Tafenoquine in the Prophylaxis and Treatment of Malaria in Australian Defence Force Personnel

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I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from published or unpublished works of others has been acknowledged in the text and a list of references is given.

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STATEMENT ON THE CONTRIBUTION OF OTHERS

This is to certify that this thesis embodies the original work undertaken by the candidate, except where the contribution of others has been acknowledged. None of the papers presented here have been submitted in support of any other award of this or any other University or institution, except where this has been acknowledged.

The original concept of the utilisation of tafenoquine for the prophylaxis and post exposure prophylaxis was developed by the research group of the Australian Army Malaria Institute (AMI) and was particularly guided by the Institute's then Director, Professor Karl Reickmann and LTCOL (Dr) Mike Edstein. Support for the investigation of this potential use of tafenoquine was given by the then Drugs for the Developing World (DDW) area of GlaxoWellcome and eventually to GlaxoSmithKline (GSK). The concept of a soak treatment for recurring vivax malaria was originally proposed by my co-investigator in all these activities, Associate Professor Scott Kitchener.

Design of the studies described within this thesis was by necessity undertaken both with colleagues within AMI and with the drug development personnel at both the United States Army Medical Materiels Development Activity (USAMMDA) and Dr Keith Barker and Dr Philip Pickford of GSK. In addition, the particular contribution of Professor Bruce Charles and his group from the School of Pharmacy, University of Queensland, who, with the pharmacology personnel of AMI, modelled the pharmacokinetics of mefloquine and tafenoquine are recognised.

As the studies described were subject to the production of Clinical Study Reports (CSR) for submission to regulatory authorities, the development of Statistical Analysis Plans and the subsequent analysis of much of the data described in Chapter 3 was undertaken in conjunction with the Statistics Department of GSK. Their contribution and patience is acknowledged.

The descriptive text introduced into the Chapters 3-5 has, where possible, been drawn from the respective CSRs to avoid any possibility of incorrect interpretation and to ensure consistency between the descriptions presented in this thesis and the

information provided to the regulatory authorities. The contribution of Ms Caron Kerr and Ms Rachael Moate of GSK in co-drafting the CSRs for the studies described in Chapters 3 and 4 of this thesis is gratefully acknowledged. The co-drafting of the CSR for Chapter 5 was undertaken by Associate Professor Scott Kitchener who was also the listed Chief Investigator of this study.

Clinical studies of this nature require extensively trained and competent research teams to deliver. Particular acknowledgment is therefore given to the men and women professionals of the AMI for their commitment and dedication, often in adverse conditions, without whom the studies could not have been delivered.

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DECLARATION ON ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Humans (*1999*)*, the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics*. *Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (1997)*.* The proposed research methodology received clearance from the Australian Defence Human Research Ethics Committee (approval numbers 165/98, 216/00 and 267/01).

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ABSTRACT

Background

The Australian Defence Force has a long history of exposure to malaria and frequently deploys into the immediate area of the Pacific Rim where drug resistance has been noted to be problematic. In the late 1990s failures of established malaria prophylaxis regimens were beginning to become more prevalent within the ADF and a search was commenced to identify alternative or promising emerging prophylaxis and treatment regimens. In this context the work presented within this thesis was undertaken with a new 8-aminoquinolone antimalarial, initially formulated by the United States Army's Walter Reed Army Institute of Research (WRAIR) and identified as investigative compound ***WR 238605.*** The thesis investigates its utility as both prophylaxis and treatment for malaria infection. The compound was subsequently identified in a joint development arrangement between the US Army and GlaxoSmithKline as ***etaquine***, before a final naming of the compound as ***tafenoquine***. The thesis presents three distinct challenges in the development of this promising antimalarial drug and describes the early human use of tafenoquine in the following settings:

- Prophylaxis against malaria infection during deployment to a malarious area;
- Post exposure prophylaxis of malaria on return from a malarious area; and
- Treatment of recurrences of malaria infection.

Methods

The thesis is developed through the description of three distinct human clinical trials. Each of these will be developed as individual chapters within the thesis although the reality is that there was some overlap between the activities with developments observed in early activity being used to define both later stages of long term trials and inform the development of the newer activities, some of which are now ongoing in other countries and research institutions.

The first double blind comparative study investigates the use of tafenoquine and mefloquine for the longer term (6 months) prophylaxis of malaria in Australian Defence personnel on deployment to Timor Leste. The second, an open label comparative study of the use of tafenoquine and primaquine in the post exposure prophylaxis of vivax malaria in a defence population in Bougainville, Papua New Guinea and in Timor Leste, and the third looks at the treatment of recurring vivax malaria with tafenoquine in an open label study in a non randomised population of defence personnel.

Results

Prophylaxis against malaria infection during deployment to a malarious area:
Tafenoquine at a weekly dose of 200mg and mefloquine at a dose of 250mg were well tolerated amongst subjects in a military deployment. No malaria occurred in either the tafenoquine and mefloquine arms during the prophylactic phase of this Phase III study. During the relapse follow-up phase, <1% of subjects in either treatment group developed *Plasmodium vivax* malaria.

The incidence and nature of adverse events was similar between the two treatment groups. The most common adverse events were gastroenteritis and unrelated injury. Tafenoquine was associated with the development of vortex keratopathy (secondary to phospholipidosis) in 69/74 (93.2%) subjects tested (compared to no mefloquine subjects). This effect was benign and reversible, with resolution in >90% subjects at 6 months and complete resolution in all subjects by 1 year post-treatment.

No significant changes were seen in most laboratory indices during the study. Increases in methaemoglobin in the tafenoquine group were small. Renal follow-up confirmed a lack of long-term renal effects of tafenoquine.

Post exposure prophylaxis of malaria on return from a malarious area:
A 3-day dosing regimen of tafenoquine (400 mg od, 200 mg bd or 200 mg od) was effective as a post-exposure prophylaxis agent in this study, demonstrating similar

efficacy to 14-day primaquine. Tafenoquine, with a shorter dosing regimen (3 days compared to 14 days primaquine), could be used as a more convenient, yet effective, post-exposure prophylaxis agent.

Tafenoquine was well tolerated, with no subjects being withdrawn due to adverse events. The most common adverse events were gastrointestinal events.

Treatment of recurrences of malaria infection:

This small scale study showed that tafenoquine is safe and effective (following chloroquine treatment) in prevention of relapse of multi-relapsing vivax malaria.

The management of relapsing vivax malaria with chloroquine/tafenoquine may be more effective and convenient in preventing further relapses than the standard chloroquine/primaquine treatment regimen. Larger studies are required to address the effectiveness and tolerability of chloroquine/tafenoquine for the treatment of vivax malaria. There is also a requirement to more extensively address tafenoquine used on its own for the treatment of recurring vivax malaria. There remains a need to investigate this regimen in other ethnic populations, including special risk groups such as children and pregnant women.

Conclusions

Tafenoquine displays the properties required of a promising antimalarial compound. It has, in two phase III clinical trials, established prophylaxis properties; a demonstrated advantage over the classical 14 days of primaquine treatment for post exposure prophylaxis against *P. vivax* in its reduced treatment time of 3 days; and has a suggested role in the management of recurrences of vivax malaria, although further research will be required to firmly establish this role. It has an acceptable adverse event profile in the limited treatments undertaken to date, when compared to other available antimalarial compounds. Additionally, it has the advantage of once weekly dosing and shorter post exposure prophylaxis regimens when compared to other available treatments.

ACKNOWLEDGEMENTS

My appreciation is extended to the Pro Vice Chancellor, Medicine, Health and Molecular Sciences, Professor Ian Wronski, and the Head of the School of Public Health, Tropical Medicine and Rehabilitation Sciences, Professor Ross Spark, for the opportunity to enrol in this degree and submit this thesis.

I wish to sincerely thank my two supervisors, firstly Professor Karl Rieckmann, Director of the Australian Army Malaria Institute. His wealth of experience in the field of malariology and in the conduct of clinical studies awakened my interest and set me off on the path of pursuing better solutions to a time old problem. Secondly, Professor Peter Leggat, who has driven the process to completion more than any other and provided the guidance and mentorship necessary to bring this thesis to a close. With the retirement of Professor Reickmann in late 2006, I wish to acknowledge Professor Rick Speare in providing further supervisory support.

To all my friends and colleagues at the Australian Army Malaria Institute I owe much gratitude for allowing me the freedom to pursue what I felt was important as we all endured the disruptions to domestic life conducting studies throughout the Pacific Rim. Particularly, I would like to thank my close friend, Associate Professor (Colonel) Scott Kitchener for sharing his drive but more importantly his direct support in this and many other projects. His contribution as an investigator, friend and sounding board, along with his ability to fix the issues directly impacted on the activities described within this thesis. Additionally, I would like to specifically acknowledge the support given by Dr Mike Edstein and Lieutenant Colonel Bob Cooper who were also instrumental in the development of not only this project, but in my general development in the field.

The contribution of the fine men and women, both officers and enlisted personnel, of the Australian Defence Force, who volunteered for inclusion into the research activities described, cannot be overstated. The contribution particularly of the Command group and Commanding Officers and their key staff personnel in facilitating access and providing the necessary leadership to “get things off the ground" was instrumental in the success of these studies. Without the commitment of these individuals these studies would not have been possible.

Acknowledgement is also given to the various agencies and organisations which have helped to sponsor this work, including SmithKline Beecham and GlaxoWellcome in the early days and then GlaxoSmithKline, who provided study medications, advice and directly contributed to study design phases as well as supporting travel scholarships to present the work from this thesis. Equally important was the direct funding stream provided by the United States Army, through the US Army Medical Materiels Development Activity, to directly support the main prophylaxis study. Without these financial contributions this work would not have been possible.

Lastly, to Tracey my spouse and best friend, who in darker times encouraged me to develop a new focus and exercise my brain, and who is singularly responsible for driving me towards the enrolment process, I express my love and gratitude. Her patience and support as I disappeared on multiple overseas deployments with the Defence Force and her commitment to keeping the dream alive while running a busy career and family is the reason that the project can finally be completed. Tracey, it's been a long time coming, but this one's for you and the boys.

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LIST OF ABBREVIATIONS

Abbreviation	**Unabridged Term**
ADF	Australian Defence Force
ADMEC	Australian Defence Medical Ethics Committee
ADHREC	Australian Defence Human Research Ethics Committee
AE	adverse event
ALT (SGPT)	alanine transaminase (serum glutamic-pyruvic transaminase)
AMI	Australian Army Malaria Institute
ART	adverse event terminology
AST (SGOT)	Aspartate transaminase (serum glutamic-oxaloacetic transaminase)
ATC	Anatomical Therapeutic Chemical
Bid	twice a day
B/l	Baseline
Bd/bid	Twice daily (bis in die)
BMI	Body Mass Index
BP	blood pressure
°C	degrees Celsius
CFR	Code of Federal Regulations
CI	confidence interval
CL/F	oral clearance
Cm	centimetre
CRF	case report form
CRO	contract research organisation
D	Day
dL	decilitre
DDW	Diseases of the Developing World
DLCO	diffusion capacity of the lungs to carbon monoxide
DMPK	Drug Metabolism and Pharmacokinetics
EDTA	ethylenediaminetetraacetic acid
EU CPMP	European Union Committee for Proprietary Medicinal Products
FDA	Food and Drug Administration
FEV_1	forced expired volume in 1 minute
g	Gram
G6PD	Glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GGT	gamma-glutamyl transferase
GSK	GlaxoSmithKline
Hb	Haemoglobin
HCG	human chorionic gonadothrophin
HSRRB	Human Subjects Research Review Board
ICH	International Committee on Harmonisation
IDMC	Independent Data Monitoring Committee
ITT	intent-to-treat
ka	first-order absorption rate constant
kg	Kilogram
L	Litre
Max	Maximum

MCHC	mean corpuscular haemoglobin concentration
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
mmHg	millimetres of mercury
NAMRU-2	Naval Medical Research Unit 2, Jakarta
ng	Nanogram
NRH	normal range high
NRL	normal range low
NRS	normal range span
od/ qd	once daily
PCR	polymerase chain reaction
PD	Post-dose
PNG	Papua New Guinea
PP	per protocol
PQ	Primaquine
ROCL	Relief out of country leave
SB	SmithKline Beecham Pharmaceuticals
sd	standard deviation
SOP	standard operating procedure
spp	species
Tid/tds	Three times daily
TQ	Tafenoquine
ug	microgram
uL	microlitre
U/L	units/litre
umol	micromole
UK	United Kingdom
US/USA	United States of America
USAMMDA	United States Army Medical and Materiel Defence Activity
USAMRMC	United States Army Medical Research and Materiel Command
URTI	upper respiratory tract infection
V/F	volume of distribution
WBC	white blood cell
WHO	World Health Organisation
wks	weeks
WRAIR	Walter Reed Army Institute of Research

CHAPTER 1

- **Introduction**

1.1 Background

The World Health Organization has long considered malaria as the leading cause of morbidity and mortality in many developing countries with an estimated 300 to 500 million cases worldwide each year [WHO, 2010]. Between one and three million deaths, mainly in children, are attributable to this disease each year [WHO, 2010].

While biting a human host, an infected female *Anopheles* spp. mosquito transmits the *Plasmodium* sporozoite from its saliva into the bloodstream of its victim. Within minutes of inoculation, the sporozoites travel through the blood and into the liver where they undergo asexual division and maturation. The time period between the mosquito bite and the first appearance of plasmodia in the peripheral blood (i.e., the Pre-Patent Period) normally ranges between 9 and 12 days in humans [Sinden et al., 2002]. From the liver, merozoites are released into the blood and invade erythrocytes, where they develop into schizonts. In *Plasmodium falciparum* malaria, there are no residual parasites in the liver after the initial cycle of entry, division, maturation, and release. However, with *P. vivax* malaria, a proportion of the *P. vivax* sporozoites develop into a dormant form, known as hypnozoites, within the liver. The hypnozoites periodically re-enter the development cycle and cause clinical relapses of *P. vivax* malaria [Sinden et al., 2002]. The life cycle of the malaria parasite is presented in **Figure 1.1** below courtesy of the Centres for Disease Control.

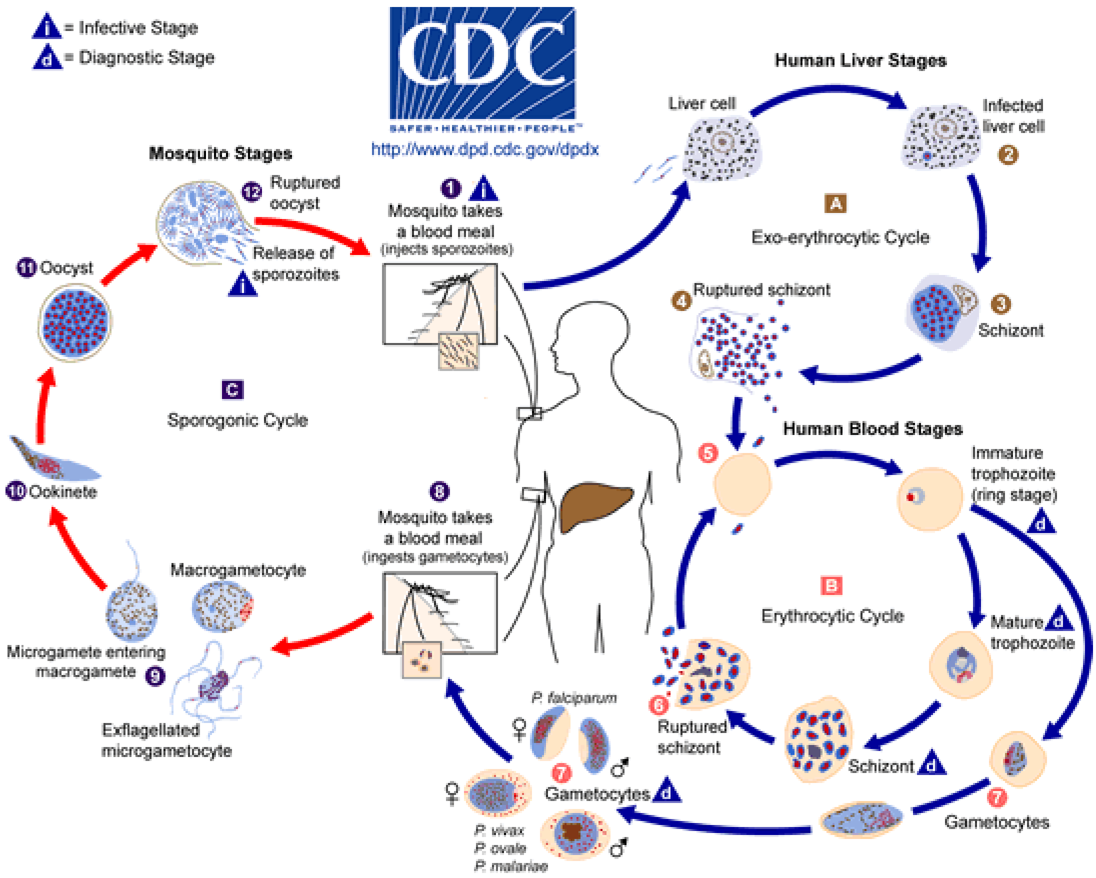


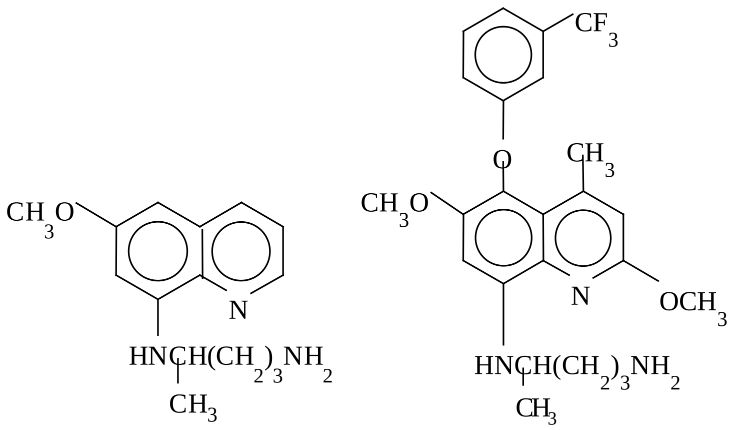
Figure 1.1 – Life cycle of the malaria parasite in vector (mosquito) and humans [accessed from CDC at http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Malaria_il.htm on 23rd June 2011].

Drugs that target the hepatic (or exoerythrocytic) stage of the parasite's life cycle are termed 'causal prophylactic drugs', and act by disrupting the life cycle of the parasite, thereby preventing parasitaemia, systemic illness, and further transmission. Drugs that target the erythrocytic schizonts, agents known as 'blood schizontocides', are used for treatment of clinically apparent malaria and as a suppressive prophylactic agent, by destroying schizonts before they cause clinical symptoms [Hoffman et al., 2011].

Current national and international guidelines for malaria recommend one of three drugs for chemoprophylaxis of malaria, namely mefloquine, doxycycline and atovaquone/proguanil (Malarone) [Antibiotic Expert Group, 2010; WHO, 2011]. In recent years, mefloquine has replaced chloroquine as single agent prophylaxis against chloroquine-resistant *P. falciparum*. For prophylaxis, mefloquine is given as a single dose of 250 mg weekly and is generally well tolerated. It is not, however, without side effects, particularly neuropsychiatric effects, such as dysphoria, dizziness and, rarely, seizures and psychosis. However, the incidence is not significantly different to that of chloroquine, when tested in a blinded manner [Boudreau et al, 1993]. The proportion of travellers complaining of disabling neuropsychiatric adverse events is less than 1%. Although mefloquine resistance has been documented in *P. falciparum* on the Thai-Cambodian and Thai-Myanmar borders, mefloquine continues to be effective elsewhere [Nosten et al., 1991]. The half maximal inhibitory concentration (IC_{50}) of mefloquine against a chloroquine-resistant, mefloquine-sensitive clone of *P. falciparum* is about 0.6 ng/mL; against a chloroquine-sensitive, mefloquine-resistant clone, it is approximately 4 ng/mL. Malarone™ (atovaquone/proguanil) is a relatively recent antimalarial registered for the Australian market and was not available at the time of this study [Leggat, 2009].

Primaquine, in combination with chloroquine, is currently recommended in national and international guidelines and widely used for the post-exposure prophylaxis or radical cure of *P. vivax* malaria [Antibiotic Expert Group, 2010; WHO, 2011]. *P. vivax* malaria is also a neglected disease of considerable public health importance. There are 70-80 million cases annually, and the disease is a source of considerable morbidity and has a significant economic impact in endemic countries [Mendis et al, 2001]. Primaquine is generally required to be administered over 14 days [Antibiotic Expert Group, 2010] and this can result in poor compliance and reduced effectiveness, which may in turn result in relapse of *P. vivax*.

Tafenoquine is an 8-aminoquinoline with an additional methoxy group at the 2 position, a methyl group at the 4 position, and a 3-trifluoromethylphenoxy substitution at the 5 position of the quinoline ring. It is administered as a succinate salt and not as a free base. It is closely related to primaquine. The structures of primaquine and tafenoquine are presented in **Figure 1.2** below:



Primaquine **Tafenoquine**

Figure 1.2 – The structural relationship between primaquine and tafenoquine.

Tafenoquine has been undergoing clinical evaluation as:

a) a causal prophylactic agent; and/or
b) a blood schizontocidal drug against human malaria parasites, including polyresistant *P. falciparum* and chloroquine-resistant *P. vivax*; and/or
c) in post-exposure prophylaxis [Warrell et al., 2002].

At the time of the current study series, a comprehensive program of clinical pharmacology and Phase II studies had been completed, with over 2000 subjects having been exposed to tafenoquine in these trials. Details of these studies are not presented in this thesis except as they relate to the discussion sections of the presented peer reviewed papers.

1.2 Presentation of the research and the thesis

In all, 13 papers, comprising 12 research papers and one commentary support this thesis (**see Table 1.1**). The work spans a period of greater than five years, with most papers being published in the past eight years. All of the 12 research papers have been published in high quality, peer-reviewed international journals, which are leading journals in the field of tropical medicine or pharmacology. Three of these papers are first author, seven are second author and two are third author papers. The commentary is a third author paper published in a locally relevant peer-reviewed military medicine journal. A broad statement of authorship of each of the papers has been given in the introductory pages and specific contributions to each paper lead the introduction of each of the chapters.

Chapter 2 provides a brief background to the study, in particular the study sites and the incidence of malaria in the ADF. The background has also been substantially published in three papers, two research papers and one commentary.

Chapter 3 considers tafenoquine as a prophylaxis against malaria in Australian soldiers deployed to Timor Leste. This study compared the chemosuppressive effectiveness of weekly tafenoquine and mefloquine as a randomised double blind clinical trial over a six month deployment period. Subjects were followed-up for three months post deployment to ascertain relapses. The study examined the efficacy, safety and pharmacokinetics of both drugs and five research papers are presented in support of this study, including the pivotal first author paper (labelled paper 3.1).

Chapter 4 focuses on tafenoquine for post-exposure prophylaxis against vivax malaria in Australian soldiers deployed to Papua New Guinea and Timor Leste. This study compared the terminal prophylaxic ability of tafenoquine and primaquine as an open-label randomised comparative trial. Subjects were followed-up for 12 months post deployment to ascertain relapses. The study also examined different dosing regimens of tafenoquine for post-exposure prophylaxis and four research papers are presented in support of these studies, including a pivotal first author paper (labelled paper 4.1).

Chapter 5 examines the findings of patients with acute vivax malaria treated with tafenoquine. This treatment was enabled under special authority from the Therapeutic Goods Authority (TGA). One research paper has been presented in support of this study, including a pivotal second-author paper (labelled paper 5.1).

The final chapter, **Chapter 6** draws the above research together providing a series of key findings, recommendations and suggestions for future research directions.

The instructions for authors for the various journals in which the material appearing in this thesis has been published has been provided in **Appendix 1.**

Table 1.1. Bibliographic data for chapters and papers presented in thesis

Reference	Indexing	Impact Factor (IF)*	#Other information
Chapter 2. Background			
2.1 Elmes NJ, Bennett SM, **Nasveld PE**. (2004) Malaria in the Australian Defence Force: the Bougainville experience. *ADF Health*. 5: 69-72.	N/A	N/A	ERA Category C; published by the *Medical Journal of Australia* group
2.2 Kitchener S, **Nasveld P**, Russell B, Elmes N. (2003) An outbreak of malaria in a forward battalion on active service in East Timor. *Military Medicine*. 168: 457-459.	PubMed	0.6	ERA Category A; 8 citations
2.3 Kitchener SJ, **Nasveld PE**, Gregory RM, Edstein MD. (2005) Mefloquine and doxycycline malaria prophylaxis in the Australian soldiers in East Timor. *Medical Journal of Australia*. 182: 168-171.	PubMed	2.1	ERA Category A (Invited paper); 10 citations
2.4 Bragonier R, **Nasveld P**, Reyburn H, Edstein M, Auliffe A. (2002) Rainy season prevalence of malaria in Bobonaro district, East Timor. *Annals of Tropical Medicine and Parasitology*. 96: 739-743.	PubMed	1.0	ERA Category C

Chapter 3. A randomised, double-blind, comparative study to evaluate the safety, tolerability and effectiveness of tafenoquine and mefloquine for the prophylaxis of malaria in non-immune Australian soldiers deployed to Timor Leste			
3.1 **Nasveld PE**, Edstein MD, Reid M, et al, for the Tafenoquine Study Team. (2010) Randomized, double-blind study of the safety, tolerability and efficacy of Tafenoquine verses mefloquine for malaria prophylaxis in non-immune subjects. *Antimicrobial Agents and Chemotherapy*. 54: 792-798.	PubMed	4.7	ERA Category A*; 7 citations
3.2 Charles BG, Miller AK, **Nasveld PE**, et al. (2007) Population pharmacokinetics of tafenoquine during malaria prophylaxis in healthy subjects. *Antimicrobial Agents and Chemotherapy*. 51: 2709-2715.	PubMed	4.4	ERA Category A*
3.3 Charles BG, Blomgren A, **Nasveld PE**, et al. (2007) Population pharacokinetics of mefloquine in military personnel for prophylaxis against malaria infection during field deployment. *European Journal of Clinical Pharmacology*. 63: 271-278. (PI; I.F.=2.2; Prov ERA Cat. A)	PubMed	2.2	ERA Category A
3.4 Edstein MD, **Nasveld PE**, Kocisko DA, et al. (2007) Gender differences in gastrointestinal disturbances and plasma concentrations of tafenoquine in healthy volunteers after tafenoquine administration for post-exposure vivax malaria prophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101: 226-230.	PubMed	2.0	ERA Category C

Chapter 4. Evaluation of Tafenoquine for the post-exposure prophylaxis of vivax malaria (Southwest Pacific Type) in non-immune Australian soldiers			
4.1 **Nasveld P**, Kitchener S, Edstein M, Rieckmann K. (2002) Comparison of tafenoquine (WR238605) and primaquine in the post-exposure (terminal prophylaxis of vivax malaria in Australian Defence Force personnel. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 96: 683-684.	PubMed	1.7	ERA Category B; 20 citations
4.2 Elmes NJ, **Nasveld PE**, Kitchener SJ, et al. (2008) The efficacy and tolerability of three different regimens of tafenoquine verses primaquine for the post exposure prophylaxis of *Plasmodium vivax* malaria in the Southwest Pacific. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 102: 1095-1101.	PubMed	2.0	ERA Category B; 5 citations
4.3 Edstein MD, **Nasveld PE**, Kocisko DA, et al. (2007) Gender differences in gastrointestinal disturbances and plasma concentrations of tafenoquine in healthy volunteers after tafenoquine administration for post-exposure vivax malaria prophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101: 226-230.	PubMed	2.0	ERA Category B
4.4 **Nasveld P**, Kitchener S. (2005) Treatment of acute vivax malaria with tafenoquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 99: 2-5.	PubMed	1.7	ERA Category B; 15 citations

Chapter 5. Treatment of acute vivax malaria with tafenoquine			
5.1 Kitchener S, **Nasveld P**, Edstein MD. (2007) Tafenoquine for the treatment of recurrent *Plasmodium vivax* Malaria. *American Journal of Tropical Medicine and Hygiene*. 76: 494-496.	PubMed	2.2	ERA Category B; 9 citations

Abbreviations: ERA-Excellence in Research Australia (Journal Classification from highest to lowest is A*, A, B, C and not ranked); ADF-Australian Defence Force

* ISI Web of Science. Journal Citation Reports

Scopus.com Journal Citation Reports

1.3 Context

The work presented in this thesis was undertaken from the late 1990s and early 2000s and has been conducted during a period of escalating deployments of the ADF in various operations in the region and further afield; areas with significant malaria transmission, such as Papua New Guinea and Timor Leste. It was also a golden age for the historic Australian Army Malaria Institute (AMI), which was able to deploy experienced researchers from regular and reserve forces into the field to overseas clinical research in various areas of operation, but particularly Bougainville, Papua New Guinea and Timor Leste.

The clinical development plan for tafenoquine was to focus initially on the treatment and relapse prevention of *P. vivax* malaria. Phase II trial data have shown that tafenoquine is effective against *P. vivax* as an anti-relapse agent, both alone and in combination with other antimalarials. These Phase II data demonstrate the potential utility of tafenoquine as a 1-3 day treatment for relapse prevention of *P. vivax* malaria. The clinical development plan aims to register a tafenoquine/ chloroquine combination regimen for the radical cure of *P. vivax* malaria. However, there will be a need to consider replacement antimalarial drugs moving forward, in particular to replace drugs such as mefloquine, where significant and increasing resistance has been reported [WHO, 2011]. It was therefore inevitable that the clinical development plan for tafenoquine would also need to examine its application for prophylaxis against malaria, especially in operational environments such as Timor Leste where there were increasing reports of malaria in deployed and returning soldiers [Kitchener et al., 2000].

1.4 References

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World Health Organization (2011) *International Travel and Health*. Geneva: WHO. URL. http://www.who.int/ith (accessed 17 June 2011)

□□□

CHAPTER 2

- **Field Settings for Tafenoquine Studies: Malaria Considerations**

- **List of peer reviewed and published papers presented in this chapter**

2.1 Elmes NJ, Bennett SM, **Nasveld PE**. (2004) Malaria in the Australian Defence Force: the Bougainville experience. *ADF Health*. 5: 69-72.

All authors participated in the conception and design of the study in this commentary and NJE drafted the manuscript. PN, NE and SB contributed to the analysis of the study. All authors gave final approval for the manuscript submitted for publication.

2.2 Kitchener S, **Nasveld P**, Russell B, Elmes N. (2003) An outbreak of malaria in a forward battalion on active service in East Timor. *Military Medicine.* 168: 457-459.

PN participated in the conception and design of the study and co-drafted the manuscript with SK. BR and NE participated in the design of the study and data collection. PN and SK participated in analysis of the study. All authors gave final approval for the manuscript submitted for publication.

2.3 Kitchener SJ, **Nasveld PE**, Gregory RM, Edstein MD. (2005) Mefloquine and doxycycline malaria prophylaxis in the Australian soldiers in East Timor. *Medical Journal of Australia.* 182: 168-171.

PN and SK participated in the conception and design of the study and drafted the manuscript. ME participated in the conception and design of the study and reviewed the manuscript. PN, SK and ME participated in the analysis. RG coordinated data collection and extraction for analysis. All authors gave final approval for the manuscript submitted for publication.

2.4 Bragonier R, **Nasveld P**, Reyburn H, Edstein M, Auliffe A. (2002) Rainy season prevalence of malaria in Bobonaro district, East Timor. *Annals of Tropical Medicine and Parasitology* 96: 739-743.

