	12	m	Yes	Yes	no	no	34	2012/03/14	Paracetamol	None	NK	Mbangweni	South Africa	37
2012/04/02	**25**	**37**	**f**	**Yes**	**Yes**	**No**	**No**	**106**	**2012/03/29**			**Pos**	**KwaNdaba**	**South Africa**	**38.7**
2012/04/13	26	29	f	Yes	Yes	No	No	72	2012/02/12	Paracetamol	None	Yes'	Ward 9	South Africa	36.8
2012/04/17	**27**	**6**	**f**	**Yes**	**Yes**	**Yes**	**Yes**	**20**	**2012/04/14**	**Paracetamol**	**None**	**Yes'**	**Mbangweni**	**South Africa**	**39**
2012/04/18	29	2	m	Yes	Yes	No	No	20	2012/04/16	Paracetamol	None	Nk	Bhekabantu	South Africa	39
2012/04/20	**30**	**2**	**f**	**Yes**	**Yes**	**No**	**No**		**2012/04/18**	**Paracetamol**	**Moz**	**NK**	**Embangweni**	**South Africa**	
2012/04/20	32	7	m	Yes	Yes	No	No	24	2012/04/18	Paracetamol	None	NK	Bhekabantu	South Africa	39.7
2012/04/20	**31**	**17**	**m**	**Yes**	**Yes**	**No**	**No**	**59**	**2012/04/15**	**Paracetamol**	**None**	**NK**	**Embangweni**	**South Africa**	**38.2**
2012/04/24	**34**	**14**	**f**	**Yes**	**Yes**	**No**	**No**	**52**	**2012/04/22**	**Paracetamol**	**None**	**NK**	**Bhekabantu**	**South Africa**	**38**
2012/04/26	48	66	f	Yes	Yes	No	No	49	2012/04/26	Paracetamol	Moz	Nk	Bhekabantu	South Africa	37.5
2012/04/30	**35**	**4**	**f**	**Yes**	**Yes**	**No**	**No**	**15.2**	**2012/04/26**	**Paracetamol**	**Moz**	**Yes'**	**Mbangweni**	**South Africa**	**38**
2012/04/30	33	23	f	Yes	Yes	No	No	50	2012/04/25	Paracetamol	None	Yes'	Bhekabantu	South Africa	36.5
2012/05/02	**38**	**3**	**m**	**Yes**	**FU form used**	**No**	**No**	**15**							**38**
2012/05/02	28	5	f	Yes	Yes	No	No	18	2012/05/01	Paracetamol	None	NK	Bhekabantu	South Africa	38.6
2012/05/02	37	5	f	Yes	FU form used	No	No	17	2012/05/02						38
2012/05/02	36	8	f	Yes	Yes	No	No	17	2012/05/01	Paracetamol	None	NK	Bhekabantu	South Africa	39
2012/05/02	44	13	m	Yes	Yes	No	No	40	2012/05/01	Paracetamol	None	NK	Bhekabantu	South Africa	37
2012/05/04	40	6	f	Yes	Yes	no	No	17	2012/05/02	Paracetamol	None	NK	Bhekabantu	South Africa	37.7
2012/05/04	43	13	m	Yes	FU form used	No	No	47							38
2012/05/04	39	14	m	Yes	Yes	No	No	32	2012/05/03	Paracetamol	None	NK	Bhekabantu	South Africa	38.1
2012/05/04	42	15	m	Yes	Yes	No	No	51	2012/05/03	Paracetamol	None	Nk	Bhekabantu	South Africa	37.7

2012/05/04	41	38	f	Yes	FU form used	No	No	57							37.2
2012/05/06	45	39	m	Yes	Yes	No	No	79	2012/05/05	Paracetamol	None	NK	Bhekabantu	South Africa	37
2012/05/07	46	12	f	Yes	Yes	no	No	32	2012/05/04	Paracetamol	None	NK	KwaNdaba	South Africa	37.5
2012/05/07	47	12	f	Yes	Yes	No	No	46	2012/05/04	Paracetamol	None	NK	Bhekabantu	South Africa	37.8
2012/05/15	49	42	m	Yes	Yes	No	No	60	2012/05/13	Paracetamol	None	Neg	Mbangweni	South Africa	36.5

Pulse rate	Blood Pressure	RDT	Thick film	Thin film (+count)	Malaria PCR	*Plasmodium* Check	Dose Coartem (tablets bd)	Due date	Date seen	Days since treatment	Temperature	Pulse rate	Blood Pressure	Compliant with medication
82	**113/72**	**Pos**		**Pos**	**Pos**	***P. falciparum***	**4**	**2012/02/08**	**2012/02/08**	**28**	**37**	**82**	**110/70**	
		Pos		Neg	Neg	Negative		2012/02/09						
		Pos		No record	Neg	Negative		2012/02/09						
		Pos		**No record**	**Pos**	***P. falciparum***	**2**	**2012/02/13**	**2012/07/25**	**191**				
80	**122/93**	**Pos**		**Neg**	**Pos**	***P. falciparum***		**2012/02/16**	**2012/07/05**	**168**				
110	**126/57**	**Pos**		**Neg**	**Pos**	***P. falciparum***	**4**	**2012/02/20**	**2012/03/12**	**49**	**36.7**	**60**	**120/89**	**Yes**
		Pos	**Neg**	**Neg**	**Pos**	***P. falciparum***		**2012/02/28**	**2012/02/29**	**29**	**36**	**102**		**Yes**
		Pos			Neg	Negative		2012/02/28						
93	104/73	Pos			Neg	Negative	4	2012/03/09	2012/03/30	**49**				
92	**95/56**	**Pos**			**Pos**	***P. falciparum***	**4**	**2012/03/25**	**2012/03/29**	**32**				
		Pos			Neg	Negative	2	2012/03/26	2012/03/23	**25**	37	96		yes
100		**Pos**			**Pos**	***P. falciparum***	**2**	**2012/03/28**	**2012/03/29**	**29**	**36**	**118**		**yes**
84	93/61	Pos			Neg	Negative	2	2012/03/29						
78	130/80	Pos			Neg	Negative	4	2012/04/06						
90	155/93	Pos			Neg	Negative	4	2012/04/06						
118		Pos			Neg	Negative	2	2012/04/09						
120		Pos			Neg	Negative	2	2012/04/09						
78	100/70	Pos			neg	Negative	4	2012/04/09						
110		**Pos**			**Pos**	***P. falciparum***	**4**	**2012/04/10**	**2012/06/27**	**106**				
100	**120/70**	**Pos**			**Pos**	***P. falciparum***	**4**	**2012/04/10**	**2012/07/18**	**127**				
		Pos			neg	Negative	4	2012/04/10						
78	140/80	Pos			neg	Negative	4	2012/04/16						
96	120/60	Pos			neg	Negative	4	2012/04/16						
96		Pos			neg	Negative	3	2012/04/23						
86	**110/77**	**Pos**			**Pos**	***P. falciparum***	**4**	**2012/04/30**	**2012/06/27**	**86**				