PN, RB and ME participated in the conception and design of the study. RB drafted the manuscript which was extensively reviewed by PN and ME. RB, HR and AA undertook field data collection and consolidation of study data. Analysis was undertaken by PN, RB and ME. All authors gave final approval for the manuscript submitted for publication.

2.1 Introduction

In the ten years before 1997, the ADF deployed in excess of 3000 personnel on operations in Africa and South East Asia. Sixteen cases of malaria were reported. In November 1997, the ADF began participating in peace monitoring in Bougainville (Papua New Guinea) and peace keeping in Timor Leste in September 1999. During the initial phase of the Timor Leste deployment over 10,000 personnel were deployed with the International Force (InterFET) until February 2000. Malaria is endemic in Bougainville and Timor Leste. As a result of increasing exposure to malaria, the ADF had 466 cases of malaria infections from November 1997 to March 2001, with approximately one fifth of all cases representing recurring vivax malaria. This indicates the persistence of the liver stages of *P. vivax* (hypnozoites) is not always eliminated by the current primaquine therapy.

P. vivax malaria among ADF personnel were treated [in accordance with Health Directive (HD) 215 – Malaria, 1994] with chloroquine and primaquine. The overall recurrence rate observed following operations in Bougainville and East Timor has been in excess of 20%. Recurrences of *P. vivax* malaria have generally been observed within two months of chloroquine and primaquine treatment (median 42 days). Commencing in 1999, the ADF began a clinical trial evaluating tafenoquine versus primaquine for the post exposure prophylaxis (PEP) of *P. vivax* malaria. Various dosing regimens for tafenoquine were evaluated (400 mg daily for 3 days, 200 mg twice daily for 3 days, and 200 mg daily for 3 days), and compared to standard regimens of primaquine 7.5 mg three times daily for 14 days. Assessment of the study findings indicated that tafenoquine given for 3 days is equally effective to 14 days of primaquine in preventing vivax malaria post-exposure. In these studies, tafenoquine was generally well tolerated at doses of 200 mg to 400 mg daily. In subsequent treatment of two failures of PEP, tafenoquine was administered without prior chloroquine. In both cases, parasitaemia was rapidly cleared and no further clearance occurred.

Other studies conducted in Thailand also indicated that tafenoquine may have significant activity against the vivax strain of malaria [Walsh et al, 1999]. These studies demonstrated that tafenoquine was more effective in preventing vivax malaria relapse following acute infection than was chloroquine alone, or primaquine.

With this information, it was hypothesised that tafenoquine may be even more effective at preventing further relapses of vivax malaria if it were given over a longer period. Initial clearance of parasites was undertaken with chloroquine, followed by a loading dose of tafenoquine 200 mg daily for 3 days and then weekly for a further 8 weekly doses. It was postulated that this would expose the hypnozoite stage of vivax malaria to adequate doses of tafenoquine to be effective in preventing the maturation of the hypnozoite and subsequent merozoite release into the blood. Eight weeks of dosing was selected for the study based on the observed median to onset between relapses of 42 days plus a margin of a further of 2 weeks.

Although the subjects in the treatment study presented in Chapter 5 received tafenoquine, it was felt to be important to identify a control population against which the study results could be compared. To this end, a population consisting of ADF members, who had been exposed to malaria in Timor Leste during the same time interval as the subjects included in the pilot study, and who had subsequently developed vivax infection were identified. No compliance data was collected for this group and it is assumed that ADF members in this group followed the requirements for primaquine post-exposure prophylaxis as outlined in ADF HD 215- Malaria. It is likely that compliance in this group may not have been complete, even though a review of the PM-40 Notification of Malaria forms (ADF malaria reporting form to AMI) for these members indicates that they had complied. The interpretation may therefore be subject to a degree of bias towards effectiveness for tafenoquine. Given that this study design was an open label pilot study, the use of a “de facto” population is considered justified to determine gross effectiveness differences.

The challenges of drug development for the prevention and management of malaria infection in man are great due to the complexity of the life cycle of the parasite in man and the mosquito vector. While the cycles represent the opportunity to target at various stages, the hypnozoite stage represents a particular challenge as clearing the parasite from the blood without addressing dormant liver stages as seen with *P.* v*ivax* infections leads to the possibility of recurrences of the infection over time despite what initially appears to be a treatment success.

2.2 Study sites

2.2.1 Bougainville

The early cohort (AMI001) of the study presented in Chapter 4 was conducted on the islands of Bougainville and Buka, North Solomons province, Papua New Guinea. Conflict in the area over the proceeding 10 years had led to a marked increase in malaria transmission, largely due to a failed health service, inadequate drug supply and a failure of public health measures designed to control vector numbers. The area was considered to be highly malarious. The principal location of ADF personnel was in the Arawa Loloho area with smaller detachments at Buin in the south, Wakanai and Buka in the north. For orientation, a map indicating the Bougainville and surroundings is at **Figure 2.1** below:

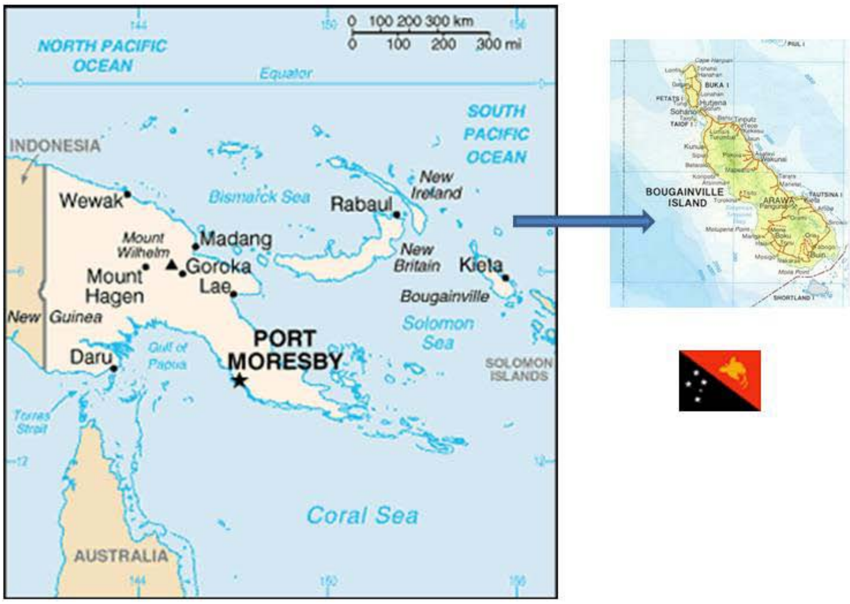

__Figure 2.1 – Orientation map of Bougainville__

2.2.2Timor Leste

Malaria is considered endemic in Timor Leste as well. Principal concentrations of Australian Defence personnel were in the capital of Dili and the Bobanaro district on the North West border with West Timor for the study presented in Chapter 3. The principal locations for Australian Defence personnel for the study presented in Chapter 4 were in Dili and surroundings, Bobonaro and the onclave of Occussi lying within West Timor. Given relatively low infection rates in study personnel evidence of malaria endemicity during the study period in the areas of study comes from several sources. An orientation map of Timor Leste is shown in **Figure 2.2**.

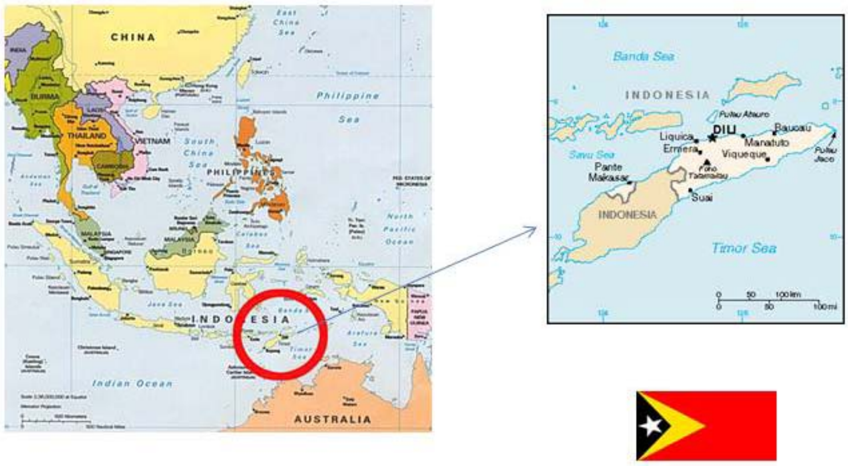

Figure 2.2 – Orientation map of Timor Leste

2.3Evidence of Malaria Endemicity

2.3.1 Cross-sectional survey in Bobonaro District

A cross-sectional survey was conducted in the indigenous population, in seven separate sites in the Bobonaro district close to where study subjects were deployed. Results showed that malaria (*P. falciparum* and *P. vivax*) was prevalent in 6 of the 7 sites studied during both phases of the survey. In areas where transmission was occurring, point prevalence rates of parasitaemia were between 1 and 19.7% overall during phase 1, with *P. vivax* being most prevalent followed by *P. falciparum*. No cases of *P. malariae* were seen in this phase. In general, rates of parasitaemia had increased by phase 2 of the survey, ranging between 1.5 and 35.3% overall. Again, *P. vivax* was the most prevalent followed by *P. falciparum* [Bragonier et al, 2002].

2.3.2 ADF Malaria Register

Data has been published from related ADF deployments [Kitchener, 2001]. Troops were routinely given doxycycline or mefloquine during deployment and treated with primaquine as terminal prophylaxis. Six months after 5500 ADF troops had returned to Australia, 267 malaria infections had been reported (5%). One third of infections were first reported during deployment (mostly *P. falciparum*) while two thirds were *P. vivax* infections which became symptomatic after return to Australia. More recent data suggests that malaria continues to be a problem for Australian troops stationed in Timor Leste. Data on infections reported to the Central Malaria Registry, Australian Army Malaria Institute up to and including the study periods are presented at **Table 2.1** below.

Table 2.1 CMR Reporting Timor Leste and Bougainville till study completion

Malaria reported to the Malaria Registry, Army Malaria Institute			
Species	**1 January – 30 June 2001**	**Total at 30 June 2001 – Timor Leste**	**Total at 30 June 2001 - Bougainville**
P. falciparum	3	51	3
P. vivax	7	335	47
Mixed	-	7	-
Uncertain	-	15	1
P. malariae	-	1	-
Total	**10**	**409**	**51**
Total from commencement of operations (September 1999 Timor Leste; November 1998 Bougainville)			

2.3.3 Mosquito field studies

2.3.3.1 Bougainville

Bougainville is highly malarious with transmission rates rivaling that found in sub-Saharan Africa. The main vector in Bougainville is *Anopheles farauti* which is a very efficient vector of malaria throughout Papua New Guinea, Solomon Islands and Vanuatu. Transmission studies conducted in Bougainville in March 1999 indicated sporozoite rates in *An. farauti* of 0.0104 for *P. falciparum* and 0.0061 for *P. vivax*, the human biting rate was 385 bites/person/night and thus the entomological inoculation rates were 4 infectious bites/person/night (1457/yr) for *P. falciparum* and 2.3 infectious bites/person/night (850/yr) for *P. vivax* [Cooper and Frances, 2002]. These studies were conducted in areas where ADF personnel were deployed during Op Bel Isi.

2.3.3.2 Timor Leste

Mosquitoes were collected in Timor Leste during their night biting phase from ADF installations and from local bodies of water. In fact (due to resource constraints) only

5% of the planned mosquito collection was performed. Of 277 *An. barbirostris* collected (known to be a malaria vector), 1 was found to be positive for both *P. falciparum* and *P. vivax* sporozoites. The low mosquito collection rate means that it is not possible to estimate the level of transmission from these data.

2.3.4 WHO Weekly Epidemiology Reports

Weekly WHO epidemiological bulletins detail reports of malaria cases from 13 districts of Timor Leste [WHO, 2011 Data from bulletins from week 41 2000 to week 17 2001 (the period of the study) show that across Timor Leste, there was a gradual increase in cases from 598 in week 41/2000 to 3063 cases in week 16/2001. Malaria continues to be a problem in Timor Leste with many of the regions experiencing malaria incidence of > 50 cases per 1000 of population reported in 2009 (WHO, 2011).

2.3.5 Malaria reported in study subjects themselves

In this study a small number of subjects in each treatment group developed post-exposure *P. vivax* malaria between 7-24 weeks after returning from the endemic area. While it is impossible to calculate a malaria attack rate from this information, it does indicate that the study population was exposed to malaria parasites.

This evidence suggests that Australian troops in this study were exposed to malaria during deployments to Bougainville and Timor Leste.

2.4 Current issues with primaquine eradication

More than 30% of *P. vivax* infections acquired in the Southwest Pacific area are not cured by the standard primaquine eradication course of 15mg base daily for 14 days. About 30 years ago, the daily adult dose of primaquine in these regions was increased to 22.5 mg daily (7.5 mg three times a day) for 14 days [Kitchener et al., 2000]. The ADF has maintained this dose regimen, reserving a higher 30 mg primaquine daily treatment course for established treatment failures.

The higher dose of primaquine had been increasingly less effective in preventing or curing vivax malaria in this part of the world. As far back as 1989, 20-25% of Australian soldiers developed malaria after returning to Australia following 3-4 week training exercises in Papua New Guinea (PNG) [Rieckmann et al., 1993]. Some of these breakthroughs were due to primaquine-refractory parasites (Chesson strain) and others were due to inadequate compliance with the cumbersome 14-day eradication regimen. More recent experience in Bougainville and other areas of PNG suggests that the ineffectiveness of the primaquine course remains a major health problem after the return of ADF personnel from malarious areas of the Southwest Pacific region. Chemoprophylaxis with daily doxycycline is able to prevent both falciparum and vivax malaria during deployments in these malarious areas. However, the persistence of cases of vivax malaria relapse after return to Australia demonstrates that the hypnozoites (liver stages of *P. vivax*) are not always eliminated by the current primaquine eradication course [Kitchener et al., 2000]. This is probably the result of a combination of the following factors:

- Insensitivity of parasites to primaquine due to the development of drug resistance
- Problems with compliance with the primaquine regimen (3 tablets a day for 14 days) after soldiers return to Australia, who are usually proceeding on leave.

Tafenoquine is a new 8-aminoquinoline drug developed by the Walter Reed Army Institute of Research (WRAIR) which, in pre-clinical models, is more active and generally less toxic than primaquine. Preliminary data from studies in Kenya suggest that tafenoquine will induce haemolysis in Glucose-6-Phosphate Dehydrogenase (G6PD) deficient individuals in the same way as primaquine [Shanks et al., 2001]. However, it is well tolerated at single doses of 400 mg of base (500 mg salt) (compared to 15 to 30 mg for primaquine) and it can be taken for a much shorter period of time than primaquine, because of its much longer duration of action. This should improve drug compliance and make it considerably more effective than primaquine in the prevention and treatment of vivax infections. In a recent clinical study in Thailand, single dose or short 3-day courses of treatment were able to achieve the radical cure of vivax infections in almost all treated patients [Walsh et al., 1999]. Therefore, tafenoquine may be more effective than primaquine in preventing vivax malaria because:

- The liver stages of *P. vivax* (hypnozoites) may be more susceptible to a higher dose of tafenoquine (400 mg daily for 3 days) than that of primaquine (22.5 mg daily for 14 days);
- Compliance with a three day course of tafenoquine should be better than the 14 day course of primaquine.

There exists an acute military and civilian need for new antimalarial drugs for chemoprophylaxis. Therefore this study was designed to compare the efficacy and tolerability of tafenoquine with primaquine in preventing *P. vivax* malaria after leaving a malarious area in the Southwest Pacific region. G6PD remains a significant issue as testing needs to be done to every study subject prior to dosing them with tafenoquine.

2.5 Key messages from this chapter

- The ADF were experiencing failures of then current prophylaxis and post-exposure prophylaxis during the period of conduct for these studies.
- There was significant exposure of ADF personnel to malaria in both Bougainville, PNG and in Timor Leste.
- The primaquine eradication schedules for the ADF required modification over the study period in response to increased case reports of malaria.
- Both primaquine and tafenoquine produce haemolysis in individuals who are G6PD deficit.

2.6 References

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World Health Organisation. (2011) URL. http://www.who.int/hac/crises/tls/en/index.html (accessed on 23rd June 2011).

Chapter 2 Paper 2.1

Elmes NJ, Bennett SM, **Nasveld PE**. (2004) Malaria in the Australian Defence Force: the Bougainville experience. *ADF Health*. 5: 69-72.

Chapter 2 Paper 2.2

Kitchener S, **Nasveld P**, Russell B, Elmes N. (2003) An outbreak of malaria in a forward battalion on active service in East Timor. *Military Medicine.* 168: 457-459.

Chapter 2 Paper 2.3

Kitchener SJ, **Nasveld PE**, Gregory RM, Edstein MD. (2005) Mefloquine and doxycycline malaria prophylaxis in the Australian soldiers in East Timor. *Medical Journal of Australia*. 182: 168-171.

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Chapter 2 Paper 2.4

Bragonier R, **Nasveld P**, Reyburn H, Edstein M, Auliffe A. (2002) Rainy season prevalence of malaria in Bobonaro district, East Timor. *Annals of Tropical Medicine and Parasitology* 96: 739-743.

CHAPTER 3

- **A randomised, double-blind, comparative study to evaluate the safety, tolerability and effectiveness of tafenoquine and mefloquine for the prophylaxis of malaria in non-immune Australian soldiers deployed to Timor Leste**
- **List of Peer-reviewed and published papers presented in this chapter**

3.1 **Nasveld PE**, Edstein MD, Reid M, et al. (2010) for the Tafenoquine Study Team. Randomized, double-blind study of the safety, tolerability and efficacy of Tafenoquine verses mefloquine for malaria prophylaxis in non-immune subjects. *Antimicrobial Agents and Chemotherapy*. 54: 792-798.

PN and ME participated in the conception and design of the study and PN drafted the manuscript. ME provided significant editing assistance with the final paper. PN, ME, MR and the statistical contributors of the Tafenoquine Study Team contributed to the analysis of the study. All authors gave final approval for the manuscript submitted for publication.

3.2 Charles BG, Miller AK, **Nasveld PE**, et al. (2007) Population pharmacokinetics of tafenoquine during malaria prophylaxis in healthy subjects. *Antimicrobial Agents and Chemotherapy.* 51: 2709-2715.

All authors participated in the conception and design of the study and BGC drafted the manuscript. BG, AM and ME contributed to the analysis of the study. PN provided the clinical input to the paper. All authors gave final approval for the manuscript submitted for publication.

3.3 Charles BG, Blomgren A, **Nasveld PE**, et al. (2007) Population pharmacokinetics of mefloquine in military personnel for prophylaxis against malaria infection during field deployment. *European Journal of Clinical Pharmacology*. 63: 271-278.

All authors participated in the conception and design of the study and BC drafted the manuscript. BC, AB and ME contributed to the analysis of the study. PN provided the clinical input to the paper. All authors gave final approval for the manuscript submitted for publication.

3.4 Edstein MD, **Nasveld PE**, Kocisko DA, et al. (2007) Gender differences in gastrointestinal disturbances and plasma concentrations of tafenoquine in healthy volunteers after tafenoquine administration for post-exposure vivax malaria prophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101: 226-230.

All authors participated in the conception and design of the study and ME drafted the manuscript. PN, ME and DK contributed to the analysis of the study. PN provided the clinical input to the paper. All authors gave final approval for the manuscript submitted for publication.

3.1 Introduction

This study compared the chemosuppressive effectiveness of weekly tafenoquine and mefloquine and to obtain side effect data on both drugs over a six month period (plus three months of the relapse follow-up phase). Mefloquine is one of the most widely used drugs for the chemoprophylaxis of malaria and is recommended by the World Health Organization and the Centers for Disease Control and Prevention (CDC, USA) for protection against chloroquine-resistant falciparum malaria. Unfortunately, because of concerns about neuropsychiatric side effects, compliance with and therefore effectiveness of mefloquine has suffered. Tafenoquine, like mefloquine allows convenient dosing, and is expected to be highly efficacious against all strains of malaria, including mefloquine resistant and multidrug-resistant malaria. In addition, tafenoquine potentially offers an advantage as a prophylaxis agent that can prevent relapse caused by *P. vivax* and *P. ovale* malaria.

3.2 Objectives

3.2.1 Primary Objective

The primary study objective was to compare the safety and tolerability of tafenoquine and mefloquine over a 6 month treatment period.

3.2.2 Secondary Objectives

The secondary study objectives were as follows:

- To assess the effectiveness of tafenoquine and mefloquine for chemoprophylaxis of *P. falciparum* and *P. vivax*
- To assess the effectiveness of tafenoquine and primaquine in preventing post-exposure malaria
- To characterise the population pharmacokinetics of tafenoquine and evaluate the effects of various subject characteristics on tafenoquine pharmacokinetics
- To monitor for phospholipidosis or effects of phospholipidosis in humans

3.3 Ethics

This study was conducted under approvals from the Australian Defence Medical Ethics Committee – ADMEC (now known as the Australian Defence Human Research Ethics Committee – ADHREC). Initial approval was given on the 14th June 2000 and recorded as ADMEC 216/00. Additionally, approval was required from the United States Army Sponsors.

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki, as amended in Somerset West, Republic of South Africa 1996. The protocol and statement of informed consent were approved by an Institutional Review Board prior to study initiation. The protocol was initially approved on 18th May 2000 and amended 3 times prior to the study start. The final protocol plus amendments 1, 2 and 3 were approved by the Australian Defence Medical Ethics Committee (ADMEC) prior to study start. Further amendments were made during the study, and details are set out below.

- **Amendment 1, dated 22nd June 2000**

This amendment covered a number of typographical changes and textual clarifications, along with some minor changes to the protocol as a result of discussion with the Principal Investigator.

- **Amendment 2, dated 2nd August 2000**

The majority of the changes referred to in this Amendment were the result of review of the protocol by the US Army Medical Research and Material Command's (USAMRMC) Human Subjects Research Review Board (HSRRB). There were two major changes to the protocol that were not the result of HSRRB recommendations.

- The visit schedule for the study was changed to visits at 4, 8, 16 and 26 weeks, with a 'window' of ± 4 weeks for the week 26 visit. This allowed for certain subjects being deployed for shorter or longer periods than the majority.
- The inclusion of ECGs on the 100 subjects selected for additional phospholipidosis and met haemoglobin assessments. ECGs were done at screening and at the final prophylaxis visit for these subjects, to monitor for any possible prolongation of the QT interval.

- **Amendment 3, dated 28th September 2000**

This amendment was produced following the unexpected results seen in the Phase III Kenya study, 252263/030. It was considered appropriate to change the primary efficacy end-point of this study from a single positive smear (either with or without symptoms of malaria) to a single point positive smear *with* signs and symptoms consistent with malaria.

- **Amendment 4, dated 23rd November 2000**

This amendment was produced by the investigator as a result of a 'corrective action report' produced at a monitoring visit in Timor Leste by USAMMDA. It has already been submitted to ADMEC before GSK staff were made aware of its existence. It deals with a number of minor alterations to the protocol, which would normally have been dealt with in the main by 'notes to file'.

- **Amendment 5, dated 9th February 2001**

This amendment dealt with two changes to the protocol.

- The definition of 'hospitalisation' was updated in line with a revised study SOP to ensure that serious adverse events were not over reported, as many soldiers were hospitalised for reasons that, in a non-military situation, they would not otherwise have been. Principally these were routine hospital appointments where soldiers stayed at the hospital facility overnight while waiting for transport back to their base units in the field the following day.
- The second change dealt with the re-scheduling of the first visit of the Relapse Follow-up Phase from Week 6 to Week 12 to ensure that all subjects would be available for the visit.

- **Amendment 6, dated 6th April 2001**

As all subjects were not scheduled to receive their final weekly dose on the same day as leaving the malarious area, this amendment introduced an additional dose of study medication for those subjects leaving Timor Leste more than 24 hours after their last weekly dose. This was considered necessary to ensure that drug levels for those subjects receiving tafenoquine would remain sufficiently high to afford protection against breakthrough *P. falciparum* infection in the 2 to 3 week period after leaving the malarious area.

Written informed consent was obtained from each subject prior to entry into the study. Case Report Forms (CRFs) were provided for each subject's data to be recorded.

3.4 Methods

3.4.1 Study design

The study was divided into two phases. The first phase ('prophylactic phase') was randomised, double-blind and "double-dummy" and consisted of a 26 week (± 4 weeks) period where subjects received prophylactic study medication (tafenoquine or mefloquine in a ratio of 3:1). This phase compared the safety, tolerability and effectiveness of weekly regimens of the two drugs for the prophylaxis of malaria. It took place during a military deployment of the Australian Defence Force (ADF) to Timor Leste. Subjects who met the eligibility criteria were randomised to receive a loading dose of either tafenoquine 200 mg or mefloquine 250 mg per day for three days, followed by study treatment (tafenoquine 200 mg or mefloquine 250mg) once a week throughout the period of deployment.

Those subjects who completed the prophylactic phase entered a 24-week 'relapse follow-up phase'. At the end of the deployment, once the subjects had returned to barracks in Townsville, Australia, they received a 14day double-blinded supervised primaquine (15 mg bid) or primaquine placebo regimen. Those who took mefloquine during the prophylactic phase received primaquine 15 mg bid, whilst those who had taken tafenoquine received placebo capsules twice daily during this period. The 'relapse follow-up phase' took place in Australia, after subjects had returned to their normal duties. This phase was designed to monitor the efficacy of tafenoquine and primaquine in preventing post-exposure relapse of malaria. Subjects were followed up over 12 weeks (for safety) involving 2 visits, followed by a further 12 weeks (for malaria relapse) involving 2 further visits or contact by telephone. The study schedule is outlined in **Figure 3-1** below.

Table 3-1 Outline of study procedures and assessments

Prophylactic Phase	**Days**				**Weeks**				*Relapse Follow-up Phase*	**Weeks (after end of prophylactic phase)**			
	-14 to -1	0	1	2	4	8	16	26±4		6	12	18* (phone)	24* (phone)
Eligibility	*												
Physical exam	*							*	**Physical exam**		*		
Medical history	*												
ECG †	*							*					
Blood smear¶	*				*	*	*	*	**Malaria status**	*	*	*	*
Haematology / Biochemistry	*			*	*	*	*	*	**Biochemistry**		*		
Plasma drug concentration				*	*	*	*	*					
Pregnancy test	*				*	*	*	*	**Pregnancy test**		*		
Baseline signs and symptoms	*	*											
Concomitant medication	*	*	*	*	*	*	*	*	**Concomitant medication**	*	*		
Adverse events			*	*	*	*	*	*	**Adverse events**	*	*		
Phospholipidosis assessments †	*							*					
Methaemoglobin †	*							*					

† Phospholipidosis, methaemoglobin and ECG measurements performed on a sample of approximately 100 subjects only.

* Originally designated as telephone contact only, but for some subjects took the form of a visit in person

3.4.2 Participants

Participants were healthy, as defined by Medical Class 1 or 2 (Australian Army standard), males or females aged between 18 and 55 years inclusive. Subjects with demonstrated G6PD deficiency, a history of allergy or intolerance to study medication, a history of psychiatric disorders and/or seizures, or a history of drug or alcohol abuse were to be excluded. In addition subjects with clinically significant medical history, concurrent conditions, or laboratory test results were also to be excluded.

In total, 663 participants were screened for entry into the study: 9 of these subjects did not proceed to dosing. As a result 654 subjects were randomised in a 3:1 ratio; i.e. 474 subjects in the tafenoquine group and 158 subjects in the mefloquine group, to prophylactic study medication. Demographic details for the intent-to-treat and per protocol populations are summarised in **Table 3-2**. It was planned that a sub-group of approximately 100 subjects would undergo extra safety assessments in order to investigate any potential phospholipidosis effects. In total 98 subjects formed this sub-group; 77 subjects from the tafenoquine group and 21 subjects form the mefloquine group.

Table 3-2 Demographic characteristics: intent-to-treat and per protocol populations

Demographic Characteristic	Intent-to-treat population		Per Protocol population	
	Tafenoquine 200 mg N=492	Mefloquine 250 mg N=162	Tafenoquine 200 mg N=462	Mefloquine 250 mg N=153
Gender N (%)				
Male	478 (97.2%)	154 (95.1%)	448 (97.0%)	147 (96.1%)
Female	14 (2.8%)	8 (4.9%)	14 (3.0%)	6 (3.9%)
Age (years)				
18-25	286 (58.1%)	97 (59.9%)	269 (58.2%)	91 (59.5%)
26-35	178 (36.2%)	48 (29.6%)	166 (35.9%)	47 (30.7%)
36-45	27 (5.5%)	16 (9.9%)	26 (5.6%)	14 (9.2%)
46-55	1 (0.2%)	1 (0.6%)	1 (0.2%)	1 (0.7%)
mean (SD)	25.4 (5.3)	26.0 (6.5)	25.4 (5.2)	25.9 (6.5)
Range	18 – 47	18 – 51	18 – 47	18 – 51
Race N (%)				
White	484 (98.4%)	160 (98.8%)	455 (98.5%)	151 (98.7%)
ATSI*	4 (0.8%)	1 (0.6%)	4 (0.9%)	1 (0.7%)
Other	4 (0.8%)	1 (0.6%)	3 (0.6%)	1 (0.7%)
Weight (kg)				
mean (sd)	80.9 (11.9)	81.3 (12.2)	81.0 (11.9)	81.4 (12.3)
Range	50 – 135	53 – 135	50 – 135	53 – 135
Height (cm)				
mean (sd)	177.8 (7.0)	177.1 (6.7)	177.9 (7.0)	177.2 (6.7)
Range	155 – 198	157 – 192	155 – 198	157 – 192
Company				
A (Rifle Company)	65 (13.2%)	22 (13.6%)	64 (13.9%)	21 (13.7%)
B (Rifle Company)	90 (18.3%)	25 (15.4%)	84 (18.2%)	24 (15.7%)
C (Rifle Company)	67 (13.6%)	21 (13.0%)	65 (14.1%)	20 (13.1%)
D (Rifle Company)	80 (16.3%)	27 (16.7%)	71 (15.4%)	26 (17.0%)
E (HQ)	92 (18.7%)	32 (19.8%)	87 (18.8%)	30 (19.6%)
F (Others)	98 (19.9%)	35 (21.6%)	91 (19.7%)	32 (20.9%)

*ATSI = Aboriginal or Torres Strait Islander

As expected from this military population, the majority of subjects were young white males. The majority of subjects in the study were male; 478/492 (97.2%) in the tafenoquine group and 154/163 (95.1%) in the mefloquine group. The mean age was 25.4 yrs in the tafenoquine group and 26.0 years in the mefloquine group. The overall age range was 18-51 years. The majority of subjects (>98%) were white, with <1% subjects in each group of Australian Aboriginal or Pacific island origin. There were no marked differences between the groups in demographic characteristics.

There were no marked differences in the demographic characteristics of subjects with additional safety assessments from those for the intent-to-treat and per protocol populations. All the subjects with additional safety assessments were male and all but one was White. The mean age was slightly lower than in the intent-to-treat population, at around 23 years.

3.4.3 Treatment administration

Subjects received a loading dose of either tafenoquine 200 mg or mefloquine 250 mg per day for three days, followed by study treatment (tafenoquine 200 mg or mefloquine 250 mg) once a week throughout the period of deployment.

At the end of the prophylactic phase, subjects received twice daily primaquine 15mg, or twice daily placebo, for 14 days. Those who took mefloquine during the prophylactic phase received primaquine, whilst those who had taken tafenoquine received placebo during this period.

Batch nos: N99354 (tafenoquine); N00061(tafenoquine-placebo); N00212 (mefloquine); N99330 (mefloquine-placebo); N00223, N00228 (primaquine); N00061 (primaquine-placebo).

3.4.4 Criteria for evaluation

3.4.4.1 Efficacy

The primary efficacy variable was prophylactic outcome (success/failure) during the prophylactic phase, up to and including the first day of primaquine eradication medication. The subjects were monitored for any clinical signs and symptoms of malaria at each visit. In addition, blood smears were taken at baseline and at each visit during the prophylaxis phase. During the relapse follow-up phase subjects were to report any clinical signs or symptoms of malaria, at which time a blood smear was to be taken.

Prophylaxis success/failure was defined as follows:

- **Prophylactic Success**: No clinical malaria (single positive smear (any species) with concurrent clinical signs and symptoms consistent with malaria infection) during prophylactic study drug administration up to and including the day of the first dose of eradication medication.
- **Prophylactic Failure**: Clinical malaria (single positive smear (any species) with concurrent clinical signs and symptoms consistent with malaria infection) during

prophylactic study drug administration up to and including the day of the first dose of eradication medication.

The secondary efficacy variables analysed were:

- number of subjects experiencing clinical malaria at any time during the study (prophylactic phase plus 6 months relapse follow-up phase);
- number of subjects with a single positive smear (any species, with or without clinical signs/symptoms) during prophylactic study drug administration;
- time to clinical malaria (all species) at any time during the study (prophylactic phase plus 6 months relapse follow-up phase);
- time to single positive smear (all species) with or without clinical signs/symptoms during prophylactic study drug administration.

Planned analyses involving occurrence of clinical malaria and a single positive smear (*P. falciparum* only and *P. vivax* only) were not performed as there were no subjects with clinical malaria or a positive smear during prophylactic treatment.

Malaria prevalence at the time of the study was estimated by performing a cross-sectional survey and an entomology study. Published data sources were used to support this evidence (see Chapter 2).

3.4.4.2 Safety

Adverse events were collected at each visit during the prophylactic phase and the relapse follow-up phase. Blood was taken for haematology and clinical chemistry analysis at baseline, at each visit during the prophylactic phase and at the 12 week visit of the relapse follow-up phase.

In order to assess any phospholipidosis effects, more detailed safety assessments were carried out in a sub-group of approximately 100 subjects. These examinations included ophthalmic examination, lung function assessment, electron microscopy of peripheral blood lymphocytes and methaemoglobin assessment. ECGs were also performed to assess any effect on QTc interval.

As a result of laboratory findings in this study and across the tafenoquine program, a long-term renal follow-up was conducted in a cohort of subjects with serum creatinine concentrations ≥0.02 mmol/L (0.23 mg/dL) above baseline at the end of the end of the prophylactic phase and/or at follow-up.

3.4.4.3 Pharmacokinetics

Blood samples for assessment of plasma drug levels were collected at predetermined randomised times/days on or after dosing on day 2 and weeks 4, 8, 16 and 26 of the prophylactic phase. Any subject diagnosed with clinical malaria during the prophylactic phase would have two additional samples taken: one at the time of diagnosis and the second after 12 weeks of follow-up.

The concentration-time data from this study were to be pooled with those from other phase III studies in order to obtain a pooled population PK analysis, and reported separately. However, a population pharmacokinetic analysis of the data from this study was ultimately undertaken by the Australian Army Malaria Institute, and University of Queensland, Brisbane, Australia, in collaboration with GSK.

3.4.5 Statistical methods

3.4.5.1 Sample Size

In order to allow comparisons of safety to be made between tafenoquine and mefloquine given over 6 months, with a reasonable precision, at least 450 tafenoquine and 150 mefloquine subjects would need to complete the 6 month prophylactic phase. Approximately 5% of subjects randomised were expected to drop-out, so 632 subjects were to be randomised in a 3:1 ratio, with 474 subjects randomised to tafenoquine and 158 to mefloquine.

For efficacy, with 450 subjects on tafenoquine and 150 on mefloquine, the study had 94% power to detect that the upper limit of the two- sided 95% confidence interval for the difference in failure rates (tafenoquine – mefloquine) at the end of the prophylactic phase was no more than 10%, assuming an underlying failure rate of 10% in each treatment group.

3.4.5.2 Principal Analysis

Treatment groups were compared for prophylactic outcome by calculating the difference in the proportion of prophylactic failures (tafenoquine-mefloquine) with a 95% confidence interval (CI) within the Per Protocol Population (PPP). The CI was calculated for the difference in two binomial proportions using standard normal approximation theory. A conclusion of non-inferiority of tafenoquine was to be drawn if the upper limit of the CI was no more than 10%.

As many subjects did not stay with the company to which they had originally been allocated, the analysis stratified by Company of Battalion was not performed. The primary analysis was based on all species of malaria parasitaemia.

3.4.5.3 Confirmatory Analyses

These were carried out using:

- the intent-to-treat (ITT) population; and
- a worst-case analysis in which subjects withdrawing during the prophylactic phase were included as failures.

A planned covariate analysis to investigate the effect of weight was not performed because there were insufficient failures in this study.

3.4.5.4 Analysis of Secondary Efficacy Variables

For secondary variables involving numbers of subjects, treatment differences in proportions with 95% CIs were calculated.

3.4.4.3 Pharmacokinetics

Modelling for tafenoquine was performed using NONMEM, utilising a single compartment model. Mefloquine modelling was undertaken using NONMEM with the samples from this study being pooled with the samples from a further 950 subjects on mefloquine (ADHREC 249/01) using a two-compartment model (see paper 3.4 for detail of methods).

3.5 Results

3.5.1 Subject disposition and demographic data

A total of 654 subjects participated in this study; 492 in the tafenoquine group and 162 in the mefloquine group. The number of withdrawals was low in both treatment groups (< 5%). There were no withdrawals due to prophylaxis failure during the prophylactic phase. The proportion of subjects withdrawn due to adverse events was similar in both treatment groups (2.4-2.5%).

Overall, 2.5-3% of subjects had a history of malaria, with 0.6-1.8% reporting an attack in the last 6 months. As expected, the mean duration of deployment was 26-27 weeks. Most subjects left Timor Leste temporarily for Relief out of Country Leave (ROCL), so the mean time spent in east Timor was just under 26 weeks.

In general the treatment groups were similar at screening with respect to active conditions. The most commonly reported prior condition was arthropod-borne disease (other), reported by 2.4% of subjects in the tafenoquine group and 2.5% of subjects in the mefloquine group. The most commonly occurring baseline events were respiratory tract infections and dyspepsia. In general, the treatment groups were similar at screening with respect to baseline signs and symptoms. All subjects were to receive ivermectin as standard pre-deployment to prevent lymphatic filariasis. Ivermectin was also given post-deployment, as well as albendazole (standard post-deployment anti-helminthic treatment). Apart from this, the most common medications taken during the study were paracetamol and codeine.

In the prophylactic phase, >98% of subjects were compliant with study medication; 99.8% in the tafenoquine group and 98.8% in the mefloquine group. The majority of subjects (334/492 (67.9%) in the tafenoquine group and 107/162 (66%) in the mefloquine group) took their last dose on the day they left Timor Leste. Most of the remaining subjects (142/492 (28.9%) in the tafenoquine group and 49.162 (30.2%) in the mefloquine group) took their last dose within 3 days of leaving east Timor. Following the prophylactic phase, > 96% of subjects in both treatment groups were compliant with primaquine eradication medication or placebo.

3.5.2 Efficacy results

The principal efficacy analysis was based on the PPP and the ITT population was used to confirm the findings of the principal analysis.

3.5.2.1 Primary efficacy analysis

Prophylactic outcome for each treatment group during prophylactic treatment is summarised in **Table 3-3** for the per protocol population. All subjects were prophylactic successes during the prophylactic phase and all were known successes.

Table 3-3 Prophylactic outcome based on clinical malaria (all species) during prophylactic treatment phase: per protocol population and intent-to-treat population

Population	Per protocol population		Intent to treat population	
	Tafenoquine	Mefloquine	Tafenoquine	Mefloquine
	N=462	N=153	N=490	N=161
Prophylactic success (total)	462 (100%)	153 (100%)	490 (100%)	161 (100%)
Prophylactic success (known)	462 (100%)	153 (100%)	473 (96.5%)	157 (97.5%)
Prophylactic success (assumed)	0	0	17 (3.5%)	4 (2.5%)
Prophylactic failure	0	0	0	0

Assumed success = no malaria during participation in the study for subjects withdrawn during prophylactic phase

3.5.2.1.1 Worst Case Analysis

The analysis was repeated assuming all subjects who withdrew during the prophylactic phase to be prophylactic failures (i.e. ‘assumed successes’ considered as failures). Even in this worst case analysis, prophylactic success at the end of the prophylactic treatment period was >96% in both groups with no difference between the treatment groups.

3.5.2.2 Secondary efficacy analysis

Prophylactic outcome for each treatment group during prophylactic treatment plus relapse follow-up phases is summarised in **Table 3-4**.

Table 3-4 Prophylactic outcome based on clinical malaria (all species) at any time during the study: per protocol and intent-to-treat populations

Population	Per protocol population		Intent to treat population	
	Tafenoquine + Placebo	Mefloquine + Primaquine	Tafenoquine + Placebo	Mefloquine + Primaquine
	N=462	N=153	N=490	N=161
Prophylactic success (total)	458 (99.1%)	152 (99.3%)	486 (99.2%)	160 (99.4%)
Prophylactic success (known)	458 (99.1%)	152 (99.3%)	469 (95.7%)	156 (96.9%)
Prophylactic success (assumed)	0	0	17 (3.5%)	4 (2.5%)
Prophylactic failure	4 (0.9%)	1 (0.7%)	4 (0.8%)	1 (0.6%)
Treatment difference (tafenoquine-mefloquine)	0.21		0.20	
95% CI	-1.32, 1.74		-1.26, 1.65	

There were four cases of malaria in the tafenoquine group (0.9%) and one case in the mefloquine group (0.7%). All were cases of *P. vivax* malaria occurring during the relapse follow-up phase. The four tafenoquine subjects all received their last dose of study medication on leaving the endemic area. The mefloquine subject received their last dose of study medication three days before leaving the endemic area. There were no differences between the groups and there were no reports of mixed species malaria infections. Similar results are seen for the ITT population.

A number of planned analyses could not be conducted due to no subjects developing clinical malaria during the study period. These analyses are detailed below:

- Clinical malaria and single positive smear (*P. falciparum* only and *P. vivax* only) during prophylactic treatment;
- Time to single positive smear (all species) during prophylactic treatment;
- Clinical malaria by species and time;
- Single positive smear by species and time

3.5.3 Safety results

3.5.3.1 Extent of exposure

3.5.3.1.1 Prophylactic study medication exposure

More than 95% of subjects in both treatment groups received at least 26 weeks of prophylactic study therapy. Mean exposure was similar in each group: 189 days in the tafenoquine group and 191 days in the mefloquine group.

3.5.3.1.2 Eradication medication exposure

More than 94% of subjects in both treatment groups received at least 14 days of eradication therapy. Mean exposure was 14 days in each group.

3.5.3.2 Adverse events

The most commonly reported adverse events during the prophylactic phase (occurring in ≥ 10% subjects in either treatment group) are shown below (Table 3.4). The majority of events were of mild or moderate intensity and occurred for the first time within the first 8 weeks of the study.

Table 3-4 Number of subjects with the most frequently reported adverse events during prophylactic phase: safety population

Adverse Event	Treatment group	
	Tafenoquine N=492	Mefloquine N=162
At least one adverse event	454 (92.3%)	143 (88.3%)
Gastroenteritis	182 (37.0%)	51 (31.5%)
Injury	178 (36.2%)	49 (30.2%)
Upper respiratory tract infection	101 (20.5%)	32 (19.8%)
Diarrhoea	77 (15.7%)	30 (18.5%)
Back pain	74 (15.0%)	26 (16.0%)
Rash	70 (14.2%)	21 (13.0%)
Headache	61 (12.4%)	20 (12.3%)
Arthralgia	55 (11.2%)	18 (11.1%)

In general the incidence and nature of adverse events during the prophylactic phase was similar across the two treatment groups. The most commonly occurring events, with an incidence of >30% in both treatment groups, were gastroenteritis and injury. Among the injuries reported were soft tissue injuries, animal bites and headache or nausea caused by inhalation of toxic fumes. Approximately 20% of subjects in each treatment group had an upper respiratory tract infection. There were no statistically significant difference between the groups in the incidence of adverse events.

Adverse events occurring during the relapse follow-up phase were generally similar to those occurring during the prophylactic phase. During the relapse follow-up phase, 203/492 (41.3%) subjects in the tafenoquine group and 53/162 (32.7%) subjects in the mefloquine group reported an adverse event. With the exception of eye abnormalities (see below), no individual event occurred in ≥10% subjects in either treatment group. The most common events were upper respiratory infection and injury. All other events occurred in < 3% subjects in either treatment group.

A total of 66 subjects (13.4%) in the tafenoquine group and 19 (11.7%) in the mefloquine group had adverse events in the prophylactic phase with a suspected/probable relationship to study treatment. The most commonly reported events were nausea and vertigo (<3%). No other event occurred in ≥ 2% of subjects in either treatment group.

3.5.3.3 Serious Adverse Events and Withdrawals

A total of 23 subjects experienced serious adverse events (SAE) during the prophylactic phase: 18/492 (3.7%) subjects in the tafenoquine group and 5/162 (3.1%) subjects in the mefloquine group. In addition, 10 subjects experienced serious adverse events during the relapse follow-up phase; 8/492 (1.6%) subjects in the tafenoquine/placebo group and 2/162 (1.2%) subjects in the mefloquine/primaquine group. In 7 subjects in the tafenoquine group, these were 7 subjects with eye abnormalities. Of the 69/74 subjects with eye anomalies subsequently reported, these initial findings 7 subjects were notified as SAE at the request of the study sponsor in order to initiate a Safety Alert to all sites using tafenoquine at the time. SAE are presented at **Table 3-5**. There were no deaths reported during the prophylactic phase or during relapse follow-up phase.

Table 3-5 Number of subjects with serious adverse events during the prophylactic and relapse follow-up phase

Serious Adverse event	Treatment Group	
	Tafenoquine 200 mg N=492	Mefloquine 250 mg N=162
Prophylactic Phase		
At least one serious AE	18 (3.7%)	5 (3.1%)
Injury	3 (0.6%)	2 (1.2%)
Colitis	3 (0.6%)	0
Gastroenteritis	3 (0.6%)	0
Abdominal pain	2 (0.4%)	1 (0.6%)
Nail disorder	2 (0.4%)	0
Epididymitis	1 (0.2%)	1 (0.6%)
Diarrhoea	1 (0.2%)	0
Gastrointestinal disorder NOS	1 (0.2%)	0
Irritable bowel syndrome	1 (0.2%)	0
Pharyngitis	1 (0.2%)	0
Viral infection	1 (0.2%)	0
Rash	0	1 (0.6%)
	Tafenoquine 200 mg Placebo N=492	Mefloquine 250 mg Primaquine 15 mg N=162
Relapse Follow-up Phase		
At least one serious AE	8 (1.6%)	2 (1.2%)
Eye abnormality	5 (1.0%)	0
Retinal disorder	2 (0.4%)	1 (0.6%)
Injury	1 (0.2%)	0
Upper respiratory tract infection	0	1 (0.6%)

A total of 14 subjects withdrew during the prophylactic phase: 11/492 (2.2%) in the tafenoquine group and 3/162 (1.9%) in the mefloquine group. Most of the events leading to withdrawal were injuries or arthralgia, none of which was reported as related to study treatment. Three subjects, all in the tafenoquine group, had events reported with a suspected relationship to study treatment: abdominal pain, depression and hyperaesthesia.

3.5.3.4 Phospholipidosis Assessments

3.5.3.4.1 Lung Function tests

There was a mean reduction in percentage predicted diffusion capacity of carbon monoxide (DLCO) at the end of the prophylactic phase in both groups. Mean FEV_1 also showed a reduction in both groups. There were no differences between the treatment groups in the change in percent predicted DLCO or FEV_1 from baseline.

3.5.3.4.1 Ophthalmic Assessments

Detailed ophthalmic assessments to monitor the possible effects of phospholipidosis were performed in a sub-group of study participants. A total of 74 tafenoquine subjects and 21 mefloquine subjects underwent ophthalmic examination at baseline, including visual acuity and field tests, colour vision tests and physical examination. No subjects had a clinically significant abnormality at baseline. At the end of prophylaxis visit, corneal deposits (vortex keratopathy) or suspected corneal deposits were reported in 69/74 (93.2%) of subjects in the tafenoquine group and 0/21 subjects in the mefloquine group. Due to this unexpected finding, more detailed examinations were carried out than had been planned in the protocol, including detailed retinal and corneal examination and photography. Some of these examinations were conducted with the knowledge that the subject had corneal deposits and were therefore unblinded.

There were no notable changes from baseline or differences between the treatment groups in visual field tests (Amsler Grid and Humphrey Perimetry), visual acuity (Snellen) or colour vision (Ishihara, SPP2 plates, FM100 test).

Subjects with corneal deposits were followed up beyond the scheduled 3-month follow-up visit during the relapse follow-up period. At each follow-up corneal deposits were noted to have improved, with all subjects having resolved within 1 year of stopping study medication. Results are shown in **Table 3-6** below.

Table 3-6 Number of subjects with corneal deposits during the follow-up period

	End of Prophylaxis	3 Months*	6 Months*	1 year*
No. of subjects with vortex keratopathy	69/74 (93.2%)	32/74 (43.2%)	6/74 (8.1%)	0
No. subjects with vortex keratopathy resolved		37/69 (53.6%)	63/69 (91.3%)	69/69 (100%)
* Timings are approximate				

Fundoscopy examinations were carried out on 86 subjects at baseline and at the 3 month post prophylaxis follow-up visit. Examiners were aware of corneal deposits (if present), and were therefore unblinded in that respect. Fundoscopy examinations revealed abnormalities (e.g. granularity/pigmentation of retinal pigment epithelium, hard drusen) in 27/69 (39.1%) of tafenoquine subjects and 4/17 (23.5%) of mefloquine subjects. Fundus fluoroscein angiograms (FFA) were performed in 14 tafenoquine subjects and 1 mefloquine subject in whom possible retinal findings had been observed; of these 4/14 (28.6%) subjects in the tafenoquine group and 1/1 (100%) subject in the mefloquine group were considered to have abnormal findings. As a result of these findings, an expert ophthalmology board were asked to review the data from this study. They concluded that the corneal changes were benign, fully reversible and similar to those seen with other drugs, such as chloroquine. The expert ophthalmology advisory board advised that vision had not been affected in any of these subjects. Lack of baseline retinal photography data meant that the relevance of the retinal findings (observed on fundoscopy and fundus fluoroscein angiograms) could not be ascertained. They noted that the results observed could reflect normal variability and the subjective nature of the examinations. They did not consider that the fundus fluorescein angiograms results provided evidence of a drug effect.

3.5.3.4.1 Electron microscopy of peripheral blood lymphocytes

In the peripheral blood leucocytes, there were no clear differences between either the tafenoquine or mefloquine treated group. Both compounds introduced a low level of ultrastructural changes consistent with phospholipid accumulation within the peripheral leucocytes in 24% and 50% of the subjects respectively. However, given the low numbers of peripheral blood leucocytes affected (up to 3% per subject); each containing mainly single lysosomal lamellar inclusion bodies, it is not considered that these ultrastructural changes were of any clinical significance.

3.5.3.5 Electrocardiograph data

ECGs were performed at baseline and at end of the prophylactic phase in 77 tafenoquine subjects and 21 mefloquine subjects. In the tafenoquine group, mean QTc interval showed a small reduction (-4.5 msec) from baseline whereas mean QTc interval showed a small increase (1.6 msec) from baseline in the mefloquine group. These changes were not considered to be clinically relevant.

There were two subjects with a prolonged QTc interval at baseline in the tafenoquine group, with no subjects with a prolonged QTc interval at the end of the prophylactic phase. However, the proportion of subjects with a borderline result was larger in the tafenoquine group than the mefloquine group at the end of the prophylactic phase.

3.5.3.6 Laboratory Data

At the end of the prophylactic phase there were generally only small changes from baseline in laboratory test results and few differences between the treatment groups (**Table 3.7**). The change from baseline at each visit (mean increase and number of subjects with a significant increase) in creatinine and bilirubin was slightly larger in the tafenoquine than in the mefloquine group. There was also a more noticeable decrease from baseline in haematocrit values in the tafenoquine group compared to the mefloquine group. Conversely, the increase from baseline in platelets at each visit was larger in the mefloquine than in the tafenoquine group.

Only a small number of subjects (~5) had haematology assessments performed at follow up, so it is not possible to draw any conclusions from this group. At follow up the difference between the treatment groups for biochemistry indices had mostly resolved. The mean change in bilirubin at the follow-up visits was 4.1 μmol/L in the tafenoquine group and 2.5 μmol/L in the mefloquine group.

Table 3-7 Clinical chemistry changes from baseline for bilirubin and creatinine and haematology changes from baseline for haematocrit and platelets: prophylactic phase

Bilirubin							
	Base-line	Days 0-10	2-6 weeks	7-12 weeks	13-21 weeks	22-30 weeks	Follow-up
Mean change from baseline (μmol/L)							
Tafenoquine	9.0	0	3.8	3.0	3.6	3.8	4.1
(N)	413	408	408	411	401	390	391
Mefloquine	9.5	0.1	0.1	0.4	1.1	1.6	2.5
(N)	135	131	134	135	135	132	132
Creatinine							
Mean change from baseline (μmol/L)							
Tafenoquine	88.7	6.1	16.2	13.2	9.6	12.1	7.0
(N)	479	475	474	477	468	454	454
Mefloquine	88.7	5.3	10.7	8.0	5.0	8.8	6.7
(N)	156	152	155	156	155	151	152
Hematocrit							
	Base-line	Days 0-10	2-6 weeks	7-12 weeks	13-21 weeks	22-30 weeks	Follow-up
Mean change from baseline (%)							
Tafenoquine	48.1	-0.6	-4.4	-3.5	-4.1	-2.8	*
(N)	492	479	486	488	483	474	
Mefloquine	47.8	0.1	-2.8	-2.4	-4.0	-2.3	*
(N)	162	158	160	161	160	159	
Platelets							
	Base-line	Days 0-10	2-6 weeks	7-12 weeks	13-21 weeks	22-30 weeks	Follow-up
Mean change from baseline ($x10^9$/L)							
Tafenoquine	263.8	5.3	-3.0	-2.9	-7.0	12.0	*
(N)	492	480	485	489	483	474	
Mefloquine	264.5	15.4	19.0	19.8	15.3	30.8	*
(N)	162	158	160	161	160	159	

For most laboratory variables the proportion of subjects in either group with results which were flagged as changing from during the prophylactic phase was similar. There were some differences between the treatment groups for creatinine, bilirubin and platelets, reflecting the mean changes from baseline. However, for bilirubin and creatinine, there was a higher incidence of shift in laboratory values in the tafenoquine compared to the mefloquine group: for creatinine 11.3% subjects had an flagged shift at final prophylaxis visit in the tafenoquine group compared to 7.1% of subjects in the mefloquine group; for total bilirubin 33.8% subjects had an flagged high shift at final prophylaxis in the tafenoquine group compared to 20.5% of subjects in the mefloquine group.

At follow-up, the differences between the treatment groups for creatinine had resolved: 6% of subjects had a flagged shift in the tafenoquine group compared to 8.2% of subjects in the mefloquine group. For total bilirubin 34.4% subjects had a high flagged shift at final prophylaxis in the tafenoquine group compared to 24.7% of subjects in the mefloquine group.

For all laboratory indices, < 5% of subjects in either group had post-treatment results flagged as clinically significant.

3.5.3.6 Methaemoglobin assessments

Methaemoglobin was measured at baseline, at the end of the prophylactic phase and at the 3-month follow-up visit. At the end of the prophylactic phase, the mean increase from baseline in methaemoglobin was larger in the tafenoquine group (1.8%) than in the mefloquine group (0.1%). At the end of the 3 month follow-up, however, the increase from baseline was small and similar in both treatment groups (0.1-0.2%).

3.5.4 Pharmacokinetic evaluation

The population pharmacokinetics of tafenoquine are well described by a one compartment model with first order absorption. Typical values of the first-order absorption rate constant (ka), oral clearance (CL/F), and apparent volume of distribution (V/F) were 0.243 h^{-1}, 0.056 L/h/kg, and 23.7 L/kg, respectively. The inter-subject variability (coefficient of variation) in CL/F and V/F was 18% and 22%, respectively. The inter-occasion variability in CL/F was 18%, and the mean elimination half-life was 12.7 days. A positive linear association between weight and both CL/F and V/F was found, but this had insufficient impact to warrant dosage adjustments. Model robustness was assessed by a nonparametric bootstrap (200 samples). A degenerate visual predictive check indicated that the raw data mirrored the postdose concentration- time profiles simulated (n =1000) from the final model. Individual pharmacokinetic estimates for tafenoquine did not predict the prophylactic outcome with the drug for four subjects who relapsed with *P. vivax* malaria during the relapse follow-up phase. These subjects had similar pharmacokinetics to those who were free of malaria infection. No obvious pattern existed between the plasma tafenoquine concentration and the pharmacokinetic values for subjects with and without drug-associated moderate or severe adverse events. This validated population pharmacokinetic model satisfactorily describes the disposition and variability of tafenoquine used in the long-term malaria prophylaxis in Australian soldiers on military deployment [Charles et al, 2007a].

For mefloquine, samples were pooled with those of a further 950 subjects on a separate mefloquine tolerability study. Mefloquine concentrations in the sub population contributed by this specific study were 762 ng/ml (range 248-1914) which compared favourably with the overall pooled results of 778 (62-2549) ng/ml. Wide ranges are largely explained by sampling times to ensure both peak and trough values were obtained across the study group. Mefloquine pharmacokinetics is well represented by a two compartment model allowing for inter-occassion variability (IOV) for clearance. Typical values of the absorption rate constant (ka), oral clearance (CL/F), and central volume of distribution (V^1/F) were 0.24 h^{-1}, 2.09 L/h, and 528 L, respectively. The intersubject variability (coefficient of variation) in CL/F and V^1/F was 24.4% and 29.6%, respectively. The inter-occasion variability in CL/F was 17.8%, and the mean elimination half-life was 14.0 days [Charles, 2007b].

3.6 Discussion

This study was designed to compare the safety and tolerability of weekly tafenoquine 200 mg and weekly mefloquine 250 mg over a 6 month period during a military deployment of the Australian Defence Force to Timor Leste. The chemosuppressive effectiveness of tafenoquine and mefloquine were also assessed. After subjects had returned to Australia, they were followed-up for a further three months to monitor the tolerability and effectiveness of tafenoquine/placebo and mefloquine/primaquine in preventing post-exposure relapses of malaria.

The two treatment groups were similar at baseline with respect to demographic characteristics. Compliance with study medication was high with at least 98% of subjects in each treatment group compliant with the prophylactic medication regimen. The treatment groups were similar with respect to their duration of deployment in Timor Leste and most subjects received their last dose of prophylactic medication on or up to three days before the day of leaving Timor Leste.

The primary objective of this study was to compare the safety and tolerability of tafenoquine and mefloquine over a 6 month treatment period. Both treatments were generally well tolerated. As expected in such a long study, the incidence of adverse events was high, with adverse events reported in 92% of subjects receiving tafenoquine and 88% of subjects receiving mefloquine during the prophylactic phase of the study. However, most events were mild or moderate in severity. The most commonly occurring events in both treatment groups were gastroenteritis and injury; these events are not unexpected in a military population deployed in the field. Overall, the groups were similar with respect to the incidence of individual events, and there were no statistically significant differences between the groups in the incidence of adverse events for those events occurring in at least 10% of subjects.

During the relapse follow-up phase, adverse event occurred in 41% of subjects treated with tafenoquine/placebo and 33% of subjects treated with mefloquine/primaquine. Events were generally similar to those during the prophylactic phase and, considering the numbers of subjects with events, the groups were similar with respect to the incidence of individual events.

The only noticeable difference between the treatment groups was the incidence of eye abnormalities: corneal deposits / vortex keratopathy. To monitor phospholipidosis and its effects, a sub-group of 98 subjects (77 in the tafenoquine group and 21 in the mefloquine group) underwent additional safety assessments including eye examinations, lung function tests and chest X-rays. No subjects had a clinically significant abnormality at baseline. At the end of prophylaxis visit, corneal deposits (vortex keratopathy) or suspected corneal deposits were reported in 69/74 (93.2%) subjects in the tafenoquine group and 0/21 subjects in the mefloquine group. Subjects with corneal deposits were asymptomatic and there were no notable changes from baseline or differences between the treatment groups in visual field tests (Amsler Grid and Humphrey Perimetry), visual acuity (Snellen) or colour vision (Ishihara, SPP2 plates, FM100 test).

Due to this unexpected finding, more detailed examinations were carried out than had been planned in the protocol, including detailed retinal and corneal examination and photography. Some of these examinations were conducted with the knowledge that the subject had corneal deposits and were therefore unblinded. Subjects with corneal deposits were followed up beyond the scheduled 3-month follow-up visit during the relapse follow-up period. At each follow-up corneal deposits were noted to have improved, with all subjects having resolved within 1 year of stopping study medication.

Fundoscopy examinations were carried out on 86 subjects, at the 3 month post prophylaxis follow-up. Examiners were aware of corneal deposits (if present), and were therefore unblinded in that respect. Fundoscopy examinations revealed abnormalities (e.g. granularity/pigmentation of retinal pigment epithelium, hard drusen) in 27/69 (39.1%) of tafenoquine subjects and 4/17 (23.5%) of mefloquine subjects. Fundus fluoroscein angiograms (FFA) were performed in 14 tafenoquine subjects and 1 mefloquine subject in whom possible retinal findings had been observed; of these 4/14 (28.6%) subjects in the tafenoquine group and 1/1 (100%) subject in the mefloquine group were considered to have abnormal findings.

As a result of these findings, an expert ophthalmology board were asked to review the data from this study. They concluded that the corneal changes were benign, fully reversible and similar to those seen with other drugs, such as chloroquine. The expert ophthalmology advisory board advised that vision had not been affected in any of these subjects. Lack of baseline retinal photography data meant that the relevance of the retinal findings (observed on fundoscopy and fundus fluoroscein angiograms) could not be ascertained. They noted that the results observed could reflect normal variability and the subjective nature of the examinations. They did not consider that the FFA results provided evidence of a drug effect.

Lung function tests, chest x-rays, and electron microscopic examination of blood leukocytes were also conducted to investigate potential phospholipidosis effects, but no clinically significant findings were observed and there were no differences between the treatment groups.

Methaemoglobinaemia is a known side-effect of anti-malarial compounds such as chloroquine and primaquine and had been reported in previous tafenoquine studies. In this study, methaemoglobin was assessed in a sub-group of 98 subjects (77 tafenoquine and 21 mefloquine subjects). Although there was a greater increase from baseline in mean methaemoglobin levels in the tafenoquine group than the mefloquine group (1.8% vs 0.1%, respectively), this had resolved at follow-up. The maximum methaemoglobin value for any tafenoquine subject was 5.0%, which is not considered to be clinically significant.

There were no deaths reported during the study. The incidence of serious adverse events during the prophylactic phase (3.7% vs 3.1% for the tafenoquine and mefloquine groups respectively) and the relapse follow-up phase (1.6% vs 1.2%) was very low. In total, 7 subjects, all in the tafenoquine group, had serious adverse events with a suspected relationship to study medication. These were 5 subjects with eye abnormalities and 2 subjects with gastrointestinal symptoms: one with abdominal pain and one with abdominal pain and diarrhoea.