		Pos		No record	Neg	Negative		2012/02/09								
		Pos		**No record**	**Pos**	***P. falciparum***	**2**	**2012/02/13**	**2012/07/25**	**191**						
80	**122/93**	**Pos**		**Neg**	**Pos**	***P. falciparum***		**2012/02/16**	**2012/07/05**	**168**						
110	**126/57**	**Pos**		**Neg**	**Pos**	***P. falciparum***	**4**	**2012/02/20**	**2012/03/12**	**49**	**36.7**	**60**	**120/89**	**Yes**		
		Pos	**Neg**	**Neg**	**Pos**	***P. falciparum***		**2012/02/28**	**2012/02/29**	**29**	**36**	**102**		**Yes**		
		Pos			Neg	Negative		2012/02/28								
93	104/73	Pos			Neg	Negative	4	2012/03/09	2012/03/30	**49**						
92	**95/56**	**Pos**			**Pos**	***P. falciparum***	**4**	**2012/03/25**	**2012/03/29**	**32**						
		Pos			Neg	Negative	2	2012/03/26	2012/03/23	**25**	37	96		yes		
100		**Pos**			**Pos**	***P. falciparum***	**2**	**2012/03/28**	**2012/03/29**	**29**	**36**	**118**		**yes**		
84	93/61	Pos			Neg	Negative	2	2012/03/29								
78	130/80	Pos			Neg	Negative	4	2012/04/06								
90	155/93	Pos			Neg	Negative	4	2012/04/06								
118		Pos			Neg	Negative	2	2012/04/09								
120		Pos			Neg	Negative	2	2012/04/09								
78	100/70	Pos			neg	Negative	4	2012/04/09								
110		**Pos**			**Pos**	***P. falciparum***	**4**	**2012/04/10**	**2012/06/27**	**106**						
100	**120/70**	**Pos**			**Pos**	***P. falciparum***	**4**	**2012/04/10**	**2012/07/18**	**127**						
		Pos			neg	Negative	4	2012/04/10								
78	140/80	Pos			neg	Negative	4	2012/04/16								
96	120/60	Pos			neg	Negative	4	2012/04/16								
96		Pos			neg	Negative	3	2012/04/23								
86	**110/77**	**Pos**			**Pos**	***P. falciparum***	**4**	**2012/04/30**	**2012/06/27**	**86**						
90	133/87	Pos			neg	Negative	4	2012/05/11								
112		**Pos**			**Pos**	***P. falciparum***	**2**	**2012/05/15**	**2012/05/15**	**28**	**36**	**116**		**yes**		

156		Pos				Negative	1	2012/05/16							
					Pos	***P. falciparum***	**?**	**2012/05/18**	**Gone to Moz; unobtainable**						
102		Pos	NS		Neg	Negative	2	2012/05/18							
106	**105/62**	**Pos**			**Pos**	***P. falciparum***	**4**	**2012/05/18**	**2012/06/28**	**69**					
		Pos			**Pos**	***P. falciparum***	**4**	**2012/05/22**	**2012/07/25**	**92**					
96	136/87	Pos			Neg	Negative	4	2012/05/24							
120		**Pos**			**Pos**	***P. falciparum***	**1**	**2012/05/28**	**2012/06/28**	**59**					
94	117/66	Pos			Neg	Negative	4	2012/05/28							
100		**Pos**			**Pos**	***P. falciparum***	**2**	**2012/05/30**	**2012/06/28**	**57**					
110		Pos		NS		Negative	2	2012/05/30							
104		Pos			Neg	Negative	2	2012/05/30							
120		Pos		NS	Neg	Negative	2	2012/05/30							
100	110/70	Pos			Neg	Negative	2	2012/05/30							
120		Pos		NS	Neg	Negative	2	2012/06/01							
102	100/60	Pos		NS	Neg	Negative	4	2012/06/01							
100	100/70	Pos		NS	Neg	Negative	3	2012/06/01							
100	110/70	Pos		NS	Neg	Negative	4	2012/06/01							
100	130/80	Pos		NS	Neg	Negative	2	2012/06/01							
88	110/70	Pos		NS	Neg	Negative	4	2012/06/03							
100	100/60	Pos		NS	Neg	Negative	3	2012/06/04							
102	100/70	Pos		NS	Neg	Negative	4	2012/06/04							
92	120/70	Pos			Neg	Negative	4	2012/06/12							