Fourteen subjects (11 (2.2%) in the tafenoquine group and three (1.9%) in the mefloquine group) were withdrawn from the study during the prophylactic phase and one subject, in the tafenoquine/placebo group, was withdrawn during the relapse follow-up phase as a result of their adverse events. Most of the events leading to withdrawal were injuries or arthralgia, none of which was reported as related to study medication. Three subjects, all in the tafenoquine group, had events reported with a suspected relationship to study medication: abdominal pain, depression and hyperaesthesia.

At the end of the prophylactic phase there were generally only small changes from baseline in laboratory test results and few marked differences between the treatment groups. The change from baseline at each visit (mean increase and number of subjects with a significant increase) in creatinine and bilirubin was slightly larger in the tafenoquine than in the mefloquine group. The differences between the treatment groups for biochemistry indices had mostly resolved at follow-up and were not considered to be clinically significant.

There was a more noticeable decrease from baseline in hematocrit values in the tafenoquine group compared to the mefloquine group. Conversely, the increase from baseline in platelets at each visit was larger in the mefloquine than in the tafenoquine group. Only a small number of subjects (~5) had haematology assessments performed at follow-up, so it is not possible to draw any conclusions from the follow-up data. For all laboratory indices, <5% of subjects in either group had any post-treatment results flagged as clinically significant (F3).

Following the end of this study, renal toxicity findings in a 2-year rat carcinogenicity study resulted in a review of renal data for tafenoquine. This included a review of all renal marker data (urinalysis, serum creatinine, serum urea etc) for all studies. These data were reviewed by a panel of clinical nephrologists who concluded that, while tafenoquine did not seem to be nephrotoxic, there was a trend towards increased creatinine values during treatment that warranted further investigation. This led to a long term follow-up being instituted for Study 033.

In total, there were 246 subjects with an increased serum creatinine concentration at end of prophylaxis and/or follow-up. Twenty-nine of these were subsequently discharged from the ADF, though none for renally related medical conditions. In total, 186 subjects were contacted and 183 subjects consented to take part in the follow-up.. Of these, 147 subjects were from the tafenoquine treatment group and 36 subjects were from the mefloquine treatment group. The demographics of this group were very similar to those of the overall study population. The follow-up was conducted between Sep 2002 and May 2003, approximately 17-26 months after the end of the main study treatment period.

A total of 173/183 (95%) subjects had normal renal function tests at their first or second follow-up visit; 140/147 (95.2%) subjects in the tafenoquine group and 30/33 (91.7%) subjects in the mefloquine group.

Overall, 10 subjects were referred for follow-up with a renal consultant, 7/147 (4.8%) subjects in the tafenoquine group and 3/36 (8.3%) subjects in the mefloquine group. All 10 subjects were confirmed by a renal physician as having no clinical evidence of chronic renal damage and it was concluded that this long term renal follow-up did not demonstrate any evidence of long-term renal damage in healthy subjects who had received tafenoquine or mefloquine for 6 months.

In the tafenoquine group, mean QTc interval showed a small reduction from baseline at the end of the prophylactic phase. The proportion of subjects with a borderline result was larger in the tafenoquine group than the mefloquine group at the end of the prophylactic phase. QTc interval changes observed in this study were considered not clinically significant. The effect on the QTc interval of tafenoquine alone and in combination with other antimalarials will be investigated in more detail as part of future clinical studies.

No prophylactic failures were seen during the prophylactic phase of the study in either group. While definite exposure to malaria cannot be confirmed, cross-sectional surveys conducted at the time of the trial and other epidemiological data suggest that the subjects would have been exposed to both *P. falciparum* and *P. vivax* malaria during their deployment.

Following return from Timor Leste, subjects in the mefloquine group were treated with supervised primaquine (15 mg bid) as terminal prophylaxis against *P.vivax* relapse. Subjects in the tafenoquine group received matching placebo, as tafenoquine is proposed to be active against the liver hypnozoites that cause *P. vivax* relapse. The rate of *P. vivax* relapse was very low in both treatment groups, with 4 subjects (0.9%) in the tafenoquine group and one subject (0.7%) in the primaquine group developing *P. vivax* malaria. Relapse occurred between 12 and 20 weeks following return from Timor Leste. All 5 subjects were reported to be 100% compliant with both their prophylaxis and eradication medication during the study.

The occurrence of *P.vivax* relapse after return of subjects to Australia was not unexpected and could be due to a number of causes. Compliance with post-exposure prophylaxis medication such as primaquine is often a problematic, however this would not be a factor for the tafenoquine treated subjects and does not seem to be the issue for the mefloquine subject who reported 100% compliance with primaquine therapy. More likely is the presence of primaquine-tolerant *P. vivax* in Timor Leste. Primaquine tolerance was a well recognised phenomenon in Papua New Guinea and other Melanesian countries at the time of this study and the presence of primaquine-tolerant *P. vivax* (Chesson strain) parasites had recently been reported in Timor Leste as well. The incidence of *P. vivax* relapse in this study was very low (<1% in either treatment group) and much lower than that reported for previous deployments (Kitchener et al., 2003).

3.7 Key messages from this chapter

- Tafenoquine at a weekly dose of 200 mg and mefloquine at a dose of 250 mg were well tolerated amongst subjects in a military deployment.
- The incidence and nature of adverse events was similar between the two treatment groups. The most common adverse events were gastroenteritis and injury.
- Tafenoquine was associated with the development of vortex keratopathy (secondary to phospholipidosis) in 69/74 (93.2%) subjects tested (compared to no mefloquine subjects). This effect was benign and reversible, with resolution in >90% subjects at 6 months and complete resolution in all subjects by 1 year post-treatment.
- No significant changes were seen in most laboratory indices during the study. Increases in methaemoglobin in the tafenoquine group were small. Renal follow-up confirmed a lack of long-term renal effects of tafenoquine.
- No malaria occurred in either the tafenoquine and mefloquine arms during the prophylactic phase of this Phase III study. During the relapse follow-up phase, <1% of subjects in either treatment group developed *P. vivax* malaria.

3.8 References

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Chapter 3 Paper 3.1

Nasveld PE, Edstein MD, Reid M, et al, for the Tafenoquine Study Team. (2010) Randomized, double-blind study of the safety, tolerability and efficacy of Tafenoquine verses mefloquine for malaria prophylaxis in non-immune subjects. *Antimicrobial Agents and Chemotherapy*. 54: 792-798.

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Randomized, Double-Blind Study of the Safety, Tolerability, and Efficacy of Tafenoquine versus Mefloquine for Malaria Prophylaxis in Nonimmune Subjects[▽]

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This study represents the first phase III trial of the safety, tolerability, and effectiveness of tafenoquine for malaria prophylaxis. In a randomized (3:1), double-blinded study, Australian soldiers received weekly malaria prophylaxis with 200 mg tafenoquine (492 subjects) or 250 mg mefloquine (162 subjects) for 6 months on a peacekeeping deployment to East Timor. After returning to Australia, tafenoquine-receiving subjects received a placebo and mefloquine-receiving subjects received 30 mg primaquine daily for 14 days. There were no clinically significant differences between hematological and biochemical parameters of the treatment groups. Treatment-related adverse events for the two groups were similar (tafenoquine, 13.4%; mefloquine, 11.7%). Three subjects on tafenoquine (0.6%) and none on mefloquine discontinued prophylaxis because of possible drug-related adverse events. No diagnoses of malaria occurred for either group during deployment, but 4 cases (0.9%) and 1 case (0.7%) of *Plasmodium vivax* infection occurred among the tafenoquine and mefloquine groups, respectively, up to 20 weeks after discontinuation of medication. In a subset of subjects recruited for detailed safety assessments, treatment-related mild vortex keratopathy was detected in 93% (69 of 74) of tafenoquine subjects but none of the 21 mefloquine subjects. The vortex keratopathy was not associated with any effect on visual acuity and was fully resolved in all subjects by 1 year. Tafenoquine appears to be safe and well tolerated as malaria prophylaxis. Although the volunteers' precise exposure to malaria could not be proven in this study, tafenoquine appears to be a highly efficacious drug for malaria prophylaxis.

The continuing spread of multidrug-resistant *Plasmodium* species and concerns about adverse effects associated with antimalarial drugs has made the prevention of malaria problematic for nonimmune subjects, such as tourists and soldiers who travel to malaria endemic areas. No antimalarial drug is completely effective in preventing malaria (10); however, an ideal prophylactic drug would be highly effective against all malaria-inducing species, very well tolerated, and taken infrequently to enhance compliance (21). Currently, mefloquine, doxycycline, and atovaquone-proguanil are recommended for malaria prophylaxis (5, 23). These drugs are highly effective in preventing malaria but have shortcomings that limit their effectiveness, such as adverse effects, expense, and the difficulty of monitoring daily compliance within deployed military populations. Furthermore, none of these recommended drugs prevents the development and relapse of *Plasmodium vivax* and *P. ovale* dormant liver stages (hypnozoites).

Tafenoquine, a long-acting 8-aminoquinoline, is currently being codeveloped by GlaxoSmithKline (GSK) Research & Development Limited and the Walter Reed Army Institute of Research as a replacement for primaquine and for the prevention of malaria. Like primaquine, tafenoquine produces hemolysis in glucose-6-phosphate dehydrogenase (G6PD)-deficient recipients (21). Tafenoquine acts on all stages of the malaria parasite, with the potential to protect against all species of malaria parasites. Previous studies with a challenge model (4) and of indigenous populations in areas in which malaria is endemic have shown that tafenoquine was highly efficacious in preventing *P. falciparum* malaria and well tolerated (9, 13, 21). Tafenoquine was also shown to be efficacious in preventing both *P. falciparum* and *P. vivax* malaria for up to 6 months in Thai soldiers (22).

This first phase III study of tafenoquine for malaria prophylaxis was a randomized, double-blind, active controlled study carried out with healthy Australian soldiers deployed to East Timor as part of a United Nations (UN) peacekeeping mission. The primary study objective was to compare the safety and tolerability of tafenoquine with those of mefloquine in malaria prophylaxis for 6 months. A subset of 98 subjects underwent extra safety assessments to investigate the possible effects of phospholipidosis, methemoglobin, and cardiac safety. Since a

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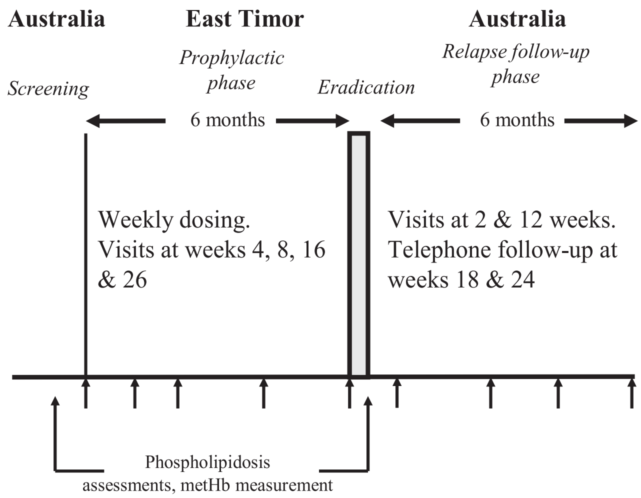

FIG. 1. Drug administration and safety analysis schedule for tafenoquine and mefloquine. metHb, methemoglobin.

placebo arm to document exposure was not possible, the key secondary objective was to assess the efficacy of tafenoquine in preventing *P. falciparum* and *P. vivax* malaria during and following deployment.

(This study was presented in part at the 51st Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, CO, November 2002.)

MATERIALS AND METHODS

Study site and subjects. The subjects were Australian soldiers deployed on UN peacekeeping duties to East Timor from October 2000 to April 2001. The soldiers were deployed to the Bobonaro District, on the western border of East Timor. The study included male and female subjects who were between 18 and 55 years of age, judged to be healthy by a medical history and physical examination with normal hematological and biochemical values, G6PD normal, and willing and able to give written informed consent and comply with the study protocol. Females were excluded if they were pregnant, lactating, or unwilling/unable to comply with recognized contraceptive methods. Subjects with a history of psychiatric disorders and/or seizures were also excluded. All subjects gave written informed consent, and the study protocol was approved by the Australian Defence Human Research Ethics Committee (ADHREC protocol no. 216/00) and the U.S. Army Human Subject Research Review Board.

Study design and drug administration. This comparative, randomized, double-blind, active controlled study had 4 phases: screening, loading, prophylactic phase, and relapse follow-up (Fig. 1). Following a loading-dose regimen of 200 mg tafenoquine or 250 mg mefloquine daily for 3 consecutive days, the subjects then received an oral weekly maintenance dose of 200 mg tafenoquine or 250 mg mefloquine for 26 ± 4 weeks, respectively. Subjects were directed to take their study medication at the same time each week with food (breakfast/dinner) to enhance drug bioavailability. Upon their return to Australia, subjects commenced a hypnozoite eradication regimen, receiving primaquine 15 mg twice a day (for the mefloquine group) or matched placebo twice a day (for the tafenoquine group) for 14 days. Drug compliance was observed and recorded for each subject by using medication logs.

Randomization. A coding memo block randomization system (block size = 8) to provide a 3:1 ratio of tafenoquine-receiving subjects to mefloquine-receiving subjects was used to assign the subjects to a treatment group. Study drugs were prepackaged and prelabeled with a unique study number.

Drug sources. Tafenoquine was supplied by GlaxoSmithKline in an opaque, hard gelatin capsule (Capsugel), each containing a 200-mg tafenoquine base. Placebo tafenoquine capsules were of identical appearance. Mefloquine (Lariam; 250-mg base tablet) was obtained from Hoffman-La Roche, and primaquine (15-mg base tablet) was supplied by GlaxoSmithKline. The matched placebos for mefloquine and primaquine were identical in external appearance to active capsules. All medication was provided in blinded individual foil blister packs and stored between 15°C to 30°C.

Safety and tolerability. Assessment of adverse events and sample collection for hematological and blood chemistry parameters were carried out at the loading stage and then at weeks 4, 8, 16, and 26 during the prophylactic phase and at weeks 2 and 12 during the relapse follow-up phase. Adverse event monitoring was supplemented by review of subjects' medical records. For a subset of 98 subjects (77 on tafenoquine and 21 on mefloquine), more-detailed safety assessments were performed. These subjects were assessed for phospholipidosis and its effects (by ophthalmic assessments, lung function tests, and electron microscopy of peripheral blood lymphocytes) and methemoglobin assessment and an electrocardiogram were performed (to assess QT interval) at screening and at the end of the prophylactic phase. Following the identification of corneal deposits at the end of this study, a wider range of ophthalmic assessments was included at follow-up.

Disclosure of adverse events was elicited by the investigator asking the subject the nonleading question, "Do you feel differently in any way since starting the new treatment?" A study physician assessed the level of relationship of any adverse event on the basis of the subject's response and any temporal association and/or known adverse responses to the drug. The physician graded the severity of adverse events as mild (not affecting daily activities), moderate (with some interference in daily activities), and severe (when daily duties could not be completed). A causal relationship to the study drug was judged by the physician to be not related, unlikely, suspected, or probable.

Efficacy assessment. Thick and thin blood smears were collected from all subjects at screening, at weeks 4, 8, 16, and 26 during the prophylactic phase, and at weeks 2 and 12 during the relapse follow-up phase or if symptoms suggestive of malaria developed. Telephone interviews with all subjects were carried out at weeks 18 and 24 during the relapse follow-up phase to determine their general health status. The Giemsa stain-treated blood smears were each read twice for malaria parasites by blinded microscopists at 2 separate institutions. A blood slide was considered negative if an examination of 200 oil immersion thick fields (magnification, ×1,000) showed no parasites. Any discrepant findings were to have been read by a third blinded expert microscopist and were to be used to define a prophylaxis failure if symptoms consistent with malaria were present.

Statistical analysis. With at least 450 subjects on tafenoquine and 150 subjects on mefloquine, the study had 94% power to detect a 10% difference in failure rates, assuming an underlying failure rate of 10% in each treatment group (15). Safety and tolerability analyses were performed on data from all subjects who took at least one dose of prophylactic study medication (tafenoquine or mefloquine). Hematological/blood chemistry values for the two groups were compared by a paired Student's t test, and 95% confidence intervals (CIs) were calculated. The efficacy analysis was performed for the per-protocol population, which was defined as the subjects who met the inclusion criteria, were protocol compliant, and completed the prophylactic and relapse follow-up phases. Proportions were examined by using a χ^2 test with Yates' correction or by Fisher's exact test. No adjustment was made for multiple testing.

RESULTS

Subject population. In total, 663 subjects were screened, and of these, 9 subjects failed the inclusion criteria. Of the remaining eligible subjects, 492 subjects were randomized to receive tafenoquine, and 162 subjects were randomized to receive mefloquine. Thirty-nine subjects (30 [6.1%] of the 492 tafenoquine subjects and 9 [5.6%] of the 162 mefloquine subjects) violated the protocol or did not complete the study, due to adverse events or other withdrawal reasons (Fig. 2). There were no marked differences between the groups in the proportions of subjects with protocol violations or withdrawals from the study (data not shown). The treatment groups were well balanced with respect to baseline demographic characteristics and history of malaria (Table 1), with the majority of subjects being white, male, and <35 years of age.

Compliance. As a result of observed therapy, compliance was high in both treatment groups (100% for the loading dose, 99% for the weekly regimens, and 96% for the follow-up antihypnozoite regimen).

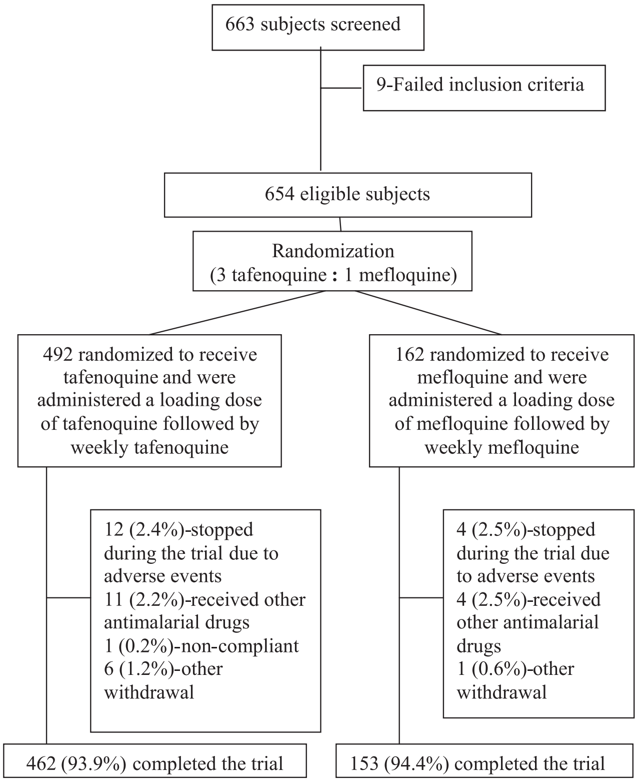

FIG. 2. Flow diagram of subject accountability during the study.

Routine laboratory tests. For most laboratory variables, the proportion of subjects with results that fell outside an extended normal range during the prophylactic phase was <5% (data not shown). In addition, the proportions of subjects with clinically significant changes from baseline values were similar across the treatment groups for most laboratory parameters. The parameters that were exceptions were hematocrit, bilirubin, and creatinine.

Decreases in hematocrits were seen in both subjects on tafenoquine and subjects on mefloquine, with up to 98 (20%) of the 492 tafenoquine subjects having a 15% decrease from the baseline at any one visit, compared to 23 (14.4%) of the 162 mefloquine subjects. However, only 2 subjects, both on tafenoquine, had a clinically significant hematocrit value (<85% of the lower limit of normal range) during the study. A higher proportion of tafenoquine subjects was reported to have an increase in bilirubin (>2 μmol/liter from the baseline) at any one visit during the study (10% of tafenoquine subjects versus 3.2% of mefloquine subjects). Of these, only 13 (2.6%) tafenoquine subjects and 1 (0.6%) mefloquine subject had a clinically significant bilirubin value (>150% of the upper limit of normal range) at some point during the study. Serum creatinine increases (>125% baseline value) were seen in both the tafenoquine and mefloquine groups, with an increase in serum creatinine in up to 19% of tafenoquine subjects at any one visit versus 10% of mefloquine subjects. At the follow-up, 6 to 8% of subjects in both groups had creatinine values that were still 25% above the baseline; however, few subjects had values outside the normal range, and none of these values was considered clinically significant.

TABLE 1. Baseline demographic characteristics and previous malarial histories of subjects on tafenoquine and mefloquine for malaria prophylaxis

Characteristic	Value for subjects who received:	
	Tafenoquine (n = 492)	Mefloquine (n = 162)
No. (%) of subjects		
Gender		
Male	478 (97.2)	154 (95.1)
Female	14 (2.8)	8 (4.9)
Age (yr)		
18–25	286 (58.1)	97 (59.9)
26–35	178 (36.2)	48 (29.6)
36–45	27 (5.5)	16 (9.9)
46–55	1 (0.2)	1 (0.6)
Race		
White	484 (98.4)	160 (98.8)
Aboriginal/Torres Strait Islander	4 (0.8)	1 (0.6)
Other	4 (0.8)	1 (0.6)
Previous history of malaria	15 (3.0)	4 (2.5)
Having malaria attacks in 6 mo prior to deployment	9 (1.8)	1 (0.6)
Age		
Mean (SD)	25.4 (5.3)	26.0 (6.5)
Range	18–47	18–51
Weight (kg)		
Mean (SD)	80.9 (11.9)	81.3 (12.2)
Range	50–135	53–135
Height (cm)		
Mean (SD)	177.8 (7.0)	177.1 (6.7)
Range	155–198	157–192

Safety evaluation subgroup. The ophthalmic assessments in the subgroup of subjects on tafenoquine and mefloquine are summarized in Table 2. At the end of prophylaxis, vortex keratopathy (corneal deposits) was found in 69 (93.2%) of 74

TABLE 2. Ophthalmic assessments of a subgroup of subjects on tafenoquine or mefloquine

Activity	Screening	Posttreatment assessment
Visual field tests	Amsler grid	Amsler grid Humphrey perimetry
Visual acuity	Snellen chart	Snellen chart
Color vision	Ishihara test	Ishihara test Standard pseudoisochromatic plates part 2 Farnsworth-Munsell 100 hue test
Physical examination	Fundoscopy Corneal examination	Fundoscopy Corneal examination Digital retinal photography Digital corneal photography Fundus fluorescein angiogram[a]

[a] Small number of subjects with possible retinal findings only.

TABLE 3. Adverse events occurring in >5% of subjects on tafenoquine or mefloquine (prophylactic phase)[a]

Adverse event	No. (%) of subjects by AE severity and treatment group							
	Mild		Moderate		Severe		Total	
	Tafenoquine	Mefloquine	Tafenoquine	Mefloquine	Tafenoquine	Mefloquine	Tafenoquine	Mefloquine
At least one AE	431 (88)	140 (86)	194 (39)	46 (28)	18 (4)	3 (2)	454 (92)	143 (88)
Gastrointestinal								
Gastroenteritis	109 (22)	36 (22)	80 (16)	17 (11)	6 (1)	0	182 (37)	51 (32)
Diarrhea	77 (16)	28 (17)	0	2 (1)	1 (<1)	0	77 (16)	30 (19)
Nausea	27 (6)	13 (8)	1 (<1)	0	0	0	28 (6)	13 (8)
Abdominal pain	19 (4)	11 (7)	5 (1)	3 (2)	1 (<1)	0	24 (5)	13 (8)
Vomiting	19 (4)	8 (5)	2 (<1)	1 (<1)	0	0	21 (4)	8 (5)
Musculoskeletal								
Injury	149 (30)	46 (28)	45 (9)	4 (3)	3 (<1)	2 (1)	178 (36)	49 (30)
Back pain	65 (13)	24 (15)	12 (2)	2 (1)	0	0	74 (15)	26 (16)
Arthralgia	52 (11)	17 (11)	9 (2)	1 (<1)	0	0	55 (11)	18 (11)
Respiratory								
URTI	97 (20)	30 (19)	6 (1)	2 (1)	0	0	101 (21)	32 (20)
Pharyngitis	24 (5)	2 (1)	2 (<1)	1 (<1)	0	0	25 (5)	3 (2)
Dermatological								
Rash	70 (14)	20 (12)	1 (<1)	1 (<1)	0	0	70 (14)	21 (13)
Fungal dermatitis	43 (9)	8 (5)	1 (<1)	0	0	0	44 (9)	8 (5)
Headache (constitutional AE)	59 (12)	18 (11)	2 (<1)	2 (1)	0	0	61 (12)	20 (12)
Viral infection	23 (5)	7 (4)	16 (3)	6 (4)	1 (<1)	0	39 (8)	13 (8)

[a] In total, there were 492 tafenoquine subjects and 162 mefloquine subjects. AE, adverse event; URTI, upper respiratory tract infection.

tafenoquine subjects but was absent in the 21 mefloquine subjects (Table 2). These changes were not associated with any visual disturbances and there were no differences between the groups in visual acuity, Amsler grid score, or Ishihara (color vision) score. All subjects with vortex keratopathy were followed up until resolution, with the incidence reducing to 39% at 3 months and 10% at 6 months; there was complete resolution by all subjects by 1 year. Based on the initial findings, fundoscopic examinations were carried out on 86 subjects at the 3-month postprophylaxis follow-up. Abnormalities (e.g., granularity/pigmentation of retinal pigment epithelium or hard drusen) were noted for 27 (39.1%) of 69 tafenoquine subjects and 4 (23.5%) of 17 mefloquine subjects. Retinal fluoroscein angiograms were performed on 14 tafenoquine subjects and 1 mefloquine subject for whom possible retinal findings had been observed. Of these, 4 (28.6%) tafenoquine subjects and 1 (100%) mefloquine subject were considered possibly abnormal. However, review by an expert ophthalmology review board concluded that the retinal findings may well have been normal variations and that there was no evidence to support drug-related visual disturbances. It should be noted that fundoscopic examination of the retina at follow-up was not blinded, because the examination was carried out with the knowledge that corneal deposits were present and no baseline data were available for comparison.

In addition to undergoing phospholipidosis assessments, the safety subgroup also underwent methemoglobin assessment and electrocardiograms for assessment of QT interval. Mean methemoglobin levels increased by 1.8% in the tafenoquine group and by 0.1% in the mefloquine group at the end of prophylaxis, but by week 12 of follow-up, the increase in methemoglobin had resolved. In the tafenoquine group, there was a small reduction in the mean QT interval (difference of −4.5 ms; 95% CI, −9.7 to 0.7 ms), whereas a small increase in the interval was seen in the mefloquine group (difference of 1.6 ms; 95% CI, −12.1 to 15.4 ms) at the end of prophylaxis. There were no subjects for which there was a clinically dangerous prolongation of the QT interval. None of the safety findings impacted participants' well-being or was considered clinically significant.

Tolerability. During the prophylactic phase, 454 (91.9%) of 492 tafenoquine subjects and 143 (88.3%) of 162 mefloquine subjects reported at least one adverse event. The most common adverse events (occurring in >5% of subjects) are summarized in Table 3. There was no significant difference between the 2 treatment groups in the number or type of adverse events, with the most common events being gastroenteritis and injury, which occurred in >30% of subjects in both treatment groups. The majority of adverse events were mild or moderate in severity. In total, there were 21 severe adverse events (18 [4%] tafenoquine subjects and 3 [2%] mefloquine subjects). The most common severe events were gastroenteritis (6 [1.2%] tafenoquine subjects and 0 mefloquine subjects) and injury (3 [0.6%] tafenoquine subjects and 2 [1.2%] mefloquine subjects). During the relapse follow-up phase, 203 (41.3%) tafenoquine/placebo subjects and 53 (33.9%) mefloquine/primaquine subjects reported adverse events; however, there was no notable difference between the treatment groups in the incidence or nature of events.

In total, 64 (13.0%) tafenoquine subjects and 23 (14.2%)

TABLE 4. Neuropsychiatric events in subjects on tafeoquine or mefloquine (prophylactic phase)[a]

Adverse event	No. (%) of subjects by AE severity and treatment group					
	Mild		Moderate		Total	
	Tafenoquine	Mefloquine	Tafenoquine	Mefloquine	Tafenoquine	Mefloquine
Vertigo	22 (5)	7 (4)	0	1 (<1)	22 (5)	8 (5)
Somnolence	12 (2)	6 (4)	0	0	12 (2)	6 (4)
Abnormal dreams	7 (1)	2 (1)	0	0	7 (1)	2 (1)
Dizziness	5 (1)	2 (1)	0	0	5 (1)	2 (1)
Insomnia	4 (<1)	3 (2)	1 (<1)	0	5 (1)	3 (2)
Abnormal coordination	2 (<1)	1 (<1)	0	0	2 (<1)	1 (<1)
Anxiety	2 (<1)	0	0	0	2 (<1)	0
Agitation	2 (<1)	0	0	0	2 (<1)	0
Euphoria	2 (<1)	0	0	0	2 (<1)	0
Tremor	2 (<1)	0	0	0	2 (<1)	0
Depression	0	0	1 (<1)	1 (<1)	1 (<1)	1 (<1)
Paroniria	1 (<1)	0	0	0	1 (<1)	0
Amnesia	1 (<1)	0	0	0	1 (<1)	0

[a] In total, there were 492 tafenoquine subjects and 162 mefloquine subjects. There were no severe adverse events (AEs) of this type.

mefloquine subjects reported neuropsychiatric adverse events, the most common being vertigo, dizziness and various sleep disorders (Table 4). There was no significant difference between the treatment groups in the incidence and type of neuropsychiatric events, and all were reported as mild or moderate.

Fifteen subjects withdrew from the study as a result of adverse events (12 [2.4%] tafenoquine subjects and 3 [1.9%] mefloquine subjects). Four tafenoquine subjects sustained injuries requiring evacuation from the study area, while 2 experienced arthralgia (1 subject on each drug). Three tafenoquine subjects withdrew for possible treatment-related adverse events, namely, abdominal pain (severe), depression (moderate), and hyperesthesia (moderate). The incidences of severe adverse events in the 2 groups were comparable (18 [3.7%] tafenoquine subjects and 5 [3.1%] mefloquine subjects).

In total, during the prophylactic phase, 66 (13.4%) tafenoquine subjects and 19 (11.7%) mefloquine subjects had adverse events with a suspected/probable relationship to treatment (Table 5). There were no significant differences between the treatment groups in the incidence or nature of treatment-related adverse events during the prophylactic phase. Only 1 subject on tafenoquine reported a severe adverse event (diarrhea and abdominal pain) suspected to be related to treatment.

TABLE 5. Table of adverse events attributed as related to study drug during prophylactic phase in the safety population[a]

Adverse event	No. (%) of patients in treatment group	
	Tafenoquine (n = 492)	Mefloquine (n = 162)
At least one AE	66 (13.4)	19 (11.7)
Nausea	14 (2.8)	4 (2.5)
Vertigo	10 (2.0)	2 (1.2)
Diarrhea	9 (1.8)	3 (1.9)
Abdominal pain	7 (1.4)	2 (1.2)
Abnormal dreaming	6 (1.2)	1 (0.6)
Somnolence	6 (1.2)	1 (0.6)
Headache	3 (0.6)	2 (1.2)
Insomnia	3 (0.6)	2 (1.2)

[a] Events occurring in >1% of subjects are shown. AE, adverse event.

Efficacy. No symptomatic malarial infections occurred during the prophylactic phase in either treatment group. Smears collected from symptomatic subjects and during routine screening for malaria diagnosis were all negative. There were 4 cases (0.9%) of malarial infection in the tafenoquine group and a single case (0.7%) in the mefloquine group during the relapse follow-up phase (95% CI, −1.32 to 1.74; P = 1.0). All cases corresponded to *P. vivax* infection, which occurred between 16 and 20 weeks following the return from East Timor.

DISCUSSION

This phase III study describes the safety and tolerability of tafenoquine administered for malaria prevention in a nonimmune population of predominately young Caucasian males. Both tafenoquine and mefloquine were well tolerated. There were no clinically significant differences between hematological and blood chemistry results for the 2 treatment groups.

Assessment for phospholipidosis and its effects in a subgroup of 98 subjects showed at the end of the prophylactic phase a high incidence (93.2%) of mild vortex keratopathy (corneal deposits) in the tafenoquine group. Based on these findings, an independent expert ophthalmology board was asked to review the data. It concluded that the corneal changes were benign, fully reversible, and similar to those seen with several other drugs, including chloroquine, for which it is not considered to be a contraindication for continuous use (1). It also advised us that vision had not been impaired in any subject. A lack of baseline retinal photography data meant that the relevance of retinal findings could not be ascertained, but they reflected normal variability. Further assessment of the eye changes observed with tafenoquine will need to be undertaken to determine with certainty the overall significance of the observed changes and to clarify the retinal issues raised during the review.

As would be expected in a long-term study, the incidence of adverse events was high, with 92% of tafenoquine subjects and 88% of mefloquine subjects reporting one or more adverse events during the 6 months of prophylaxis. The majority of these events was mild or moderate in severity, and the events were typical of the type of events expected in a population of soldiers on active duty (e.g., injury or gastroenteritis). The number of withdrawals from the study was low for a long-term study, also reflecting the nature of the study population. There were no significant differences in the occurrence of treatment-related adverse events, including gastrointestinal and neuropsychiatric disturbances between the 2 treatment groups.

Limited comparative data on the tolerability of tafenoquine used for prophylaxis are available. In adult black Kenyans, the incidences of adverse events for subjects on placebo and on weekly 200 mg tafenoquine for 13 weeks were similar (21). Relative to our findings, the study of the Kenyans reported a higher incidence of headache (24% versus 12.4%) but lower incidences of diarrhea (7% versus 15.7%) and rashes (4% versus 14.2%) with the same maintenance dose. However, such comparisons are difficult to make when the subject populations differ so markedly in ethnicity, nutritional status, culture, employment, and tolerance to medication.

Mefloquine was well tolerated by the Australian soldiers, which is in accordance with the results of other randomized, double-blind studies of military populations (2, 6, 17). No soldiers on mefloquine withdrew from the study due to treatment-related adverse events, and no more than 2% of the soldiers on either tafenoquine or mefloquine experienced drug-associated neuropsychiatric disturbances. Severe neuropsychiatric adverse events in European travelers on mefloquine have been reported (18, 20), but such events were not observed in the present study. Neuropsychiatric adverse events related to mefloquine use are reported to be more common in females (20), and the somewhat atypical distribution of participants in this study should be considered when generalizing these findings.

Without a placebo control, the exposure to malaria experienced by the Australian soldiers could not be directly estimated. As an indication of the malaria exposure that the soldiers probably encountered, 2 malaria prevalence surveys were conducted (January 2001 and April 2001) in 7 East Timorese villages (about 200 residents in each village), all within 1 km of where the soldiers were located (3). The surveys showed that malaria was present in 6 of the 7 locations, with point prevalence rates ranging from 0 to 35.3% (*P. falciparum*, 0 to 14.4%; *P. vivax*, 0 to 16%). In addition to this evidence, several studies have confirmed a high incidence of malaria in East Timor (8, 11–12, 14, 19). While these studies are not conclusive proof that subjects in the present study were exposed to malaria, it is highly likely that the soldiers were exposed to both *P. falciparum* and *P. vivax* malaria. Because no prophylactic failures occurred during the treatment phase in East Timor, both treatments appeared to be effective in suppressing malaria infections. During the 6-month relapse follow-up period, 4 (0.9%) subjects on tafenoquine/placebo and 1 (0.7%) subject on mefloquine/primaquine developed *P. vivax* infections. These findings indicate that tafenoquine and primaquine are equally effective in preventing *P. vivax* relapse when primaquine compliance is monitored and confirm the results of previous studies in Papua New Guinea (16) and East Timor (7). Although the relapse rates for primaquine and tafenoquine appear to be similar, tafenoquine offers a major advantage in that there is no need to take additional medication after leaving the endemic area if tafenoquine is used for prophylaxis.

In summary, tafenoquine at 200 mg weekly is safe and well tolerated in nonimmune Caucasian subjects following 6 months of prophylaxis. Although mild vortex keratopathy was seen in the subjects on tafenoquine, this was benign and fully reversible. The most frequently recorded treatment-related adverse events for both tafenoquine and mefloquine were gastrointestinal disturbances, and these tended to be mild or moderate. Both treatments fully suppressed malarial infections during prophylaxis, and less than 1% of subjects developed postexposure malaria after either completion of tafenoquine prophylaxis or primaquine treatment. Tafenoquine is an effective alternative weekly antimalarial that can be used without the need for further medication after leaving an endemic area.

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Chapter 3 Paper 3.2

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Population Pharmacokinetics of Tafenoquine during Malaria Prophylaxis in Healthy Subjects[▽]

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The population pharmacokinetics of tafenoquine were studied in Australian soldiers taking tafenoquine for malarial prophylaxis. The subjects (476 males and 14 females) received a loading dose of 200 mg tafenoquine base daily for 3 days, followed by a weekly dose of 200 mg tafenoquine for 6 months. Blood samples were collected from each subject after the last loading dose and then at weeks 4, 8, and 16. Plasma tafenoquine concentrations were determined by liquid chromatography-tandem mass spectrometry. Population modeling was performed with NONMEM, using a one-compartment model. Typical values of the first-order absorption rate constant (K_a), clearance (CL/*F*), and volume of distribution (*V*/*F*) were 0.243 h^{-1}, 0.056 liters/h/kg, and 23.7 liters/kg, respectively. The intersubject variability (coefficient of variation) in CL/*F* and *V*/*F* was 18% and 22%, respectively. The interoccasion variability in CL/*F* was 18%, and the mean elimination half-life was 12.7 days. A positive linear association between weight and both CL/*F* and *V*/*F* was found, but this had insufficient impact to warrant dosage adjustments. Model robustness was assessed by a nonparametric bootstrap (200 samples). A degenerate visual predictive check indicated that the raw data mirrored the postdose concentration-time profiles simulated (n = 1,000) from the final model. Individual pharmacokinetic estimates for tafenoquine did not predict the prophylactic outcome with the drug for four subjects who relapsed with *Plasmodium vivax* malaria, as they had similar pharmacokinetics to those who were free of malaria infection. No obvious pattern existed between the plasma tafenoquine concentration and the pharmacokinetic parameter values for subjects with and without drug-associated moderate or severe adverse events. This validated population pharmacokinetic model satisfactorily describes the disposition and variability of tafenoquine used for long-term malaria prophylaxis in a large cohort of soldiers on military deployment.

Tafenoquine, a synthetic analog of primaquine, is a new 8-aminoquinoline antimalarial drug being codeveloped by GlaxoSmithKline Pharmaceuticals and the Walter Reed Army Institute of Research (1). Clinical trials have shown tafenoquine to be an effective antimalarial agent that has been generally well tolerated, with transient gastrointestinal discomfort being the most commonly reported adverse event (8, 10, 11, 13, 15). To date, it has been evaluated in more than 2,000 subjects in six phase II clinical studies. Since tafenoquine acts on all malaria stages, it has potential in the chemoprophylaxis of malaria, in radical cure/relapse prevention of *Plasmodium vivax* infections, and as a transmission-blocking agent (gametocytocidal activity).

The pharmacokinetics of tafenoquine in humans have been derived from studies after oral administration, as no parenteral formulation exists. Tafenoquine is slowly absorbed following oral administration, with maximum plasma concentrations observed at about 12-h postdose in fasted subjects (1). Plasma tafenoquine concentration-time data have been described by a one-compartment model with first-order absorption and elimination (1, 2). The elimination half-life of tafenoquine is about 2 weeks. It is extensively distributed to tissues, with a large volume of distribution and a low clearance, but data on the metabolism of tafenoquine in humans are limited. Although animal studies have shown that absorbed tafenoquine secreted via the bile is found predominantly in the form of metabolites, which accounted for the majority of the drug-related material eliminated in the urine and feces, unchanged tafenoquine was the only drug-related component detected in human plasma by high-performance liquid chromatography–mass spectrometry (HPLC-MS) and HPLC with fluorescence detection (GlaxoSmithKline Pharmaceuticals, unpublished data).

Tafenoquine is highly effective in preventing malaria infections following a weekly dose of either 200 mg or 400 mg for 13 weeks (13) or 400 mg monthly for 6 months (15). In developing the dosage regimen for malaria prophylaxis, a phase III study was conducted to assess the safety, tolerability, and effectiveness of tafenoquine in Australian soldiers deployed for 6 months on peacekeeping duties to an area where malaria is endemic. The full clinical results of that study will be published elsewhere. The soldiers were on a weekly regimen of 200 mg of tafenoquine, and blood samples were collected on four occasions for drug analysis. No malaria infections occurred during the prophylactic phase, but four soldiers were diagnosed with *P. vivax* infection after returning to Australia.

The primary aim of the present study was to use these data to develop a population pharmacokinetic model for tafenoquine and to estimate the disposition of this drug in the target population of soldiers on military deployment. Secondary aims

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were to determine whether individual pharmacokinetic estimates for tafenoquine would predict prophylactic outcomes and to investigate if there was any relationship between tafenoquine concentrations and drug-associated adverse events.

MATERIALS AND METHODS

Study design and subjects. The clinical trial was designed as a prospective, randomized, double-blind comparative study of the safety, tolerability, and effectiveness of tafenoquine and mefloquine in Australian soldiers on weekly malaria prophylaxis. The subjects were deployed on peacekeeping duties to East Timor for 6 months. They all were judged to be healthy by a complete medical history, physical examination, and normal hematological and biochemical values. They had to be glucose-6-phosphate dehydrogenase normal and willing and able to give written informed consent and comply with the study protocol. Females were excluded if they were pregnant, lactating, or unwilling/unable to comply with recognized contraceptive methods. The study protocol received prior written approval by the Australian Defence Human Research Ethics Committee and the U.S. Army Human Subject Research Review Board.

Tafenoquine dosing regimen. Following a loading dose regimen of 200 mg tafenoquine base daily for three consecutive days, the subjects then received an oral weekly maintenance dose of 200 mg tafenoquine over approximately 6 months. An opaque Swedish Orange size 1 hard gelatin capsule (Capsugel) containing tafenoquine at 200 mg (pure free base) was used as the dosage form. Subjects were directed to take their tafenoquine with food (breakfast or dinner) at the same time each week. Dosage administration was observed and recorded for each subject.

Pharmacokinetic sampling. The sampling design was guided by the results from a previous smaller study of Thai soldiers (2) and also by logistical issues of the field operations. Blood samples were collected at prerandomized times after the last loading dose and then at prerandomized times at weeks 4, 8, and 16. Samples were collected on predetermined days after dosing on each of the assessment weeks. The predetermined days included day 1 (early postdose; absorption phase), days 3 and 5 (72 to 120 h postdose), and day 7 (predose; trough phase). For example, on week 4, one group of soldiers (about 125 subjects) was bled on day 1, one group was bled on day 3, one group was bled on day 5, and one group was bled on day 7. Thereafter, the groups of soldiers were bled in a cyclical fashion such that at the end of the study each group had been bled on at least one occasion on days 1, 3, 5, and 7. However, the sample for day 2 of the study (1 to 12 h; post-final loading dose) was collected from the study subjects.

Blood (7 ml) was drawn by venipuncture into EDTA tubes and transported on ice bricks to the field laboratory within 3 h of collection. Whole-blood samples were centrifuged at ~1,200 × *g* for 15 min (Sigma, Quantum, Australia), and plasmas were separated and stored in liquid nitrogen (<4 weeks) and then air freighted on dry ice to Quintiles Limited (Edinburgh, United Kingdom) for storage at −70°C until analysis. Tafenoquine was stable under these handling and storage conditions.

Measurement of tafenoquine. Plasma tafenoquine concentrations were determined using a validated HPLC method with a triple-quadrupole mass spectrometer. Briefly, plasma (0.05 ml) was spiked with [$^2H_4$$^{15}N$]tafenoquine as a stable-isotope-labeled internal standard, and the protein was precipitated with methanol, followed by centrifugation and then injection of 4 μl of the supernatant fluid onto a reversed-phase HPLC column (4-μm-diameter particles; Genesis C_{18} column; 30 mm × 2.1-mm internal diameter) held at 40°C. The mobile phase was methanol-1 mM ammonium acetate buffer, pH 2.5 (70:30 [vol/vol]), pumped at 1 ml/min and split approximately 1 to 4 into the TurboIonSpray interface of a PE-Sciex API 3000 LC/MS/MS system (Applied Biosystems) operated in positive-ion multiple-reaction monitoring mode. A chromatographic cycle time of 1.3 min was used, with the peaks being eluted at 0.4 min. The multiple-reaction monitoring transitions monitored were 464 to 379 *m/z* for tafenoquine and 469 to 379 *m/z* for stable-isotope-labeled tafenoquine. Linear responses in analyte/internal standard peak area ratios were observed for tafenoquine concentrations ranging from 5 to 500 ng/ml, using a weighted ($1/C^2$) linear regression. Results of a three-run validation gave an intra-assay imprecision (coefficient of variation [CV%]) of <5.8% and an interassay imprecision of <7.3%, with an inaccuracy of 1.5 to 4.4%. The lower limit of quantification of the method was 5 ng/ml.

Population pharmacokinetic modeling. The population pharmacokinetics of tafenoquine were determined in double precision by using NONMEM (version 5, level 1.1; Globomax LLC, Hanover, MD) in conjunction with a G77 compiler. A one-compartment model with first-order absorption and elimination was fitted to the data, using first-order conditional estimation with interaction. An initial analysis was conducted by permitting NONMEM to estimate the base model parameters (i.e., no covariates). The influence of mean-centered continuous variables, i.e., age, current weight, and estimated creatinine clearance (CL_{CR} [by the Cockcroft-Gault method]), and the categorical variables, i.e., sex or evidence of phospholipidosis, was assessed by adding these to the base model in turn and noting the change in the objective function value (OFV). The inclusion of a covariate improved the fit of the data to the model if there was a decrease in the OFV. The difference between a pair of OFV values when a covariate was included (full model) and then excluded (reduced model) was tested for significance (α = 0.01), using the chi-square statistic with 1 degree of freedom ($\chi^2_{1,0.01}$ = 6.6).

The interindividual variability (IIV) was modeled, assuming a log-normal distribution, as follows:

$$CL/F_{ij} = CL/F \cdot e^{(\eta_{i,CL/F} + \kappa_{j,CL/F})}$$

$$V/F_{ij} = V/F \cdot e^{(\eta_{i,V/F} + \kappa_{j,V/F})}$$

$$K_{a_{ij}} = K_a \cdot e^{(\eta_{i,K_a} + \kappa_j K_a)}$$

where CL/F_{ij}, V/F_{ij}, and K_{aij} represent the true but unknown values of the parameters for the *i*th subject on the *j*th occasion about the typical respective population values *CL/F*, *V/F*, and K_a. The parameters $\eta_{i,CL/F}$, $\eta_{i,V/F}$, and η_{i,K_a} are random variables distributed with means of 0 and respective variances of $\omega^2_{CL/F}$, $\omega^2_{V/F}$, and $\omega^2_{K_a}$. *K* (kappa) is a random variable representing the variability of a given pharmacokinetic parameter value on different occasions, with an occasion being defined a priori as a dose or sequential doses followed by at least one observation (in this study, there were typically four occasions). The interoccasion variability (IOV) was assumed to be sampled from a normal distribution having a mean of 0 and a variance of π^2. In modeling the IOV, it was assumed that the variances of each parameter were sampled from the same distribution. The residual unexplained variability (RUV) among observed plasma tafenoquine concentrations and those predicted by the final population model were estimated by a combined proportional plus additive error model, as follows: $C_{ij} = C_{pred,ij}(1 + \varepsilon_{1,ij}) + \varepsilon_{2,ij}$, where C_{ij} is the *i*th observed concentration in the *j*th subject, $C_{pred,ij}$ is the plasma tafenoquine concentration predicted by the pharmacokinetic model, and $\varepsilon_{1,ij}$ and $\varepsilon_{2,ij}$ are randomly distributed variables having mean values of 0 and variances of σ_1^2 and σ_2^2, respectively.

Model assessment. The final model was assessed by an inspection of standard diagnostic plots of observed concentration versus population model predicted concentration and separate plots of weighted residual versus model-predicted concentration, elapsed time, subject identification, and screened covariates (3). A degenerate visual predictive check was performed by simulating from the final model 1,000 concentrations at each of 44 sampling times of up to 200 h postdose, at week 1 (after the third loading dose), and then at weeks 4, 8, and 16 during maintenance dosing. The 50th percentile concentration (as an estimator of the population-predicted concentration) and the 5th and 95th percentile concentrations were processed by ActivePerl (v.5.8.4; ActiveState) and then plotted against elapsed time for each of the above four sampling windows. Observed tafenoquine concentrations were superimposed on the plots. Model robustness was assessed by a nonparametric bootstrap, with replacement, of 200 NONMEM runs of the final model, comparing the bootstrapped median parameter values and the percentile bootstrap 90% confidence intervals (4, 5) with the respective values estimated in the final model.

Adverse events, severity rating, and association with drug. As part of the clinical phase III trial, adverse events were elicited by an investigator asking the subject a nonleading question, such as "Do you feel differently in any way since starting the new treatment?" A physician assessed the level of relationship of any adverse event on the basis of the subject's response and any temporal association and/or known adverse responses to the drug. The physician graded the severity of adverse events as follows: mild, not affecting daily activities; moderate, causing some interference with daily activities; severe, daily duties could not be completed. Attribution or relationship to tafenoquine was judged by the physician to be not related, unlikely to be related, suspected (reasonable probability) to be related, or probably related.

RESULTS

Population characteristics. The study population consisted of 476 males and 14 females, with a mean (± standard deviation [SD]) age of 25.4 ± 5.3 years (range, 18 to 47 years) and

TABLE 1. Development of structural model for pharmacokinetics of tafenoquine

Model	Parameterization[d]	ΔOFV[a]
1	$CL/F = \theta_1$; $V/F = \theta_2$; $K_a = \theta_3$	
2	$CL/F = \theta_1 \cdot (1 + \theta_4 \cdot \text{age}/25.4)$; $V/F = \theta_2$; $K_a = \theta_3$	−2
3	$CL/F = \theta_1$; $V/F = \theta_2 \cdot (1 + \theta_4 \cdot \text{age}/25.4)$; $K_a = \theta_3$	−9[b]
4	$CL/F = \theta_1 \cdot (1 + \theta_4 \cdot CL_{CR}/121)$; $V/F = \theta_2$; $K_a = \theta_3$	−4
5	$CL/F = \theta_1 \cdot \text{PHOS} + \theta_4 \cdot (1 - \text{PHOS})$; $V/F = \theta_2$; $K_a = \theta_3$	0
6	$CL/F = \theta_1$; $V/F = \theta_2 \cdot \text{PHOS} + \theta_4 \cdot (1 - \text{PHOS})$; $K_a = \theta_3$	−1[b]
7	$CL/F = \theta_1 \cdot \text{sex} + \theta_4 \cdot (1 - \text{sex})$; $V/F = \theta_2$; $K_a = \theta_3$	−3[b]
8	$CL/F = \theta_1$; $V/F = \theta_2 \cdot \text{sex} + \theta_4 \cdot (1 - \text{sex})$; $K_a = \theta_3$	−12
9[c]	$CL/F = \theta_1 \cdot (1 + \theta_4 \cdot WT/80.9)$; $V/F = \theta_2 \cdot (1 + \theta_5 \cdot WT/80.9)$; $K_a = \theta_3$	−39
10	$CL/F = \theta_1 \cdot (WT/70)^{0.75}$; $V/F = \theta_2 \cdot (WT/70)^{1.0}$; $K_a = \theta_3$	+37[b]

[a] ΔOFV, change in OFV from that of model 1 (OFV = 22,177).
[b] Rounding errors occurred during fitting.
[c] Final model.
[d] WT/80.9, body weight (kg) centered on average weight (80.9 kg); age/25.4, age (years) centered on average age (25.4 years); $CL_{CR}/121$, CL_{CR} (ml/min) centered on average CL_{CR} (121 ml/min); PHOS, phospholipidosis (tested in 77 subjects; 1 = phospholipidosis present, 0 = phospholipidosis not present); sex, male = 0 and female = 1.

a mean (± SD) weight of 80.9 ± 11.9 kg (range, 50 to 135 kg). All but eight were of Caucasian background. Of the 490 subjects, 2 subjects provided one blood sample, 3 subjects provided two blood samples, 23 subjects provided three blood samples, and the remaining 462 subjects provided four blood samples, giving a total of 1,925 plasma concentration-time points available for the pharmacokinetic analyses.

Population pharmacokinetic modeling. Summary results of the population model-building process are shown in Table 1. The data did not support the inclusion of an absorption lag time in any model. Neither age nor CL_{CR} on CL/*F* significantly improved the fit, nor did sex or phospholipidosis as indicator variables. Both age and sex effects on *V*/*F* produced small but significant decreases in the OFV, of 9 and 12, respectively. Use of an allometric size model scaled to 70 kg for CL/*F* (power, 0.75) and *V*/*F* (power, 1.0) was not supported (OFV = +37). Inclusion of centered linear weight on both CL/*F* and *V*/*F* significantly decreased the OFV, from 22,177 to 22,138. This model predicted that a 1-kg change in weight from the population average value of 80.9 kg would give a commensurate change of 0.0167 liters/h (0.38%) in CL/*F* and a change of 9.7 liters (0.51%) in *V*/*F*. The linear, positive influence of weight on both CL/*F* and *V*/*F* is shown in Fig. 1a and b, respectively.

Modeling the covariance between $\omega^2_{CL/F}$ and $\omega^2_{V/F}$ reduced the OFV from 22,265 to 22,248 compared with the corresponding model when $\omega^2_{CL/F}$ and $\omega^2_{V/F}$ were assumed to be independent. Inclusion of the IOV for CL/*F* reduced the OFV further, to 22,177. However, while the addition of IOV to *V*/*F* further reduced the OFV, the value for $\omega^2_{V/F}$ was suspiciously low and the correlation coefficient (*r*) calculated from the diagonal and off-diagonal elements of the variance matrix [$r = \omega^2_{CL/F,V/F}/(\omega^2_{CL/F} \cdot \omega^2_{CL/F})^{0.5}$] was ~1, indicating an inappropriate variance model. The RUV was best modeled by using a combined proportional and additive model, as seen by an increase in the OFV and by numerical difficulties when the additive and proportional models were used separately.

Parameter values for the final population model and the bootstrap validation are shown in Table 2. The estimated time (T_{max}) for peak concentration to occur after a dose was 21.4 ± 8.57 h, calculated from each subject's conditional estimates of K_a and K_e by the standard formula $T_{max} = \ln(K_a/K_e)/(K_a - K_e)$ for a one-compartment extravascular model. The observed mean (± SD) peak tafenoquine concentration measured in samples drawn within 5% of the time of the estimated mean population T_{max} (21.4 h) for 42 subjects at weeks 4, 8, and 16 was 321 ± 63 ng/ml. The observed mean (± SD) trough tafenoquine concentration drawn within 5% of the target 168-h-postdose sampling time for 162 subjects at weeks 4, 8, and 16 was 221 ± 57 ng/ml. The typical population CL/*F* and *V*/*F*

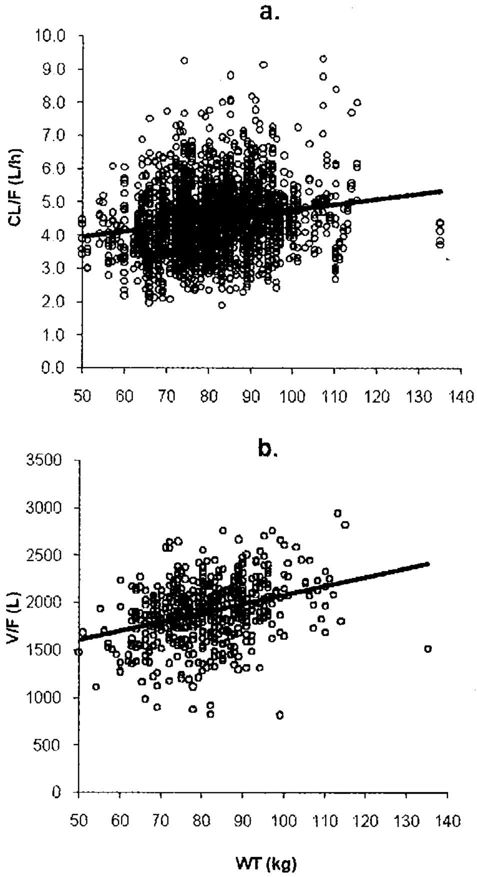

FIG. 1. Relationship of body weight (WT) to individual estimates of (a) CL/*F* and (b) *V*/*F* for tafenoquine.

TABLE 2. Comparison of parameter estimates for the population model with the results of 200 bootstrapped runs

Parameter and model	Final model value	Bootstrap value (n = 200) (median [90% CI])[b]
Structural model[a]		
CL/F (θ_1; liters/h)	3.02	3.01 (2.42–3.52)
V/F (θ_2; liters)	1,110	1,110 (874–1,382)
K_a (θ_3; h^{-1})	0.243	0.245 (0.212–0.280)
Weight centered on CL/F[c]	0.448	0.447 (0.249–0.816)
Weight centered on V/F[d]	0.713	0.713 (0.371–1.20)
Variance model		
$IIV_{CL/F}$ (CV%)	18	18 (16–20)
$IIV_{V/F}$ (CV%)	22	22 (20–25)
IIV_{K_a} (CV%)	76	75 (64–85)
$IOV_{CL/F}$ (CV%)	18	18 (16–20)
RUV (CV%)	5.9	5.9 (4.7–7.4)
RUV (ng/ml)	22.9	23.1 (18.7–26.3)

[a] $CL/F = \theta_1 \cdot (1 + \theta_4 \cdot WT/80.9)$; $V/F = \theta_2 \cdot (1 + \theta_5 \cdot WT/80.9)$; $K_a = \theta_3$.
[b] Percentile bootstrap 90% confidence interval (5th to 95th percentiles).
[c] Linear coefficient (θ_4) for weight centered on CL/F.
[d] Linear coefficient (θ_5) for weight centered on V/F.

values for all subjects, with a mean weight of 80.9 kg, were 4.37 liters/h and 1,901 liters, respectively. The typical value of K_a over all subjects was 0.243 h^{-1}. The IIV about CL/F, V/F, and K_a was 18%, 22%, and 76%, respectively. The IOV for CL/F was 18%. Mean values per kg for CL/F and V/F calculated from conditional estimates for each subject were 0.056 ± 0.013 liters/h/kg and 23.7 ± 4.5 liters/kg, respectively. The elimination half-life ($t_{1/2}$), derived from the expression $t_{1/2}$ = (0.693 · V/F)/(CL/F) with individual estimates of CL/F and V/F, was 12.7 ± 3.0 days.

Routine diagnostic weighted residuals versus population model-predicted values (data not shown) were symmetrically distributed and were mostly within about 3 units of the null ordinate, indicating a good fit of the model to the data. Plots of weighted residuals versus both subject identification and time (data not shown) were distributed symmetrically in a band with no obvious trend and were mostly within approximately 3 units of the null ordinate, indicating that no time-related factor affected the data and that no subject's data contributed to any marked deviation from the model. The bootstrapped median parameter values very closely agreed with the respective values from the final population model (Table 2). The degenerate visual predictive check showed the observed data to be symmetrically distributed about the 50th percentile profile, with approximately 10% of the data distributed outside the 5th- to 95th-percentile boundaries (Fig. 2a, b, c, and d).

Individual pharmacokinetics of tafenoquine in subjects with malaria and with drug-associated adverse events. The four subjects who had a relapse after returning to Australia had a mean (± SD) CL/F of 0.060 ± 0.014 liters/h/kg, a V/F of 23.2 ± 8.0 liters/kg, and a $t_{1/2}$ of 11.1 ± 2.3 days, calculated from conditional parameter estimates for each individual.

One or more adverse events with a suspected/probable relationship to tafenoquine were reported by 73 subjects. These were ranked as mild in 67 subjects (91.8%), moderate in 5 subjects (6.8%), and severe in 1 subject (1.4%) and encompassed the following: nausea, abdominal pain, flatulence, vomiting, vertigo, agitation, amnesia, headache, eye abnormality, reflux, dreaming abnormality, insomnia, somnolence, diarrhea, hyperesthesia, tremor, paranoia, headache, anorexia, depression, coordination abnormality, appetite increase, and thirst. Tafenoquine was not withdrawn in any of the 67 mild cases, but it was withdrawn for three subjects who reported either moderate hyperesthesia, abdominal pain, or depression. Assessment for phospholipidosis was carried out in a subgroup of 77 subjects because tafenoquine has cationic amphiphilic characteristics and, therefore, the potential to cause phospholipid accumulation. Table 3 shows adverse events reported in the five moderate cases and one severe case where tafenoquine was suspected to cause the discomfort, together with individual estimates of the pharmacokinetic responses for these subjects. All moderate adverse events were experienced 1 to 24 days after the initiation of tafenoquine, while the single subject with

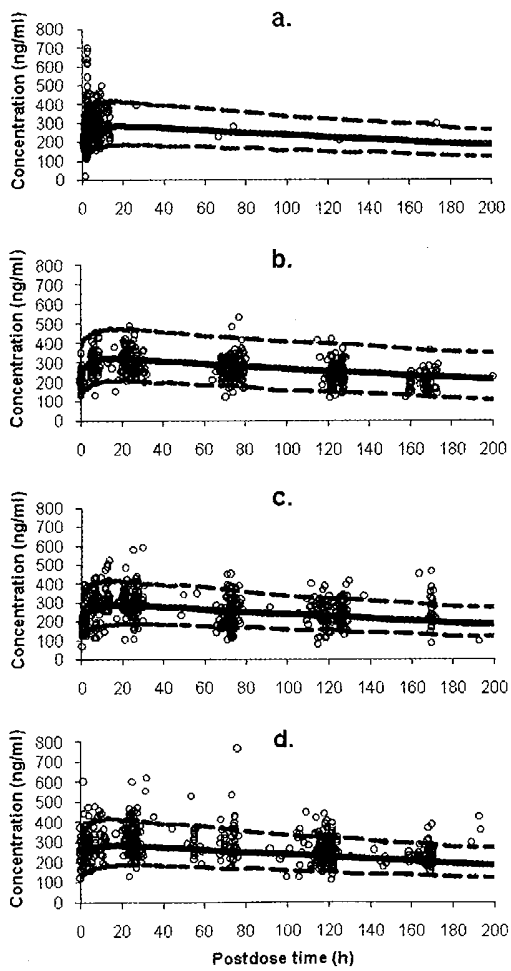

FIG. 2. Degenerate visual predictive check of the final population model for tafenoquine. Plots are shown for plasma tafenoquine concentration versus postdose time in sampling windows of (a) week 1 (post-loading dose), (b) week 4, (c) week 8, and (d) week 16. The population-predicted profile (50th percentile) is shown by the solid line, and the 90% prediction intervals estimated from 1,000 simulated concentrations over 200 h (postdose) are encompassed by the broken lines in each plot.

TABLE 3. Tafenoquine pharmacokinetic data for six subjects reporting at least one adverse effect classified as severe ($n = 1$) or moderate ($n = 5$)

Adverse event	Treatment duration (days)[a]	Cumulative dose (mg)[b]	Dosing stopped	C_{last} (ng/ml)[c]	CL/F (liters/h/kg)	V/F (liters/kg)	$t_{1/2}$ (days)
Severe event							
Diarrhea and/or abdominal pain	2	400	No	*	0.059	24.4	12.0
Moderate events							
Insomnia	1	200	No	*	0.059	23.2	11.3
Hyperesthesia	12	800	Yes	283	0.046	20.7	13.1
Abdominal pain	20	1,000	Yes	253	0.053	27.8	15.1
Depression	24	1,000	Yes	275	0.061	25.1	12.0
Vomiting and/or nausea	3	600	No	315	0.077	26.1	9.8

[a] Number of days from starting dosing until adverse event reported.
[b] Total amount of drug taken before adverse event reported.
[c] Last plasma tafenoquine concentration before adverse event reported. *, adverse event was reported before first plasma sample was drawn.

severe effects reported diarrhea and abdominal pain 2 days after commencing tafenoquine treatment.

DISCUSSION

This study of the population pharmacokinetics of tafenoquine in 490 Australian soldiers is the largest undertaken by far with this promising new oral antimalarial agent. Previously, a two-stage dose-ranging pharmacokinetic study was performed with 48 healthy adult males (Caucasian [$n = 20$], African-American [$n = 12$], and Hispanic [$n = 16$]) (1), while a subsequent population pharmacokinetic study was reported for 104 Thai soldiers on a monthly prophylactic regimen of tafenoquine (2). The present findings confirm the knowledge of tafenoquine disposition in humans and considerably extend the pharmacokinetic data to a large population of healthy, Caucasian military personnel deployed in field operations.

The apparent *V/F* was similar to that reported by Edstein et al. (2), but the systemic CL/*F* was greater (4.37 liters/h versus 3.20 liters/h). The derived typical elimination $t_{1/2}$ of 12.7 days was slightly shorter than the 14 to 16 days reported previously, which may partly reflect the fact that the last samples were drawn at only up to 1 week postdose and therefore the presumed "terminal" phase may have included some components of a distribution phase, but not substantial enough to be supported by a two-compartment model. The mean values for CL/*F* and *V/F* obtained by Brueckner et al. (1) for fasted subjects of similar average weight to that from this study were 5.7 liters/h and 2,558 liters, respectively, which are 30% to 35% higher than the present typical values. However, in the current study, the subjects took tafenoquine with food, which reportedly can increase the bioavailability (*F*) by up to one-third (R. P. Brueckner, personal communication), which brings the respective CL/*F* and *V/F* values into closer agreement when corrected for *F*. While the extent of tafenoquine absorption may be greater, food could also slow the rate of drug absorption, as evidenced by the typical K_a of 0.243 h^{-1}, compared with 0.391 h^{-1} and 0.694 h^{-1} reported by Brueckner et al. (1) and Edstein et al. (2), respectively. As a result, the average T_{max} of 21.4 h was greater than the 8.6 h to 13.8 h reported previously (1, 2), which as well as the influence of food, may reflect continuous absorption along the intestinal tract, perhaps due in part to microprecipitation and redissolution of tafenoquine, which is only slightly water soluble (1). Unpublished data on file (GlaxoSmithKline) for healthy volunteers showed mean (CV%) T_{max} values of 18.6 h (84%) and 26.3 h (126%) under fasted conditions and when administered with a standard high-fat meal, respectively, indicating that the T_{max} and its variability were increased by food. Nonetheless, it should be remembered that T_{max} is a model-dependent parameter in that the true value is likely to be overestimated when a one-compartment model is used compared with that for a two-compartment model. In agreement with previous reports (1, 2), there was marked IIV in the T_{max}, reflecting the considerable variability in both K_a and K_e, with the latter being estimated from conditional estimates of *V/F* and CL/*F* for each subject.

The variability in CL/*F* and *V/F* was not excessive, at 18% to 22%, most likely reflecting the uniformity of the military subjects. The variance model supported estimation of the IOV in CL/*F* but not that in *V/F* or K_a. While Edstein et al. (2) used a proportional (exponential) model for RUV, presently a combined additive-proportional RUV model was supported, which is the preferred model wherever possible, especially where the range of concentration data is as wide as in this study. There was a positive linear association between weight and both CL/*F* and *V/F*, but attempts to model these parameters using an allometric size model scaled to 70 kg were not supported by the data, most likely because of the reasonably narrow range of body weights. Although heavier subjects tended to have a slightly greater CL/*F* and *V/F*, this would not have any major implications for changes in the way that tafenoquine would be prescribed, at least on the basis of the pharmacokinetic data alone. Using the present steady-state plasma tafenoquine concentrations as the appropriate clinical target, a 20-kg change in weight would require changes in the loading dose and maintenance dose of only about 10% and 7.5%, respectively. Unpublished data (GlaxoSmithKline) indicated that a considerable fraction of a tafenoquine dose may be excreted unchanged, while the clinical data from the trial of which the present study was a part showed that mean serum creatinine concentrations increased 12.1 mmol/liter from baseline until the end of the prophylaxis. However, estimated creatinine clearance explained an insignificant amount of the variability about CL/*F*. Age explained a small yet significant amount of the variability in both *V*/*F* and CL/*F* but was positively correlated with weight and thus was not considered further.

In assessing performance, model robustness was evaluated via a nonparametric bootstrap, which indicated that randomly selected combinations of data gave very similar results to those obtained with the original data set. In addition, a degenerate visual predictive check showed that the raw data obtained after the third split loading dose and at week 4, 8, and 16 during maintenance dosing mirrored the corresponding profiles obtained from simulations using point estimates of the final model parameter values. This convenient approach has been shown elsewhere (16) to give a good approximation of the full posterior predictive check, in which the simulations are performed using posterior distributions of the parameter values (6), which are difficult to calculate from the NONMEM output. The predictive check showed, firstly, that the structural model was satisfactory by the symmetrical distribution of the raw data about the 50th percentile profile and, secondly, that the variance model was appropriate, with about 10% of the raw data lying outside the 5th and 95th percentiles.

The prophylactic efficacy of tafenoquine is determined by its ability to prevent parasitemia from developing, which is associated with the susceptibility of malaria parasites to tafenoquine concentrations achieved in the target population. Tafenoquine has both causal prophylactic activity against the hepatic stages of the parasite and suppressive activity, which eradicates the erythrocytic stages of the parasite (1). In the present study, no subject developed parasitemia during the 6 months of prophylaxis, but four had a relapse of *P. vivax* infection after returning to Australia. In contrast, one subject in a population of 104 Thai soldiers on 400 mg tafenoquine monthly for 6 months developed vivax malaria during prophylaxis (15). At the time of diagnosis, the Thai soldier had a plasma tafenoquine concentration of 40 ng/ml, which was >5-fold lower than the mean steady-state trough tafenoquine concentration of 221 ng/ml presently recorded. Six Australian soldiers had tafenoquine concentrations of <100 ng/ml at either week 4, 8, or 16. Of those, only one subject had consistently lower tafenoquine concentrations (<120 ng/ml) on the three occasions sampled and therefore may have had a reduced margin of suppressive protection against malaria infection. The Thai soldier who developed parasitemia also had consistently lower tafenoquine concentrations during the prophylactic phase (15). Unlike the Thai soldier, the four Australian soldiers who relapsed had comparable tafenoquine concentrations to subjects who did not have a recurrence of malaria. Although the number of subjects who relapsed was small, the individual estimates of the pharmacokinetic responses for these subjects did not provide a prediction or correlation with tafenoquine's prophylactic efficacy.

There was no apparent correlation between either the pharmacokinetic parameter values predicted for individual subjects or the last tafenoquine concentration measured in subjects reporting moderate or severe adverse events. These findings suggested that plasma tafenoquine concentrations are not the primary predictor of tafenoquine tolerability. This lack of an association between plasma drug concentrations and adverse events has also been seen with another antimalarial agent, mefloquine, which shares similar pharmacokinetic properties with tafenoquine (12) in that both are lipophilic, are slowly absorbed from the gastrointestinal tract, are extensively bound to tissues, and have elimination $t_{1/2}$ values of about 2 weeks (1, 2, 9, 14).

In conclusion, the pharmacokinetic properties of tafenoquine determined in this study support a weekly dosing regimen for prolonged periods. Although body weight influenced CL/*F* and *V*/*F*, it was not considered to have sufficient impact to warrant changing the maintenance or loading dose for any individual from such a population. Nonetheless, dose changes may be warranted for other patients who are markedly overweight or underweight compared with this homogenous group of soldiers. Any dosing requirements for markedly overweight subjects may need special consideration, as reviewed recently (7). Tafenoquine was generally well tolerated. Individual pharmacokinetic parameter estimates for subjects with malaria did not predict prophylactic outcomes, and plasma concentrations at steady state did not appear to be related to the occurrence of adverse events. Since this population was a homogenous group of healthy Australian soldiers of predominantly Caucasian background, additional pharmacokinetic studies may be required for other populations.

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Chapter 3 Paper 3.3

Charles BG, Blomgren A, **Nasveld PE**, et al. (2007) Population pharacokinetics of mefloquine in military personnel for prophylaxis against malaria infection during field deployment. *European Journal of Clinical Pharmacology*. 63: 271-278.

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Chapter 3 Paper 3.4

Edstein MD, **Nasveld PE**, Kocisko DA, et al. (2007) Gender differences in gastrointestinal disturbances and plasma concentrations of tafenoquine in healthy volunteers after tafenoquine administration for post-exposure vivax malaria prophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101: 226-230.

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CHAPTER 4

- **Evaluation of Tafenoquine for the post-exposure prophylaxis of vivax malaria (Southwest Pacific Type) in non-immune Australian soldiers**

- **List of peer reviewed and published papers presented in this chapter**

1. **Nasveld PE**, Kitchener S, Edstein M, Rieckmann K. (2002) Comparison of tafenoquine (WR238605) and primaquine in the post-exposure (terminal prophylaxis) of vivax malaria in Australian Defence Force personnel. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 96: 683-684.

All authors participated in the conception and design of the study and PN drafted the manuscript. PN, SK and ME contributed to the analysis of the study. All authors gave final approval for the manuscript submitted for publication.

2. Elmes NJ, **Nasveld PE**, Kitchener SJ, et al. (2008) The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for the post exposure prophylaxis of *Plasmodium vivax* malaria in the Southwest Pacific. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 102: 1095-1101

PN, SK and ME participated in the conception and design of the study. NE managed one of three cohorts of study subjects and drafted the manuscript. PN, NE and ME contributed to the analysis of the study. All authors gave final approval for the manuscript submitted for publication.

3. Edstein MD, **Nasveld PE**, Kocisko DA, et al. (2007) Gender differences in gastrointestinal disturbances and plasma concentrations of tafenoquine in healthy volunteers after tafenoquine administration for post-exposure vivax malaria prophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101: 226-230

All authors participated in the conception and design of the study and ME drafted the manuscript. PN, ME and DK contributed to the analysis of the study. PN provided the clinical input to the paper. All authors gave final approval for the manuscript submitted for publication.

4. **Nasveld PE** and Kitchener SJ. (2005) Treatment of acute vivax malaria with tafenoquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 99: 2-5

Both authors participated in the conception of the study and PN drafted the manuscript. PN and SK contributed to the analysis of the study. Both authors gave final approval for the manuscript submitted for publication.

4.1 Introduction

This study compared the effectiveness of a three day course of tafenoquine and the standard 14 day course of primaquine mefloquine in preventing relapses caused by *P. vivax* malaria. Primaquine is the only drug currently in use for the prevention of relapsing forms of malaria [Antibiotic Expert Group, 2010]. It is a cumbersome 14 day program administered once an exposed individual leaves a malarious area. Issues of compliance once an individual leaves a risk environment has long been thought to contribute to the apparent failure of primaquine to reliably eradicate the hynozoite (liver) stages of malaria and available data on tafenoquine suggested that similar or better results may be expected from a shorter 3 day course undertaken in the days before an exposed individual left the risk environment. Tafenoquine, unlike primaquine, allows convenient dosing, and is expected to be highly efficacious against *P. vivax* and *P. ovale* malaria. Additionally, the study sought to evaluate the tolerability and safety of tafenoquine when used as post-exposure prophylaxis as well as to characterise the pharmacokinetics of tafenoquine in the study population.

4.2 Objectives

4.2.1 Primary Objective

The primary study objective was to compare the effectiveness of tafenoquine and primaquine for post exposure prophylaxis of vivax malaria.

4.2.1 Secondary Objectives

The secondary study objectives were as follows:

- The secondary study objective was to compare the safety and tolerability of tafenoquine and primaquine for post exposure prophylaxis of vivax malaria.
- To characterise the population pharmacokinetics of tafenoquine

4.3 Ethics

This study was conducted under approvals from the Australian Defence Medical Ethics Committee – ADMEC (now known as the Australian Defence Human

Research Ethics Committee – ADHREC). The protocol and statement of informed consent were approved by the Australian Defence Medical Ethics Committee (ADMEC) prior to study initiation on the 5th November 1998 and recorded as ADMEC 165/98.

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki as amended in Somerset West, Republic of South Africa 1996. Two protocol amendments were made, and approved by ADMEC for the following changes to the protocol.

Amendment 1, approved 27th September 1999

This amendment was requested due to a higher than expected adverse event rate. ADMEC approved the requested change in tafenoquine dosing from 500 mg (equivalent to 400 mg base) once a day to 250 mg (equivalent to 200 mg base) twice a day.

Amendment 2, approved 19th April 2000

This amendment was requested because drug levels being observed were higher than that required for 2-4 weeks protection. ADMEC approved a further change in tafenoquine dosing from 250 mg (equivalent to 200 mg base) twice a day to a single daily dose of 250 mg (equivalent to 200 mg base).

Written informed consent was obtained from each subject prior to entry into the study. Subjects were recruited using non-coercive means and no inducements were offered. The subjects invited to take part in the trial were entitled to make a choice based on full and complete information presented in a manner that was both understandable and ethnically appropriate. The Consent Form was designed to assure the protection of the subject's rights. Consent was obtained from all subjects within 5 days of the start of treatment.

4.4 Methods

4.4.1 Study design

This was an open-label, randomised, parallel group study in male and female members of the Australian Defence Force who had been deployed in the Southwest Pacific. Subjects had been taking daily doxycycline as malaria prophylaxis during deployment. Three distinct cohorts were enrolled into the study - AMI 001 (Bougainville, Papua New Guinea), AMI 002 and AMI 003 (Timor Leste).

Subjects who met the entry criteria (healthy, G6PD-normal, free from malaria) were randomised to receive primaquine (PQ at 7.5 mg daily for 14 days) or tafenoquine (TQ):

- 400 mg once daily for 3 days (AMI 001 & 002)
- 200 mg twice daily for 3 days (AMI 001 & 002)
- 200 mg once daily for 3 days (AMI 003)

Randomisation was in the ratio (PQ:TQ) 1:1 for AMI 001, 1:2 for AMI 002 and 1:3 for AMI 003.

Subjects were evaluated at screening and Day 4 (last day on deployment), when blood samples were taken for haematology, biochemistry and PK analysis. Details of any adverse events or changes in concomitant medication were also recorded.

Subjects were followed up for 12 months for the development of relapse of *P. vivax*. If relapse occurred, this was treated with chloroquine (3 days) followed by tafenoquine (3 days).

4.4.2 Participants

Participants included were adults between 18 and 55 years of age, male or female, and in good health as defined by Medical Class 1 or 2 (Australian Army standard). All subjects were G6PD normal and gave written informed consent. Subjects with known hypersensitivity to any component of the study drugs; known G6PD deficiency; who were unwilling/unable to give blood samples required in the study; who were taking any other investigational drug during, or within 30 days, of taking the study drugs for this medication; and females who were pregnant or unwilling/unable to comply with recognized contraception methods for 30 days after administration of the study drug were excluded from the study.

Over all three cohorts 1559 subjects were screened for entry into the study. Twenty five of these subjects failed to meet the inclusion criteria and received no study treatment. The remaining 1534 subjects were randomised to treatment apart from one subject in Cohort AMI 003 who met the inclusion criteria but was not randomised and received no study treatment. Of these, 1512 were included in the intention-to-treat population with 22 not eligible because the date of their first dose of study medication was missing (i.e. there was insufficient evidence of at least one dose of study medication). The numbers of subjects who were screened, met the inclusion criteria and were eligible for the intention-to-treat population are shown in **Table 4.1**.

Table 4-1 Subject disposition: all study subjects AMI 001, AMI 002, AMI 003 (number of subjects)

AMI 001 Population	Treatment Group				Total
	NR	Primaquine 7.5 mg tid	Tafenoquine 200 mg bid	Tafenoquine 400 mg od	
Screened	3	213	86	290	592
Met inclusion criteria	0	213	86	290	589
ITT population	0	210	86	288	584

NR Three subjects who did not meet the inclusion criteria and were not randomised.
Prima = primaquine; Tafen = tafenoquine

AMI 002 Population	Treatment Group				Total
	NR	Primaquine 7.5 mg tid	Tafenoquine 200 mg bid	Tafenoquine 400 mg od	
Screened	18	144	77	159	398
Met inclusion criteria	0	144	77	159	380
ITT population	0	131	75	158	364

NR Eighteen subjects who did not meet the inclusion criteria and were not randomised.

AMI 003 Population	Treatment Group			Total
	NR	Primaquine 7.5 mg tid	Tafenoquine 200 mg od	
Screened	4	158	407	569
Met inclusion criteria	1	158	406	565
ITT population	0	158	406	564

NR Four subjects who did not meet the inclusion criteria and were not randomised and one subject who met the inclusion criteria but was not randomised or treated.

Demographic characteristics for the intention-to-treat populations in each cohort are summarised in **Table 4.2**. In Cohort AMI 001, the treatment groups were well matched for demographic characteristics. As expected, there were more male than female subjects, with 85-89% males across the three treatment groups. The mean age in each group was 30 years. In Cohort AMI 002, the treatment groups were well matched for demographic characteristics. All subjects were male and the mean age in each group was 24-26 years. In Cohort AMI 003, the treatment groups were well matched for demographic characteristics. The majority of subjects were male and the mean age in both groups was 26 years.

Table 4-2 Demographic characteristics AMI 001, AMI 002, AMI 003: intention-to-treat population

AMI 001 Demographic Characteristic	Treatment Group		
	Primaquine 7.5 mg tid N=210	**Tafenoquine 200 mg bid N=86**	**Tafenoquine 400 mg od N=288**
Gender n(%)			
male	186 (88.6%)	73 (84.9%)	248 (86.1%)
female	24 (11.4%)	13 (15.1%)	40 (13.9%)
Age (years)			
mean (sd)	30.9 (7.2)	30.1 (7.5)	30.8 (7.0)
range	19 – 56	20 – 58	20– 53
Weight (kg)			
mean (sd)	78.8 (11.4)	79.7 (11.8)	78.6 (12.0)
range	52.0 – 105.0	51.0 – 110.0	48.0 – 115.0
Height (cm)			
mean (sd)	178.0 (8.3)	177.7 (8.7)	178.0 (8.7)
range	152.0 – 198.0	153.0 – 196.0	151.0 – 208.0
Body Mass Index			
mean (sd)	24.8 (2.6)	25.2 (2.9)	24.8 (2.9)
range	18.5 – 34.4	18.2 – 32.9	17.6 – 36.3

AMI 002 Demographic Characteristic	Treatment Group		
	Primaquine 7.5 mg tid N=131	**Tafenoquine 200 mg bid N=75**	**Tafenoquine 400 mg od N=158**
Gender n(%)			
male	131 (100%)	75 (100%)	158 (100%)
Age (years)			
mean (sd)	25.4 (6.0)	24.3 (4.6)	26.1 (5.4)
range	18 – 47	18 – 40	18 – 45
Weight (kg)			
mean (sd)	80.2 (10.0)	78.1 (8.7)	79.5 (9.6)
range	57.0 – 110.0	60.0 – 98.0	58.0 – 105.0
Height (cm)			
mean (sd)	180.0 (7.2)	178.6 (6.7)	179.5 (7.3)
range	156.0 – 196.0	160.0 – 197.0	156.0 – 200.0
Body Mass Index			
mean (sd)	24.7 (2.5)	24.5 (2.4)	24.7 (2.4)
range	17.4 – 30.9	18.8 – 31.1	19.4 – 32.1

AMI 003 Demographic Characteristic	Treatment Group	
	Primaquine 7.5 mg tid N=158	**Tafenoquine 200 mg od N=406**
Gender n(%)		
male	158 (100%)	402 (99.0%)
female	0	4 (1.0%)
Age (years)		
mean (sd)	25.8 (5.7)	26.2 (5.6)
range	18 – 44	18 – 50
Weight (kg)		
mean (sd)	79.8 (10.0)	81.4 (10.6)
range	54.0 – 105.0	53.0 – 140.0
Height (cm)		
mean (sd)	179.8 (7.4)	179.9 (6.8)
range	154.0 – 200.0	157.0 – 199.0
Body Mass Index		
mean (sd)	24.7 (2.5)	25.1 (2.8)
range	17.4 – 31.4	17.9 – 36.2

The demographic characteristics of cohorts AMI 002 and AMI 003 were very similar; all but 4 subjects were male and the mean age ranged from 24 to 26 years. The demographic characteristics of Cohort AMI 001 were slightly different; 13% of subjects were female and the mean age was slightly older, 30 years.

4.4.3 Treatment and administration

Subjects were randomised to receive either primaquine or tafenoquine as described above. The study drug was supplied as follows:

- Primaquine: each tablet contained 7.5 mg primaquine. Primaquine was supplied by Randwick Logistic Company, Australia. The manufacturer was Boucher and Muir. For AMI 001 the batch number was 0330141 expiring in August 2007 and for AMI 002 and 003 the batch number 0330697 expiring August 2007.
- Tafenoquine: each capsule contained 250 mg (200 mg base equivalent) tafenoquine. Tafenoquine was supplied by SmithKline Beecham (SB). Batch numbers were not available following company acquisition.
- Doxycycline was supplied as Doryx™ 100 and obtained from existing Defence stock and confirmed as in date. Batch numbers were not recorded.

All study volunteers randomised to primaquine were to continue doxycycline 100mg for 2 weeks on leaving the deployment area in accordance with current ADF policy. The study schedule for this study is presented at Table 4-3 below.

Table 4-3 Outline of Study Assessments

	Screening (days –5 to 0)	Day 1	Day 3	Day 4	Day 14	Follow-up (to 12 months)
Consent	✓					
Eligibility	✓					
Physical exam	✓					
Med history / demographics	✓					
Blood smear	✓					✓(1)
Haematology / biochemistry	✓(3)			✓(3)		
Plasma drug concentration	✓(3)			✓(3)		
Pregnancy test	✓(2)					
Adverse events		✓	✓	✓	✓	
Concomitant medication	✓	✓	✓		✓	
Study medication issued		✓				
Final dose study med (tafenoquine)			✓			
Final dose study med (primaquine)					✓	

(1) Only if malaria was suspected within the 12 month follow-up – no routine smears were performed during follow-up.

(2) Female subjects only

(3) Not for primaquine subjects in AMI 002 and AMI 003

4.4.4 Criteria for evaluation

4.4.4.1 Efficacy

The primary efficacy index was the proportion of subjects with confirmed parasitaemia during the 12-month follow-up period.

The secondary efficacy variable was time to confirmed parasitaemia during the 12-month follow-up period.

4.4.4.2 Safety

Safety was assessed by collection of adverse events and laboratory data. Adverse event data were collected throughout the study period. Blood samples for laboratory analysis were collected at screening and at Day 4.

4.4.4.3 Pharmacokinetics

Tafenoquine drug levels were measured using an established methodology [Kocisko et al, 2000]. Sampling occurred at day 3 approximately 10 hours post last dose. Population pharmacokinetics are expressed as ng/ml with standard deviations calculated for the population. Internal comparisons were made between levels in subjects who experienced parasitaemia and those who did not, as well as between those experiencing adverse events and those who were adverse event free. Gender comparisons were also undertaken in Cohort AMI 001 only.

4.4.4.4 Statistical analyses

The primary efficacy analysis consisted of a chi-squared test of independence (and associated 95% confidence interval) for the number of failures in each tafenoquine group versus primaquine. A Fisher’s exact test was also performed as low failure rates were observed for some of the treatment groups.

For the secondary efficacy analysis, the median time to failure (and associated 95% Confidence Interval) was estimated for each treatment group using PROC LIFETEST in SAS. The Kaplan Meier estimate of the survival curve was plotted.

4.5 Results

4.5.1 Subject disposition

No per protocol population was defined for this study, so no formal analysis of protocol violators was performed.

A protocol violation in treatment of two subjects with relapsed *P. vivax* did occur. Two subjects from Cohort AMA 001 reported *P. vivax* relapse 157 and 150 days after initial post-exposure prophylaxis. According to the protocol, these subjects should have received chloroquine followed by tafenoquine, but actually received tafenoquine alone. A full account of this protocol violation is given in **section 4.6** and is also presented at paper 4.4.

All subjects included in the study took doxycycline (100mg daily) as prophylactic medication during their deployment, with one exception who received mefloquine (250 mg weekly).

At the time of the start of this study, Operational Deployment Orders required that soldiers take daily doxycycline (100mg 14d) and weekly chloroquine (300mg) concurrently with any post-exposure prophylaxis. During the course of the study, these requirements were changed to require concurrent doxycycline only, and subsequently to require no extra concurrent anti-malarial therapy. This affected cohort AMI 001 only, as cohorts AMI 002 and 003 were recruited after the directive was changed to require no extra concurrent anti-malarial therapy. Concurrent doxycycline and chloroquine were, where applicable, recorded as concurrent medications.

In the primaquine group, 35 subjects took concurrent doxycycline and chloroquine and 175 took concurrent doxycycline. In the tafenoquine 400mg od group, 46 subjects took concurrent doxycycline and chloroquine, 153 took concurrent doxycycline and 89 subjects took tafenoquine alone. All subjects in the tafenoquine 200mg bid group took tafenoquine alone.

With the exception of chloroquine (taken as part of the study medication regimen), few subjects received concomitant medication during the study. A total of 144 subjects (9.5%) across all cohorts received concomitant medication during the study. The most common concomitant medications were mild analgesics and treatments for gastrointestinal disturbances.

In all cohorts, subjects receiving any of the tafenoquine regimens were recorded as 100% compliant with receipt of tafenoquine. Compliance data were not recorded for subjects receiving primaquine.

4.5.2 Efficacy results

4.5.2.1 Primary efficacy analysis

The efficacy analysis was based on the intention-to-treat population.

The primary efficacy index was the proportion of subjects with confirmed parasitaemia during the 12 month follow-up period following randomisation. Results are summarised in **Tables 4.4, 4.5 and 4.6** for Cohorts AMI 001, AMI 002 and AMI 003, respectively.

Table 4-4 Number (%) of subjects with confirmed parasitaemia during the 12 month follow-up period. AMI 001: intention-to-treat population

Parasitaemia	**Treatment Group**		
	Primaquine 7.5 mg tid N=210	**Tafenoquine 200 mg bid N=86**	**Tafenoquine 400 mg od N=288**
No confirmed parasitaemia	205 (97.6%)	85 (98.8%)	282 (97.9%)
Confirmed parasitaemia	5 (2.4%)	1 (1.2%)	6 (2.1%)
Treatment Comparison (Tafenoquine – Primaquine)			
95% CI for comparison	-	-4.3%, 1.85%	-2.9%, 2.34%
P-value (chi-square)	-	0.49955	0.82341
P-value (Fisher's exact)	-	0.67581	1

In Cohort AMI 001, 12 subjects reported confirmed parasitaemia during the 12 month follow-up period: 5 (2.4%) in the primaquine group, 1 (1.2%) in the tafenoquine 200mg bid group and 6 (2.1%) n the tafenoquine 400mg od group. There was no statistically significant difference between the tafenoquine and primaquine treatment groups.

Table 4-5 Number (%) of subjects with confirmed parasitaemia during the 12 month follow-up period. AMI 002: intention-to-treat population

Parasitaemia	Treatment Group		
	Primaquine 7.5 mg tid N=131	**Tafenoquine 200 mg bid N=75**	**Tafenoquine 400 mg od N=158**
No confirmed parasitaemia	113 (86.3%)	71 (94.7%)	141 (89.2%)
Confirmed parasitaemia	18 (13.7%)	4 (5.3%)	17 (10.8%)
Treatment Comparison (Tafenoquine – Primaquine)			
95% CI for comparison	-	-17%, -1.2%	-11%, 4.06%
P-value (chi-square)	-	0.04617	0.34993
P-value (Fisher's exact)	-	0.06406	0.37544

In Cohort AMI 002, 39 subjects reported confirmed parasitaemia during the 12 month follow-up period: 18 (13.7%) in the primaquine group, 4 (5.3%) in the tafenoquine 200 mg bid group and 17 (10.8%) in the tafenoquine 400 mg od group. There was a lower incidence of confirmed parasitaemia in both the tafenoquine groups when compared to the primaquine group.

Table 4-6 Number (%) of subjects with confirmed parasitaemia during the 12 month follow-up period. AMI 003: intention-to-treat population

Parasitaemia	Treatment Group	
	Primaquine 7.5 mg tid N=158	**Tafenoquine 200 mg od N=406**
No confirmed parasitaemia	151 (95.6%)	386 (95.1%)
Confirmed parasitaemia	7 (4.4%)	20 (4.9%)
Treatment Comparison (Tafenoquine – Primaquine)		
95% CI for comparison	-	-3.3%, 4.33%
P-value (chi-square)	-	0.80442
P-value (Fisher's exact)	-	1

Data source: Tables 11.01, 11.01A in Section 12; Listing C01 in Appendix C

In Cohort AMI 003, 27 subjects reported confirmed parasitaemia during the 12 month follow-up period: 7 (4.4%) in the primaquine group and 20 (4.9%) in the tafenoquine 200 mg od group. There was no statistically significant difference between the tafenoquine and primaquine treatment groups.

4.5.2.2 Secondary efficacy analysis

The secondary efficacy variable was time to parasitaemia. In Cohorts AMI 001 and AMI 003, the survival curves for all treatments are almost superimposed on each other. For Cohort AMI 002, however, there was some separation of the survival curves showing longer times to parasitaemia in the tafenoquine 200 mg bid and tafenoquine 400 mg od groups compared to the primaquine 7.5 mg tid group.

4.5.3 Safety results

4.5.3.1 Extent of exposure

Compliance was 100% for all tafenoquine subjects in all three cohorts. Primaquine compliance was not recorded in this study. In total, 1013 subject received tafenoquine for three days during the study; 161 subjects received 200 mg bd, 446 subjects received 400mg od and 406 subjects received 200 mg od.

4.5.3.2 Adverse events

The most commonly reported experiences (i.e. those occurring in at least 5% of subjects in any treatment group) are summarised below (**Table 4.7**) for the intention-to-treat population in each cohort. Most events were of mild or moderate intensity.

In Cohort AMII 001 the lowest incidence of adverse events occurred in the primaquine group, and the highest in the tafenoquine 400 mg od group. This most commonly reported events generally concerned the gastrointestinal system, and the most frequently occurring event in all treatment groups was nausea. The incidence of diarrhoea, abdominal pain and oesophageal reflux were consistently higher in the tafenoquine groups than in the primaquine group.

In Cohort AMI 002 the overall incidence of adverse events was higher in the tafenoquine groups than in the primaquine group. The most commonly reported events all concerned the gastrointestinal system, and the proportions of subjects with nausea, abdominal pain, diarrhoea and vomiting were consistently higher in the tafenoquine groups than in the primaquine group.

In Cohort AMI 003 the overall incidence of adverse events was higher in the tafenoquine group than in the primaquine group. The most commonly reported events all concerned the gastrointestinal system, and the most frequently occurring event in both treatment groups was nausea. The proportions of subjects with abdominal cramps and diarrhoea were higher in the tafenoquine group than in the primaquine group.

The pattern of adverse events was similar in all cohorts. The overall incidence of adverse events was higher among subjects treated with tafenoquine than among those treated with primaquine. The most commonly occurring events were gastrointestinal in nature and the incidence of gastrointestinal events tended to be higher following tafenoquine treatment.

Table 4-7 Number (%) of subjects with the most frequently reported adverse events related to study treatment: intention-to-treat population

AMI 001 Adverse event (Verbatim Term) Related to Study Treatment	Treatment Group Primaquine 7.5 mg tid N=210	 Tafenoquine 200 mg bid N=86	 Tafenoquine 400 mg od N=288
Suspected Relationship to Treatment			
Nausea	27 (12.6%)	18 (20.9%)	67 (23.3%)
Diarrhoea	5 (2.4%)	17 (19.8%)	25 (8.7%)
Headache*	7 (3.3%)	4 (4.7%)	23 (8.0%)
Oesophageal reflux	6 (2.9%)	7 (8.1%)	17 (5.9%)
Abdominal pain*	6 (2.9%)	5 (5.8%)	17 (5.9%)
Probable Relationship to Treatment			
Nausea	12 (5.7%)	4 (4.7%)	31 (10.8%)

* Headache includes the verbatim term headaches. Abdominal pain includes the verbatim terms abdo pain and adbo pain

\# ≥5% of subjects in any treatment group

AMI 002 Adverse event (Verbatim Term) Related to Study Treatment	Treatment Group Primaquine 7.5 mg tid N=131	 Tafenoquine 200 mg bid N=75	 Tafenoquine 400 mg od N=158
Suspected Relationship to Treatment			
Nausea	7 (5.3%)	7 (9.3%)	24 (15.2%)
Abdominal pain*	3 (2.3%)	9 (12.0%)	24 (15.2%)
Diarrhoea	3 (2.3%)	6 (8.0%)	8 (5.1%)
Probable Relationship to Treatment			
None			

* Abdominal pain consists of the verbatim term abdo pain.

\# ≥5% of subjects in any treatment group

AMI 003 Adverse event (Verbatim Term)	Treatment Group Primaquine 7.5 mg tid N=158	 Tafenoquine 200 mg od N=406
Suspected Relationship to Treatment		
Nausea	10 (6.3%)	32 (7.9%)
Abdominal cramps*	1 (0.6%)	21 (5.2%)
Probable Relationship to Treatment		
None		

* Abdominal cramps consists of the verbatim terms abdo cramps and adbo cramps.

\# ≥5% of subjects in any treatment group

The proportion of subjects with adverse events reported by the investigator with a suspected or probable relationship to study medication, together with the most commonly reported of these experiences (i.e. those occurring in at least 5% of subjects in any treatment group) are summarised in **Table 4.8** for all cohorts.

In Cohort AMI 001, nausea was the most frequently occurring event reported as related to study treatment. The other events related to treatment generally involved the gastrointestinal system, and for most of these the incidence was higher in the tafenoquine groups than in the primaquine group.

In Cohort AMI 002, all the events reported as related to study treatment occurring in at least 5% of subjects in a group were gastrointestinal. The proportions of subjects with nausea, abdominal pain and diarrhoea were higher in the tafenoquine groups than in the primaquine group.

In Cohort AMI 003, only nausea and abdominal cramps occurred with a reported relationship to study medication in at least 5% of subjects. For both these events, the proportions of subjects were higher in the tafenoquine group than in the primaquine group.

The pattern of adverse events reported by the investigator as related to study medication was similar in all cohorts. The most commonly occurring events were gastrointestinal in nature and the frequency of gastrointestinal events tended to be higher following tafenoquine treatment.

In all treatment groups, the proportion of female subjects reporting adverse events was higher than the proportion of male subjects. In general, the most commonly occurring events were similar for both male and female subjects, although the proportions of female subjects reporting these events was higher.

Table 4-8 Number (%) of subjects with the most frequently reported (>5% in any group) adverse events. Intention-to-treat population

Cohort AMI 001	Treatment Group		
Adverse event (Verbatim Term)	**Primaquine 7.5 mg tid N=210**	**Tafenoquine 200 mg bid N=86**	**Tafenoquine 400 mg od N=288**
At least one AE	79 (37.6%)	43 (50.0%)	167 (58%)
Nausea	44 (21.0%)	22 (25.6%)	99 (34.4%)
Diarrhoea	8 (3.8%)	18 (20.9%)	36 (12.5%)
Headache	17 (8.1%)	7 (8.1%)	31 (10.8%)
Abdominal pain	8 (3.8%)	8 (9.3%)	24 (8.3%)
Oesophageal reflux	6 (2.9%)	7 (8.1%)	21 (7.3%)
Lethargy	11 (5.2%)	3 (3.5%)	18 (6.3%)
Vomiting	5 (2.4%)	1 (1.2%)	15 (5.2%)

Cohort AMI 002	Treatment Group		
Adverse event (Verbatim Term)	**Primaquine 7.5 mg tid N=131**	**Tafenoquine 200 mg bid N=75**	**Tafenoquine 400 mg od N=158**
At least one AE	20 (15.3%)	24 (32.0%)	73 (46.2%)
Nausea	8 (6.1%)	10 (13.3%)	32 (20.3%)
Abdominal pain	3 (2.3%)	9 (12.0%)	31 (19.6%)
Diarrhoea	3 (2.3%)	6 (8.0%)	15 (9.5%)
Vomiting	2 (1.5%)	4 (5.3%)	7 (4.4%)

Cohort AMI 003	Treatment Group	
Adverse event (Verbatim Term)	**Primaquine 7.5 mg tid N=158**	**Tafenoquine 200 mg od N=406**
Suspected Relationship to Treatment		
Nausea	10 (6.3%)	32 (7.9%)
Diarrhoea	1 (0.6%)	25 (6.2%)
Abdominal cramps	1 (0.6%)	21 (5.2%)

In Cohort AMI 001, the incidence of adverse events tended to decrease with increasing subject weight in all treatment groups. This may reflect the fact that the majority of females fell into the lowest three weight groups and as noted above, the incidence of adverse events was higher in females than in males. The most commonly occurring events were similar between the weight categories. Nausea remained the most frequently occurring event for almost all treatment/weight categories, although the incidence tended to be lower in the higher weight groups. In Cohort AMI 002, there was less evidence of a decrease in the overall incidence of adverse events with increasing subject weight. In general, the most frequently occurring events in all treatment/weight categories were abdominal pain and nausea. In Cohort AMI 003, there was no evidence of a consistent decrease in the overall incidence of adverse events with increasing subject weight, although as there were only small numbers of subjects in some treatment/weight categories it is difficult to draw firm conclusions. Nausea and abdominal cramps were generally the most frequently occurring events.

In Cohort AMI 001 and to some extent in Cohort AMI 002, the incidence of adverse events tended to decrease with increasing subject weight in all treatment groups. As noted above, for Cohort AMI 001, this may reflect the fact that the majority of females fell into the lowest three weight groups and the incidence of adverse events was higher in females than in males. For all cohorts, there were few differences between the weight categories in which events occurred most commonly and nausea was the most frequently occurring event in most categories. The low number of subjects in the lowest and highest weight categories means that this data should be interpreted with caution.

There was no evidence in Cohort AMI 001 of a clear relationship between overall incidence of adverse events and BMI category. There were few differences between the BMI categories in which events occurred most commonly and nausea remained the most frequently occurring event for almost all treatment/BMI categories. Similarly in Cohort AMI 002, there was no evidence of a relationship between overall incidence of adverse events and BMI. The most frequently occurring events in all treatment/BMI categories were nausea, abdominal pain, diarrhoea and vomiting. In Cohort AMI 003, there was no evidence of a relationship between overall incidence of adverse events and BMI. Nausea was the most frequently occurring event in all treatment/BMI categories.

In none of the cohorts was there evidence of a clear relationship between the incidence of adverse events and BMI. There were few differences between the BMI categories in which events occurred most commonly and nausea remained the most frequently occurring event in all categories.

4.5.3.2.1 Severity of Adverse Events

The proportion of subjects with mild, moderate and severe adverse events are summarised in Tables 4.9 for all cohorts.

Table 4-9 Number (%) of subjects with adverse events by severity AMI 001, AMI 00 and AMI 003: intention-to-treat population

AMI 001 Severity of Adverse Experiences	Treatment Group		
	Primaquine 7.5 mg tid N=210	**Tafenoquine 200 mg bid N=86**	**Tafenoquine 400 mg od N=288**
Mild	78 (37.1%)	41 (47.7%)	157 (58.7%)
Moderate	7 (3.3%)	5 (5.8%)	23 (7.0%)
Severe	0	0	3 (0.4%)

AMI 002 Severity of Adverse Experiences	Treatment Group		
	Primaquine 7.5 mg tid N=131	**Tafenoquine 200 mg bid N=75**	**Tafenoquine 400 mg od N=158**
Mild	16 (12.2%)	21 (28.0%)	63 (39.9%)
Moderate	4 (3.1%)	4 (5.3%)	9 (5.7%)
Severe	0	0	1 (0.6%)

AMI 003 Severity of Adverse Experiences	Treatment Group	
	Primaquine 7.5 mg tid N=158	**Tafenoquine 200 mg od N=406**
Mild	23 (14.6%)	109 (26.8%)
Moderate	1 (0.6%)	9 (2.2%)
Severe	0	1 (0.2%)

Most of the adverse events during the study in all cohorts were of mild severity. There were 5 subjects with severe adverse events; all received tafenoquine 400 mg od. The severe events were abdominal cramps (two subjects), nausea (two subjects), headache (one subject) and vomiting (one subject).

4.5.3.2.2 Serious adverse events and withdrawal

There were no serious adverse events during the study and no deaths during the study.

One subject was initially recorded as withdrawn due to an adverse event. In Cohort AMI 003, one subject in the tafenoquine 200 mg od group was recorded as withdrawn due to mild abdominal cramps which started on the second day of dosing and the investigator reporting this event with a suspected relationship to study treatment. However, the subject did receive all three doses of tafenoquine 200 mg od and completed the study.

4.5.3.3 Laboratory Tests

In general there were only small changes from baseline and few marked differences between the treatment groups in all cohorts. For all three cohorts there was a trend to increased creatinine values post dosing, as shown below in **Table 4.10**.

Table 4-10 Summary of creatinine changes from baseline AMI 001, AMI 002, AMI 003: intention-to-treat population

AMI 001	**Treatment Group**		
Creatinine change from baseline μmol/L	**Primaquine 7.5 mg tid N=142**	**Tafenoquine 200 mg bid N=86**	**Tafenoquine 400 mg od N=278**
mean	3.5	16.9	13.5
min	-40	-10	-80
max	40	80	50

AMI 002	**Treatment Group**		
Creatinine change from baseline μmol/L	**Primaquine 7.5 mg tid N=9**	**Tafenoquine 200 mg bid N=70**	**Tafenoquine 400 mg od N=142**
mean	0	9.3 *	4.4
min	-10	-40 *	-30
max	10	40	30

* excludes one subject whose baseline creatinine value is clearly incorrect on database (subject 049.002.00343)

AMI 003	**Treatment Group**	
Creatinine change from baseline μmol/L	**Primaquine 7.5 mg tid N=3**	**Tafenoquine 200 mg od N=395**
mean	-6.7	7.3
min	-30	-20
max	30	50

For most laboratory variables the proportion of subjects with results which had increased or decreased from baseline by more than a specified amount, were similar in all treatment groups in all cohorts. There was a higher proportion of subjects with creatinine values increased from baseline in the tafenoquine groups compared to the primaquine groups. This was most noticeable in Cohort AMI 001, where 22% and 23% of subjects in the tafenoquine 200mg and 400mg groups respectively had a high a flagged increase compared to 4% subjects in the primaquine group. For WBC, there was a suggestion of a higher proportion of subjects with post-dose values flagged higher in the tafenoquine groups than in the primaquine groups.

The numbers of subjects with laboratory results which were flagged as having significantly changed are summarised in Tables 4-11, 4.12 and 4-13 for Cohorts AMI 001, AMI 002 and AMI 003, respectively. Only those variables where there were post-dose results with significant flag are included in the tables.

Table 4-11 Number (%) of subjects with significant flagged laboratory values AMI 001: intention-to-treat population

Laboratory Variable	Treatment Group					
	Primaquine 7.5 mg tid N=210		Tafenoquine 200 mg bid N=86		Tafenoquine 400 mg od N=242	
Haematology		**n**		**n**		**n**
Haemoglobin						
B/l: low	0	149	0	85	0	286
PD: low	1 (0.5%)	199	0	86	5 (1.7%)	286
Haematocrit						
B/l: low	0	149	0	85	0	287
PD: low	1 (0.5%)	199	0	86	5 (1.7%)	286
WBC						
B/l: high	0	147	0	85	0	286
PD: high	2 (1.0%)	199	0	86	9 (3.1%)	285
Granulocytes						
B/l: low	0	147	0	85	0	239
PD: low	1 (0.5%)	196	0	86	0	237
B/l: high	0	147	0	85	0	284
PD: high	3 (1.5%)	196	0	86	5 (1.7%)	283
Lymphocytes + Monocytes						
B/l: high	0	148	0	85	2 (0.7%)	284
PD: high	0	196	0	86	6 (2.1%)	284
MCHC						
B/l: low	0	148	0	85	2 (0.7%)	285
PD: low	0	199	0	86	4 (1.4%)	286
Platelets						
B/l: low	0	148	0	85	0	286
PD: low	0	196	0	86	1 (0.3%)	283
B/l: high	0	148	0	85	2 (0.7%)	286
PD: high	0	196	0	86	1 (0.3%)	283
Clinical Chemistry		**n**		**n**		**n**
ALT						
B/l: high	0	149	0	85	0	283
PD: high	0	199	0	86	1 (0.3%)	282
AST						
B/l: high	1 (0.7%)	149	0	85	0	283
PD: high	1 (0.5%)	198	1 (1.2%)	86	0	282
Total bilirubin						
B/l: high	1 (0.7%)	149	2 (2.4%)	85	2 (0.7%)	283
PD: high	0	199	1 (1.2%)	86	1 (0.3%)	282

NB B/l = baseline; PD = post-dose
n = number of subjects with assessment, percentages are based on n for each variable/treatment group.

Table 4-12 Number (%) of subjects with significant flagged laboratory values AMI 002: intention-to-treat population

Laboratory Variable	Treatment Group Primaquine 7.5 mg tid N=131		Tafenoquine 200 mg bid N=75		Tafenoquine 400 mg od N=158	
Haematology		**n**		**n**		**n**
Haemoglobin						
B/l: high	0	12	0	72	1 (0.7%)	148
PD: high	0	13	1 (1.4%)	72	1 (0.7%)	142
Haematocrit						
B/l: high	0	12	1 (1.4%)	72	2 (1.3%)	150
PD: high	0	13	0	72	3 (2.1%)	143
WBC						
B/l: high	0	12	1 (1.4%)	72	1 (0.7%)	149
PD: high	0	13	1 (1.4%)	71	4 (2.8%)	141
Granulocytes						
B/l: high	0	12	0	65	1 (0.7%)	139
PD: high	0	8	1 (7.1%)	14	0	34
Platelets						
B/l: high	0	12	0	72	0	149
PD: high	0	13	0	71	1 (0.7%)	141
Clinical Chemistry		**n**		**n**		**n**
ALT						
B/l: high	0	13	0	74	0	152
PD: high	2 (1.8%)	109	0	71	0	147
AST						
B/l: high	0	13	0	74	0	152
PD: high	2 (1.8%)	109	0	72	0	147
GGT						
B/l: high	0	13	0	74	1 (0.7%)	152
PD: high	1 (0.9%)	109	0	71	0	146
Total bilirubin						
B/l: high	0	13	0	74	3 (2.0%)	152
PD: high	1 (0.9%)	109	0	71	2 (1.4%)	147

NB B/l = baseline; PD = post-dose
n = number of subjects with assessment, percentages are based on n for each variable/treatment group.

Table 4-13 Number (%) of subjects with significant flagged laboratory values AMI 003: intention-to-treat population

Laboratory Variable	Treatment Group			
	Primaquine 7.5 mg tid N=158		Tafenoquine 200 mg od N=406	
Haematology		**n**		**n**
Haemoglobin				
B/l: low	1 (0.6%)	158	0	405
PD: low	0	4	1 (0.2%)	403
WBC				
B/l: high	0	158	1 (0.2%)	404
PD: high	0	4	7 (1.7%)	403
Granulocytes				
B/l: low	0	158	0	404
PD: low	0	4	1 (0.2%)	403
B/l: high	0	158	4 (1.0%)	404
PD: high	1 (25.0%)	4	8 (2.0%)	403
Platelets				
B/l: low	0	158	1 (0.2%)	404
PD: low	0	4	1 (0.2%)	403
B/l: high	0	158	0	404
PD: high	0	4	3 (0.7%)	403
Clinical Chemistry		**n**		**n**
ALT				
B/l: high	0	156	0	399
PD: high	0	3	1 (0.2%)	402
Total bilirubin				
B/l: high	1 (0.6%)	156	4 (1.0%)	399
PD: high	0	3	3 (0.7%)	402

NB B/l = baseline; PD = post-dose
n = number of subjects with assessment, percentages are based on n for each variable/treatment group.

In general, the proportions of subjects with significantly altered laboratory values were very low and similar in all treatment groups in all cohorts.

4.3.4 Pharmacokinetic Evaluation

Tafenoquine levels were determined for the available population on samples collected approximately 10 hours after the last tafenoquine dose. Of the 809 participants who took tafenoquine, 86.3% (698/809) provided a day 3 sample. The remainder were unavailable due to military commitments. There was no significant differences in tafenoquine levels between those experiencing adverse events, but of interest in the 200mg bd cohort, the levels seen in those experiencing an episode of malaria infection were significantly higher than in those who were infection free. The levels of shown at **Table 4-14**.

Table 4-14 Tafenoquine levels in participants experiencing adverse events and parasitaemia

	Tafenoquine 400mg od (n=242)	Tafenoquine 200mg bd (n=161)	Tafenoquine 200mg od (n=406)
With AE	619 ± 122 (n = 92)	631 ± 136 (n = 56)	317 ± 65 (n = 72)
Without AE	609 ± 138 (n = 87)	630 ± 120 (n = 80)	321 ± 64 (n = 311)
With parasitaemia	563 ± 91 (n = 13)	749 ± 116 (n = 5)	312 ± 81 (n = 19)
Without parasitaemia	618 ± 132 (n = 166)	626 ± 125 (n = 131)	320 ± 63 (n= 364)

Gender differences were investigated in only Cohort AMI 001 and related to the occurrence of adverse events. As may be expected in a military population the number of female participants was limited (13.9%; 24/174). Of the 173 participants 148 (86%) provided a blood sample on day 3 approximately 12 hours post the last dose of tafenoquine. Dosing in this cohort was 400mg per day given as either a single dose or a split dose of 200mg bd. Females tended to achieve higher tafenoquine levels than males and this relationship appears to be at least partly independent of weight. The relationship between tafenoquine levels and gastrointestinal adverse events was also explored in this group and is summarised in Table 4-15.

Table 4-15 Gender based tafenoquine levels in participants experiencing gastrointestinal adverse events

	Male		Female	
	Single dose (n = 76)	**Split dose (n = 73)**	**Single dose (n = 11)**	**Split dose (n = 13)**
All participants	563 ± 110 (n = 67)	601 ± 114 (n = 58)	703 ± 102 (n= 11)	767 ± 128 (n = 12)
Without GI AE	542 ± 116 (n = 33)	589 ± 107 (n = 34)	585 (n = 1)	767 ± 74 (n = 5)
With GI AE	584 ± 101 (n = 34)	619 ± 123 (n = 24)	715 ± 100 (n = 10)	767 ± 162 (n = 7)

4.6 Protocol Violation

Under the ADMEC approved protocol study subjects who experienced recurrences of vivax malaria were to be treated with the standard 3 day treatment course of chloroquine of 1500mg over 3 days followed by a further treatment course of tafenoquine of 1200mg (400mg stat followed by 20mg twice daily for a further two days). Due to a drug administration error there were two subjects in Cohort 001 who did not receive the clearance regimen of chloroquine prior to commencing the follow up tafenoquine.

Both subjects presented to local supporting military hospital on the same day and were confirmed as having vivax malaria. Study investigators had attended the hospital to write up the tafenoquine dosing and provide the medication assuming other medication, including chloroquine would be prescribed by the attending physician. This did not occur and hence only tafenoquine was initiated providing an opportunity to document the actions of tafenoquine alone in the treatment of vivax malaria. When the prescribing error was detected the subjects had clinically improved and the advice of the Director, Australian Army Malaria Institute was to withhold chloroquine and manage the cases as clinically indicated.

Both subjects rapidly improved without complications and the parasite clearance and tafenoquine levels were able to be mapped. This protocol violation was reported to the ethics committee as required. Both subjects remained free of vivax malaria for at least the 2 years they were monitored post study (a review of the Central Malaria Register conducted 23rd June 2011 indicates that neither subject has been subsequently reported to the Register – a period greater than 10 years – but there is no immediate way of determining whether or not they remained in the ADF after study conclusion). The tafenoquine levels against parasite clearance is shown at **Figures 4-1 and 4-2**.

Figure 4.1 Parasitaemia and tafenoquine drug levels for case 1

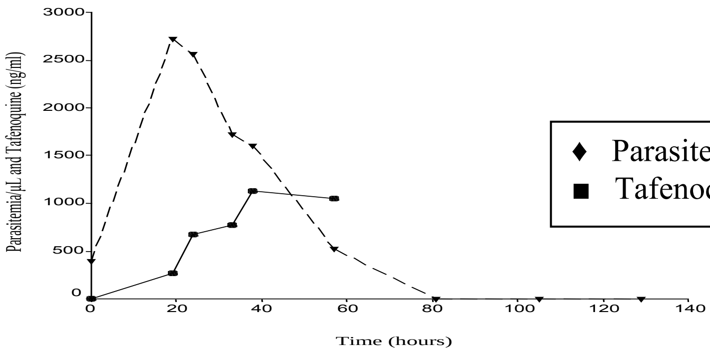
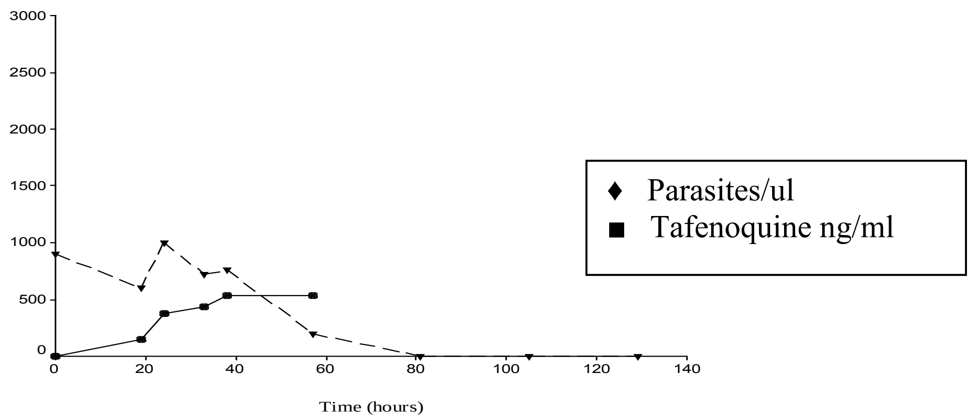


Figure 4.2 Parasitaemia and tafenoquine drug levels for case 2



The protocol violation provided the first human report of tafenoquine used alone for the treatment of vivax malaria and contributed significantly to the knowledge of the developmental drug. Both subjects were made aware of the violation but were happy with the outcome.

4.7 Discussion

This study was an open, randomised, parallel group study to evaluate the efficacy of tafenoquine for the post-exposure prophylaxis of vivax malaria. It was conducted in three cohorts of non-immune Australian soldiers deployed to Bougainville, Papua New Guinea (AMI 001), Bobonaro district, Timor Leste (AMI 002) and Occussi district, Timor Leste (AMI 003). Subjects received study medication on their last three days in the endemic area and were monitored for the development of malaria for the following 12 months.

In total, 1512 subjects received treatment in this study: 584 in Cohort AMI 001, 364 in Cohort AMI 002 and 564 in Cohort AMI 003. Across all three cohorts, 599 subjects received primaquine 7.5mg three times daily for 14 days, 446 subjects received tafenoquine 400mg once daily for 3 days, 161 subjects received tafenoquine 200mg twice daily for 3 days (3d) and 406 subjects received tafenoquine 200mg once daily for 3 days.

All three cohorts were well matched for demographic characteristics; 85-100% subjects were male and the mean age was between 24 and 31 years. Only a small proportion of subjects (<10% overall) took any concomitant mediation during the study.

The primary efficacy index was the proportion of subjects with confirmed parasitaemia during the 12-month follow-up period. Across all three cohorts, between 1.2% and 13.7% of subjects developed confirmed parasitaemia during the 12-months following return from the endemic area. The incidence was highest in Cohort AMI 002 and lowest in Cohort AMI 001 and it is presumed that this reflects differing levels of exposure to malaria between the locations of deployment for the three cohorts.

In general there was no noticeable difference in the proportion of subjects developing parasitaemia between the 3-day tafenoquine treatment regimens and the 14-day primaquine regimen in any cohort. In cohorts AMI 001 and AMI 002, there was no noticeable difference between the once daily and twice daily tafenoquine groups, with the tafenoquine 200mg bd group showing slightly lower incidence of parasitaemia development compared to both the primaquine and tafenoquine 400mg od groups. In Cohort AMI 003, the once daily 200mg dosing regimen for tafenoquine was similar in efficacy to the primaquine regimen.

Non-immune soldiers deployed to malaria endemic regions such as the Southwest Pacific are at risk of contracting malaria caused by both *P.falciparum* and *P.vivax*. Personal protection measures (permethrin-treated bednets and insect repellants) are routinely deployed, and chemoprophylaxis provided. The standard ADF chemoprophylaxis regimen is doxycycline 100mg daily, with weekly mefloquine (250mg) used where the subject is intolerant to doxycycline. Although this regimen is able to prevent both acute falciparum and vivax malaria during deployments, the problem of persistent liver hypnozoites of *P.vivax* still remains [Kitchener et al, 2000]. Commonly post-exposure prophylaxis with primaquine is used. The standard primaquine anti-relapse regimen is 15mg/day for 14 days. Previous experience in the Australian Army had shown that this regimen is not completely effective in the Pacific region, so a 22.5mg/day regimen (7.5mg three times a day for 14 days) was being routinely used at the time of this study. Subsequently, even this higher dose has proved ineffective and 30mg/day for 14 days is now used routinely by the Australian army. However, the persistence of cases of vivax malaria relapse after return to Australia demonstrates that the hypnozoites are not always eliminated by the current primaquine eradication course [Kitchener et al, 2000]This is probably the result of a combination of the following factors:

- insensitivity of parasites to primaquine
- problems of compliance with the primaquine regimen (3 tablets a day for 14 days) after soldiers return to Australia, usually proceeding on leave.

Relapses despite primaquine therapy may reflect changes in primaquine response among formerly susceptible strains, or geographic spread of strains that have long been known to be refractory. The term resistance may be misleading, as this is usually assessed by the effect a drug has on the asexual parasite density in the blood or the time to parasite recrudescence. As primaquine has little effect on asexual parasite density, it cannot be used to define resistance. There has been some controversy over whether primaquine drug resistance exists, so the term "primaquine-refractory" or "primaquine-tolerant" has been widely adopted [Baird et al, 2001; Schwartz et al, 2000; Collins et al, 1996]. There have been many reports of primaquine refractory *P.vivax* in Timor Leste [Kitchener et al, 2002] and elsewhere [Schwartz et al, 2000; Schwartz et al, 2003; Rajgor et al, 2003] - even when compliance has been reported to be good. In many cases, increased doses of primaquine (up to 30mg/day) are now being recommended [Schwartz et al, 2000; Kitchener et al, 2002; Baird et al, 2003], however this is felt to be the maximum tolerated dose of primaquine. This study shows that a 3 day regimen of tafenoquine appears to offer similar protection, when used as a post-exposure prophylaxis agent, as the increased 22.5mg/day primaquine regimen.

It is commonly acknowledged that compliance with 14 days primaquine therapy is problematic [Baird et al, 2003]This is particularly a problem when this regimen is given as post-exposure prophylaxis to a subject who is otherwise feeling fit and well. On return from deployment, soldiers will commonly begin a period of leave and will not want to take medication during this time - especially medication that can be associated with gastrointestinal side effects [Collins et al, 1996] A shorter, 3-day dosing regimen would be expected to promote improved compliance and therefore greater effectiveness outside the controlled, supervised dosing environment of a clinical study.

Tafenoquine and primaquine were generally well tolerated in this study. There were no deaths or no serious adverse events reported. The majority of adverse events in all three cohorts were mild in severity.

The incidence of adverse events varied between the three cohorts, with 49% of subjects reporting one or more adverse events in AMI 001, 32% in AMI 002 and 25% in AMI 003. While the dose of tafenoquine varied between the cohorts, the dose of primaquine remained the same, and yet even for primaquine a higher incidence of adverse event was seen in AMI 001 (38%) compared to AMI 002 and 003 (15%). It is possible that the reduced level of adverse events reflects that fact that active adverse event collection (i.e. questioning) stopped at Day 4 for cohorts AMI 002 and 003, while it continued to Day 14 (the end of the primaquine course) for AMI 001. However, there is no apparent reason for marked difference in adverse event incidence in the tafenoquine groups. It is possible that the use of concurrent doxycycline and/or chloroquine in the subjects in AMI 001 has contributed to this increased incidence. Whatever the reason, these differences mean that it is important to compare the incidence of adverse events within cohorts rather than across all subjects in the study.

The most common adverse event in all three cohorts was nausea. The incidence of nausea was lowest in the primaquine groups and notable higher in the tafenoquine 400mg groups. Diarrhoea and abdominal pain/cramps were also commonly reported. Both these events were reported more frequently in the tafenoquine groups, but there was no consistent dose-related effect. The majority of these events were considered to be related to study medication by the investigator. Headaches were reported by 8-10% of all subjects in cohort AMI 001, but by <5% of any subjects in the other two cohorts. This adverse event profile is consistent with previous studies where gastrointestinal effects were also most common [Shanks et al, 2001].

Adverse events in this study were also analysed by a number of sub-groups. The incidence of adverse events was higher in female subjects than in male subjects in AMI 001 in all treatment groups, although the nature of the events reported was similar for both sexes. This analysis was not performed for AMI 002 and 003, as the number of female subjects was very small. When analysed across weight groups, there was a tendency for the incidence of adverse events to decrease with increasing weight, but this was not consistent across cohorts and there was no notable difference between the treatment groups. There was no evidence of a relationship between BMI and incidence of adverse events across treatment groups or between cohorts.

For the laboratory data, in general there were only small changes from baseline and few marked differences between the treatment groups in all cohorts. There was a higher proportion of subjects with creatinine values increased from baseline in the tafenoquine groups compared to the primaquine groups. This was most noticeable in Cohort AMI 001, where 22% and 23% of subjects in the tafenoquine 200mg and 400mg groups respectively had a high flagged creatinine compared to 4% subjects in the primaquine group. However these data need to be carefully interpreted due to the low number of subjects in the primaquine group with laboratory results recorded.

4.8 Key messages from this chapter

- A 3-day dosing regimen of tafenoquine (400mg od, 200md bd or 200mg od) was effective as a post-exposure prophylaxis agent in this study, demonstrating similar efficacy to 14-day primaquine.
- Tafenoquine was well tolerated, with no subjects being withdrawn due to adverse events. The most common adverse events were gastrointestinal events.
- Tafenoquine, with a shorter dosing regimen (3 days compared to 14 days primaquine), could be used as a more convenient, yet effective, post-exposure prophylaxis agent.
- Tafenoquine alone appears to adequately treat vivax malaria but a more comprehensive study will be required to clearly establish the reliability of tafenoquine alone treatment regimens.

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Chapter 4 Paper 4.1

Nasveld PE, Kitchener S, Edstein M, Rieckmann K. (2002) Comparison of tafenoquine (WR238605) and primaquine in the post-exposure (terminal prophylaxis) of vivax malaria in Australian Defence Force personnel. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 96: 683-684

Chapter 4 Paper 4.2

Elmes NJ, **Nasveld PE**, et al. (2008) The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for the post exposure prophylaxis of Plasmodium vivax malaria in the Southwest Pacific. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 102: 1095-1101

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Chapter 4 Paper 4.3

Edstein MD, **Nasveld PE**, Kocisko DA, Kitchener SJ, Gatton ML and Rieckmann KH. (2007) Gender differences in gastrointestinal disturbances and plasma concentrations of tafenoquine in healthy volunteers after tafenoquine administration for post-exposure vivax malaria prophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101: 226-230

Chapter 4 Paper 4.4

Nasveld PE and Kitchener SJ. (2005) Treatment of acute vivax malaria with tafenoquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 99: 2-5

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- **CHAPTER 5**
- **Treatment of acute vivax malaria with tafenoquine**
- **List of peer reviewed and published papers presented in this chapter**

Kitchener S, **Nasveld P**, Edstein M. (2007) Tafenoquine for the treatment of recurrent *Plasmodium vivax* malaria. *American Journal of Tropical Medicine and Hygiene*. 76:494-496

PN, SK and ME participated in the conception and design of the study. PN and SK managed the study subjects and SK drafted the manuscript and led the analysis. PN and ME contributed to the analysis of the study and contributed significantly to the manuscript. All authors gave final approval for the manuscript submitted for publication.

5.1 Introduction

Information obtained from the study reported in Chapter 4 on post exposure prophylaxis with tafenoquine, along with the results of the Walsh [Walsh et al, 1999] study led to discussions on the treatment of recurring vivax malaria between investigators at AMI and the sponsors at GSK and USAMMDA. The particular question was on how to potentially optimise the observed tafenoquine activity and consequently a study proposal was developed to investigate treatment of recurrent vivax malaria with tafenoquine.

The available information suggested that tafenoquine may be even more effective at preventing further relapses of vivax malaria if it were given over a longer period. The intent was to conduct an initial clearance of parasites with chloroquine, followed by a loading dose of tafenoquine 200 mg daily for 3 days and then weekly for a further 8 weekly doses. It was postulated that this would expose the hypnozoite stage of vivax malaria to adequate doses of tafenoquine to be effective in preventing the maturation of the hypnozoite and subsequent merozoite release into the blood. Eight weeks of dosing was selected for the study based on the observed median to onset between relapses of 42 days plus a margin of a further of 2 weeks.

Ultimately it was agreed that a small pilot study of 40 personnel with documented recurrent vivax malaria would be undertaken and supported by the sponsors. The nature of the study was exploratory and it was conducted under a section 19 of the *Therapeutic Goods Act, 1989*.

Under subsections 19(5)-(9) and Section 41HC of the Act, the “Therapeutic Goods Authority (TGA) is able to grant certain medical practitioners authority to prescribe a specified unapproved therapeutic good or class of unapproved therapeutic goods to specified recipients or classes of recipients (identified by their medical condition). The medical practitioner becomes an 'Authorised Prescriber' and can prescribe that product for that condition (also known as the 'indication') to individual patients in their immediate care without further approval from the TGA.”

The conduct of the pilot study under section 19 of the Act precluded the use of a direct comparator arm as it was argued on the provision of an unapproved treatment (tafenoquine) for “patients” who had failed the available alternative of chloroquine and primaquine. It was, however, considered to be important to attempt to identify a control population against which the study results could be interpreted. To this end, a population consisting of ADF members who had been exposed to malaria in Timor Leste during the same time interval as the subjects included in the pilot study, and who had subsequently developed vivax infection were identified. No compliance data was collected for this group and it was assumed that ADF members in this group followed the requirements for primaquine post-exposure prophylaxis as outlined in HD 215- Malaria. It is possible that compliance in this group may not have been complete, even though a review of the PM-40 Notification of Malaria forms submitted as a requirement of the Australian Defence Force’s Central Malaria Register indicated that those selected for the comparator group had complied.

The interpretation may therefore be subject to a degree of bias towards effectiveness for tafenoquine. Given that this study design was an open label pilot study, the use of this de facto population is considered justified to determine gross effectiveness differences.

5.2 Objectives

5.2.1 Primary Objective

- To evaluate the effectiveness of tafenoquine in treatment of recurrent *P. vivax* malaria

5.2.2 Secondary Objectives

- To evaluate the safety and tolerability of tafenoquine in treatment of recurrent *P. vivax* malaria
- To describe the drug levels achieved during 8 weeks of tafenoquine in the subject population

5.3 Ethics

This study was conducted under subsections 19(5)-(9) and Section 41HC of the *Therapeutic Goods Act 1989*. Under these arrangements patients suffering from a life-threatening or otherwise serious illness or condition (Section 19(6) and 41HC of the *Therapeutic Goods Act 1989* and Regulation 12B (2) of the Regulations) may access unapproved therapeutic goods prescribed by an Authorised Prescriber. The prescriber has the responsibility to ensure the patient has given appropriate informed consent prior to treatment. Consent needs to include:

- that the product is not approved (i.e. registered or listed) in Australia;
- possible benefits of treatment and any risks and side effects that are known;
- the possibility of unknown risks and late side effects; and
- any alternative treatments using approved products which are available.

When Authorisation is given by the TGA, the Authorised Prescriber receives a letter of authorisation. Authorised prescribers were identified in the key Defence centres of Sydney, Townsville, Brisbane and Darwin.

Once a potential subject was identified, the authorised prescriber would notify AMI investigators and discuss the case to ensure inclusion and exclusion criteria were met. The Authorised Prescriber then applied to the TGA on an individual patient basis. Once TGA approval was obtained, patient details were provided to GSK Melbourne who initiated the supply of tafenoquine.

Despite there being no requirement to formally lodge the protocol or seek HREC approval under Section 19, the investigators did submit the study for ADMEC consideration. ADMEC approved the protocol, endorsed the Authorised Prescribers and registered the activity as ADMEC 267/01 on the 20th February 2001.

The study was conducted in accordance with Good Clinical Practices and the Declaration of Helsinki as amended in Somerset West, Republic of South Africa 1996.

Written informed consent was obtained from each subject prior to entry into the study. Subjects were recruited using non-coercive means and no inducements were offered.

The subjects invited to take part in the trial were entitled to make a choice based on full and complete information presented in a manner that was both understandable and ethnically appropriate. The Consent Form was designed to assure the protection of the subject's rights. Consent was obtained from all subjects within 5 days of the start of treatment.

5.4 Methods

5.4.1 Study design

This study was an open label trial of tafenoquine (used following chloroquine) in the treatment of recurrent cases of *P. vivax* malaria. The study was conducted entirely on ADF personnel who had a microscopically confirmed recurrence of *P. vivax* malaria. Subjects were initially treated with chloroquine 600 mg (base), followed by 300mg six hours later, 300mg the following day and a final 300 mg on the third day. Those demonstrating a reduction in parasitaemia were, within seven days, to begin a loading dose of tafenoquine. The loading dose regimen was tafenoquine 200mg once daily for three days, followed by a maintenance dose of 200 mg tafenoquine once weekly for eight weeks. A total of 11 x 200 mg tafenoquine capsules were taken per tafenoquine treatment course (total 2.2 gm).

Significant delays were experienced between the identification of the volunteers and the initiation of the tafenoquine dosing. These delays were beyond the control of the investigators, as the approval and supply system were controlled by the TGA (from whom prescribing approval for each subject was obtained) and GSK (who would transport the trial medication directly to the prescribing doctor). Delays of up to 8 weeks were experienced (mean 2.6 weeks) during which time the volunteer was maintained on a weekly dose of chloroquine 300mg to prevent interval relapse.

Subjects were followed for a period of 6 months for vivax malaria relapses. Relapses during this period were treated according the extant ADF Health Policy Directive 215 – Malaria using 3 days chloroquine (600 mg/300 mg/300 mg/300 mg) plus primaquine (7.5 mg three times daily for 14 days). Subjects were unable to be followed or were redeployed to malarious areas after this period; therefore no further active surveillance was conducted. The study schedule is presented in **Table 5.1**.

Table 5-1 Study schedule

	Clinical assessment	Blood film	Full Blood Count*	GGT, AST, ALT	Blood sample for TQ levels
At diagnosis	X	X	X	X	
24 hrs post last dose CQ	X	X	X	X	X
12 hours post third dose TQ	X	X	X	X	X
2 hours prior to week 2 TQ dose	X	X	X	X	X
12 hours after week 4 TQ dose	X	X	X	X	X
2 hours prior to week 6 TQ dose	X	X	X	X	X
12 hours after week 8 TQ dose	X	X	X	X	X

* Haemoglobin, Total White Blood Cells, WBC differential, platelets

5.4.2 Participants

Participants were healthy, as defined by Medical Class 1 or 2 (Australian Army standard), males and females aged between 18 and 55 years inclusive. Participants had a confirmed diagnosis of recurrent *P. vivax* malaria; previously established and confirmed diagnosis of *P. vivax* malaria within 6 months of relapse occurring; previous treatment of clinical vivax malaria with chloroquine or primaquine or chloroquine followed by a 3 day tafenoquine regimen; and were not intending to separate from the ADF within the subsequent 12 months. Subjects with demonstrated G6PD deficiency, a history of allergy or intolerance to any of the trial compounds or who had received another investigational drug within 30 days or 5 half lives (whichever was longer) of the study start were excluded. In addition subjects with concurrent significant illness or medical condition and females who were pregnant, lactating, intending pregnancy within the next 3 months or unwilling to comply with recognised contraception for 6 months after the first dose of the study drug were also excluded.

All subjects enrolled were members of the ADF having served in Timor Leste during either Operation Warden, with the International Force in East Timor (InterFET), or Operation Tanager with the Peace Keeping Force of the United Nations Transitional Administration in East Timor (UNTAET).

The ADF Central Malaria Register received 237 reports of primary vivax malaria fulfilling the inclusion criteria. Thirty-one subjects were enrolled in this study and commenced study medication. Twenty-seven subjects completed the full tafenoquine treatment; treatment was terminated early for 4 subjects when all tafenoquine clinical trials were stopped as a consequence of abnormal eye findings as described in Chapter 3. Of the 27 subjects who completed treatment, 17 were recruited after their first relapse of vivax malaria, 7 after their second relapse and 3 after their third relapse.

The study group included one female subject. The average age at the onset of treatment was 26 years 3 months. The oldest subject was 35 years and the youngest 19 years 1 month old at the onset of treatment. All subjects resided in Australia at the time of treatment and were treated as outpatients in Military Health facilities.

No demographics are obtained for the control group.

5.4.3 Treatment and administration

Acute parasitaemia was treated with:

1. Chloroquine 600 mg (base) stat followed by a second dose of 300mg (base) after six hours; then
2. Chloroquine 300 mg (base) once daily for a further 2 days; then
3. Chloroquine 300 mg (base) weekly until further authorisation and supply of tafenoquine achieved; then

Prevention of relapse with:

1. Loading dose of tafenoquine 200 mg (base) once daily for three consecutive days; then
2. Weekly doses of tafenoquine 200 mg (base) given on the same day (± 1) of each week for eight further weeks.

There were no rules on dosing with food, but doses that were vomited within 1 hour of ingestion were to be repeated. No doses were reported as vomited.

The control population were treated with the ADF conventional relapse prevention regimen of primaquine, 7.5 mg three times daily for 14 days, after treatment of acute parasitaemia with chloroquine, as described above.

5.4.3.1Compliance with study medication

Study medication was authorised to be taken under the supervision of the treating Medical Officer who advised dates of dosing to the Investigators. Dates of dosing were logged by study staff onto the central database held at AMI.

No compliance measures were employed in the control group except that the PM-40 Notification of Malaria form notifying the case to the ADF Central Malaria Register was checked to determine that treatment had been given.

5.4.4 Criteria for evaluation

5.4.4.1 Efficacy

The primary endpoint of this pilot study was the development of *P. vivax* parasitaemia within 6 months of commencing the tafenoquine treatment course. The absence of parasitaemia following initial chloroquine treatment was confirmed by microscopy. No further assessment was made between initial clearance and commencement of tafenoquine.

Thick and thin blood smears, for detection of malaria, were prepared at diagnosis, before the start of tafenoquine, after the tafenoquine loading dose and then every two weeks to week 8. In addition, any subject developing symptoms of malaria during treatment or follow-up phases was required to have thick and thin blood slides prepared and examined at the supporting medical facility, with slide copies sent to AMI for confirmation by a microscopist experienced in examination of slides for low level parasitaemia. Disagreements between the treating medical facility results and those of AMI were to be adjudicated by a third microscopist.

5.4.4.2 Safety

Serious adverse experiences which occurred during the clinical study or within 12 weeks of receiving the last dose of study medication, whether or not related to study drug, were to be reported. Adverse events were to be reported directly to the Principal Investigator by telephone within 24 hours of the treating medical officer becoming aware. A clinical summary with relevant results was required to be faxed to AMI within 72 hours of first notification. Instances of death, cancer or congenital abnormality if brought to the attention of the investigator AT ANY TIME after cessation of study medication AND considered by the investigator to be possibly related to study medication, were to be reported to GSK. Other adverse events possibly, probably or definitely related to the volunteer's inclusion in the study were reported back to AMI. No adverse event reporting was specifically required after the treatment phase. Any adverse events that developed were to be treated under existing medical facility arrangements, recorded in the UMR and supervised by the authorised prescriber.

At closure of the study, an AMI clinical staff member visited all sites to confirm full reporting of outcomes and adverse events, and to retrieve any remaining study medication. All adverse events were to be followed up until resolution, or completion of the study period.

A blood sample was taken for biochemistry and haematology assessments at diagnosis, before the start of tafenoquine, after the tafenoquine loading dose and then every two weeks to week 8.

5.4.4.3 Pharmacokinetics

Pharmacokinetic assessments were conducted on samples collected 24 hour after the last dose of chloroquine and during the active tafenoquine treatment phase collected 12 hours after completion of the loading dose, then alternating between two hours before or 12 hours after every second dose. Samples were collected in an EDTA tube, which was spun shortly after being drawn to separate plasma. Plasma samples were stored at room temperature or chilled (but not frozen) for transport to AMI where they were stored refrigerated.

Plasma tafenoquine concentrations were measured by High-Performance Liquid Chromatography using the method previously developed by Kocisko, Edstein and colleagues at the Pharmacy Department of AMI [Kocisko et al., 2000]. Results were expressed in nanograms per millilitre (ng/ml) of plasma.

Plasma tafenoquine levels taken two hours before treatment doses were designated as assumed true values. All values correctly labelled and available were charted against the time point of sampling, using SigmaPlot to create a pharmacokinetic curve. A comparison curve was prepared for the complete set of values available for the single break through case of malaria recorded in the study period.

5.4.5 Statistical methods

5.4.5.1 Sample size

A 20% recurrence rate was expected from subjects with vivax malaria treated previously with primaquine. For statistical power of 80%, assuming a sample of 160 cases could be identified of recurrent malaria after primaquine treatment (control group), approximately 40 cases treated with tafenoquine were determined to be necessary to discern a 0.10 risk ratio for tafenoquine treatment. A significance level of 5% was used. This sample size was also an acceptable number of patients to be treated under the regulatory TGA authorisations.

There was no randomisation in this study. All subjects were selected on the basis of consent and meeting the inclusion and exclusion criteria.

Data for the control group was collected using the existing ADF malaria notification system maintained by AMI.

Recurrence data for the study group was collected by telephone consultation with the treating medical officers and treating medical facility. It is possible that the sensitivity of recurrence identification among the study group is greater than that for the control group. However, this would form a bias towards tafenoquine treatment being less effective, and was therefore considered an acceptable potential bias.

5.4.5.2 Planned efficacy evaluation

Chi square comparison of the health outcomes were to be conducted on the proportions of recurrence of vivax malaria within six months for those volunteers receiving tafenoquine treatment and the control group of soldiers receiving primaquine treatment. The control group receiving conventional primaquine treatment served as the ‘expected’ proportion of patients experiencing recurrent vivax malaria within the time period, with the tafenoquine study group providing the ‘observed’ population. Significant efficacy was accepted if the probability of the Chi square statistic reflecting greater efficacy of tafenoquine was less than 5% ($p < 0.05$). Further description of efficacy was analysed as a relative risk ratio.

5.4.5.3 Safety evaluation

All patients for the study were required to be interviewed by the authorised prescriber at each visit. Baseline haematology and biochemistry assessments were required particularly as all recruited patients were recovering from clinical vivax malaria. During the treatment phase, the need for further investigations was determined by the clinical assessments as determined by the authorised prescriber.

5.4.5.4 Pharmocokinetic evaluation

Data from pharmacokinetic assessments were described using graphical representation of pooled results at each time point. Any cases of breakthrough malaria during the study period were also separately pooled and plotted graphically for direct comparison to all pooled results from other patients. Should the proportion of breakthrough cases be sufficiently large in the study group, a Kaplan-Meier curve and analysis was to be conducted.

5.5 Results

5.5.1 Protocol deviations

On 1st May 2001, Glaxo SmithKline withdrew approval and medication from the trial due to unexpected corneal deposits observed in a long-term prophylaxis trial being conducted by AMI in Timor Leste (see Chapter 3). In this study, this resulted in a total of 4 subjects having tafenoquine dosing terminated prematurely. One subject had received the loading dose only; another had received the loading dose and two weekly doses; while the remaining two subjects had reached week three of dosing.

5.5.2 Efficacy results

A single episode of relapsing vivax malaria (confirmed *P. vivax* parasitaemia) was recorded in the study group in the study period, an incidence of 3.7%. In comparison, 44 episodes of relapsing vivax malaria were recorded in the control group in the study period (from the identified 237 primary cases of vivax malaria), an incidence of 18.6%.

It was concluded that tafenoquine is significantly better than primaquine-doxycycline in preventing relapse of vivax malaria ($p = 0.035$, one-tailed Fischer's Exact test used as one cell [treatment failures] contained fewer than 5 cases).

5.5.2.1 Case discussion of treatment failure

One subject (25 year old male) developed malaria and was found to have parasitaemia 126 days after onset of tafenoquine treatment. He had had three previous episodes of malaria beginning with a single case of falciparum malaria after four months deployment in Timor Leste. This was treated with quinine and doxycycline. He continued doxycycline prophylaxis for a further one month during the re-deployment of his Unit to Australia. On re-deployment, he received primaquine, 7.5 mg three times a day for two weeks, an appropriate total dose of 5.25 mg/kg. His first episode of vivax malaria began within six weeks of beginning primaquine. This suggests a primaquine tolerant parasitaemia. He was treated with chloroquine and the same dose of primaquine. His records indicate compliance with treatment and prophylaxis although no independent confirmation through blood sampling was obtained. His second episode of vivax malaria began three months later. This episode was treated with chloroquine. He provided consent to enter the study and began tafenoquine treatment seven weeks later having been held on chloroquine weekly in the interim.

His recurrent post-tafenoquine parasitaemia was treated with the same initial course of chloroquine as previously (600 mg followed six hours later by 300 mg daily for three days). The parasitaemia subsided quickly and he was placed onto weekly chloroquine 300 mg for a further six months.

5.5.3 Safety results

5.5.3.1 Adverse events

The treatment failure in this study was reported as a serious adverse event. Full details are given in Section 5.5.2.1.

There were no other adverse events reported in this study and no withdrawals due to adverse events.

5.5.3.2 Laboratory tests

No laboratory data was routinely collected as the design required only abnormal findings to be reported back to the AMI investigators. No abnormal findings were noted in the Unit Medical Records of study participants when the treatment facilities were followed up with an AMI clinical staff member. There were no clinically significant changes in laboratory indices noted during the study.

5.5.4 Pharmacokinetics

The pharmacokinetic curve of tafenoquine is displayed in **Figure 6.1**. The figure provides the curves for all available data and the three subjects where complete pharmacokinetic sampling was available, indicating that levels in the order of 200ng/ml of tafenoquine could be expected to be maintained through the 8 week treatment window. A separate curve is displayed of the tafenoquine concentrations achieved by the single case of relapse within the study period.

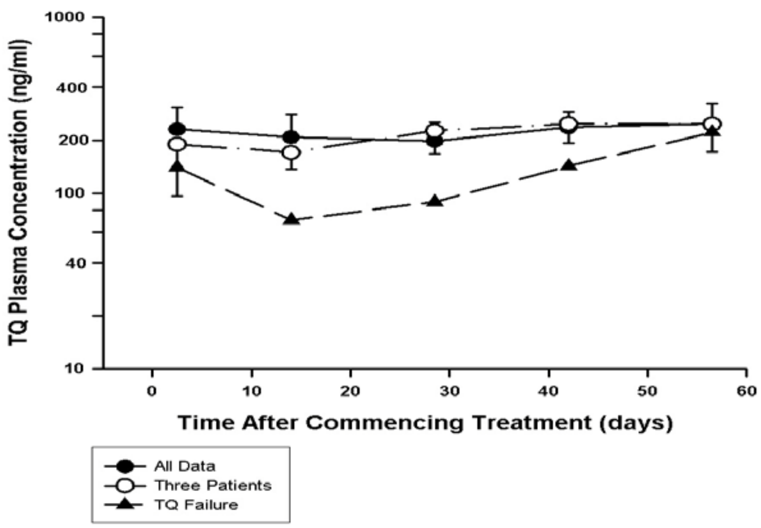

Figure 6.1 Tafenoquine concentrations in subjects receiving all 8 weeks of dosing

A mean plasma tafenoquine concentration of 208 ng/ml (Standard Deviation = 87 ng/ml) was measured following eight weeks of medication.

The single case of treatment failure was found to have **not** achieved plasma tafenoquine levels greater than 100 ng/ml (the established therapeutic threshold) until six weeks into the treatment program.

No further statistical analyses were conducted due to the low number of failures in the study group.

5.6 Discussion

This study showed that tafenoquine is safe and effective (following chloroquine treatment) in prevention of relapse of multi-relapsing vivax malaria.

The control group used in this study consisted of cases of primary vivax malaria with primary relapse only. The tafenoquine groups contained subjects who had already previously relapsed, possible indicating a greater tendency to relapse again. This is a potential bias against tafenoquine. In contrast, compliance was not monitored in the control group and poor compliance could have increased the chance of relapse in this group – representing a bias in favour of tafenoquine. Given that the primary endpoint required the demonstration of greater efficacy in preventing relapse of vivax malaria using tafenoquine, it is felt that the study results do reflect a true tafenoquine effect.

The early appearance of vivax malaria after treatment of falciparum malaria and use of primaquine terminal prophylaxis suggests the subject ultimately experiencing tafenoquine treatment failure was infected with a primaquine-tolerant parasitaemia during this first episode of vivax malaria. Conceivably, this parasite was also more likely to be tolerant of tafenoquine as the two medications have similar chemical structures. In addition, this subject has a distinctly different tafenoquine pharmacokinetic curve during the treatment phase. A delay in development of higher levels of tafenoquine may explain the treatment failure. This may represent a longer interval of time during which the vivax hypnozoites are subjected to a sub-therapeutic level of tafenoquine.

These two factors of a possible higher primaquine-tolerant infection and a delay in development of higher concentrations of tafenoquine may be independently or concurrently responsible for the treatment failure recorded in this study.

The management of relapsing vivax malaria with chloroquine/tafenoquine is more effective and convenient in preventing further relapses than the standard chloroquine/primaquine treatment regimen. Larger studies are required to address the effectiveness and tolerability of chloroquine/tafenoquine for the treatment of vivax malaria. There is also a need to investigate this regimen in other ethnic populations, including special risk groups such as children and pregnant women.

5.7 Key messages from this chapter

- Tafenoquine / Chloroquine in combination appears to be more effective than primaquine / chloroquine in eradicating the hepatic stages of vivax malaria.
- Scope remains to further investigate this combination or tafenoquine alone in the treatment of vivax malaria.
- Optimisation of dosing for tafenoquine needs to be further investigated.

5.8 References

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Chapter 5 Paper 5.1

Kitchener S, **Nasveld P**, Edstein MD. (2007) Tafenoquine for the treatment of recurrent *Plasmodium vivax* Malaria. *American Journal of Tropical Medicine and Hygiene*. 76: 494-496.

CHAPTER 6

- **General Discussion**

6.1 Overview

The thesis describes work undertaken through the Australian Army Malaria Institute in three key areas of drug development for tafenoquine and synergises the potential utilisation of this promising new anti malarial compound.

The key areas of potential use identified within the Drug Development Plan developed by GSK and the US Army focus on the use of tafenoquine as:

- A malaria chemoprophylaxis agent;
- An alternative to primaquine as a post exposure chemoprophylaxis agent for recurring forms of malaria (*P. vivax* and *P. ovale*);
- A stand alone or adjunct treatment therapy for malaria; and
- An adjunct to focussed regional malaria eradication in combination with other preventative strategies such as bed nets and insecticide / larvicide control of vectors.

The studies described within this thesis contribute significantly to knowledge of the first three of these potential uses and provides some vision into the potential role of an easily administered short course eradication drug in supporting a wider geographical eradication agenda

6.2 Contributions to the understanding of malaria within our immediate area of strategic defence interest

Chapter 2 of the thesis explored the issues of malaria within parts of the area of Australian strategic defence interest; specifically the island of Bougainville, Papua New Guinea and the fledgling nation of Timor Leste. The series of studies provided the opportunity to move research teams into these locations and through a series of supporting activities improve the general knowledge of the burden of malaria both within the general population and specifically within the exposed defence population.

Papers presented in this section of the thesis have helped to define the malaria issue experienced by our Defence personnel on deployment [Kitchener et al, 2003; Elmes et al, 2004], highlight issues with the then available prophylaxis agents [Kitchener et al, 2005] and characterise the seasonal nature of malaria in parts of Timor Leste [Bragonier et al, 2002]. They reinforce that there was significant exposure of ADF personnel to malaria in both Bougainville, PNG and in Timor Leste and that the ADF were experiencing failures of then current prophylaxis and post-exposure prophylaxis during the period of conduct for these studies.

Additional AMI activity during this time focussed on transmission studies, characterisation of antimalarial resistance patterns and vector identification. Where appropriate these have been referenced [Cooper et al, 2002], but much of the AMI activity of this time has not been published separately but has been used to extensively review key policy documents such as the Health Directive 215 – Malaria which underpins the prevention, diagnosis and treatment for malaria within our Australian Defence Force

6.3 Longer term chemoprophylaxis with tafenoquine

Chapter 3 of this thesis presented the findings of a 6 month chemoprophylaxis study which confirmed that tafenoquine at a weekly dose of 200 mg and mefloquine at a dose of 250 mg were well tolerated amongst subjects in a military deployment [Nasveld et al, 2010]. The study conducted further develops our knowledge and understanding of the safety and tolerability of both tafenoquine and mefloquine, as well as effectiveness of these compounds in preventing malaria in a high risk population [Edstein et al, 2007]. Further the study provided the means to further define and characterise our understanding of the pharmacokinetics of tafenoquine and mefloquine within a largely younger, predominantly Caucasian male population and to confirm the required therapeutic levels required for effective chemoprophylaxis with both study compounds [Charles et al, 2007a].

Significant background activity during this study ensured it has “pivotal” study status with the Drug Development Plan having satisfied the GSK Tafenoquine Development

Team that exposure was sufficient to support claims of efficacy. The significance of such a determination is that within modern ethical constraints the unnecessary exposure of large numbers of human subjects to malaria infection within a study design can be avoided by the conduct of comprehensive supporting research establishing a broad exposure picture. Given the significance of malaria globally and the concerns of ethics committees on vulnerable populations in a research setting, this style of research will do much to support the development of safe study designs where the requirement for placebo arms can be avoided, thus ensuring the correct balance between risk and benefit to study participants.

In terms of safety of tafenoquine two interesting observations were made during the conduct of this study. The first of these involved the unexpected findings of vortex keratopathy on the examination of the cortex. This finding required longer term follow-up of the sub set of individuals noted to have these changes and confirmed that the changes were in essence benign and reversible. As reported, the majority of keratopathy had resolved by 6 months post tafenoquine dosing and all by 12 months. What remained uncertain was the dose / response relationship between the administration of tafenoquine and the onset of the vortex keratopathy.

While ophthalmological review and follow-up indicated that there was no impact on visual acuity, the finding did lead to the cessation of all trial activity with the study drug for a period of 4 years. Key questions were raised by the drug co-sponsors. GSK were obviously concerned re the viability of continuing development of the drug while the US Army were more focussed on the potential for the observed eye changes to ultimately impact on vision and in particular the effective operation of night vision equipment now considered to be an essential component of the soldiers defensive and offensive armoury.

Following the end of this study, renal toxicity findings in a 2-year rat carcinogenicity study resulted in a review of renal data for tafenoquine. Small but noticeable changes in serum creatinine levels were noted on a review of the findings of this and previous studies with tafenoquine. While these failed to impress as being clinically significant in isolation the findings of an increased incidence of renal tumours in the rat population and similar creatinine changes in other tafenoquine studies prompted some concern about the renal safety of tafenoquine.

As a result of these observations it was determined that a longer term follow up of subjects with a pre-determined change from baseline creatinine values. This was not undertaken under separate ethical approval but rather at the direction of ADMEC who determined that longer term clinical follow up was in keeping with good clinical research practice and that the responsibility of the investigators to ensure the well being of study participants would be best met by continuing surveillance of the potentially at risk population.

Consequently, in addition to the protocol delivered in Chapter 3, a long-term renal follow up was conducted in a cohort of subjects with serum creatinine concentrations $\geq$ 0.02 mmol/L above baseline at end of treatment, and/or at follow up.

In total, there were 246 subjects with an increased serum creatinine concentration at end of prophylaxis and/or follow-up. Twenty-nine of these were subsequently discharged from the ADF, though none for renally related medical conditions. In total, 186 subjects were contacted and 183 subjects consented to take part in the follow-up. Of these, 147 subjects were from the tafenoquine treatment group and 36 subjects were from the mefloquine treatment group. The demographics of this group were very similar to the overall study population.

At the first follow-up visit, subjects provided a blood sample for creatinine and urea analysis and a urine sample for urinanalysis. Subjects fulfilling the criteria were recalled for further follow-up visits. If the results were confirmed at the further follow-up visit, the subject was referred for renal work-up with a nephrologist.

A total of 173/183 (95%) subjects had normal renal function tests at their first or second follow-up visit; 140/147 (95.2%) subjects in the tafenoquine group and 30/33 (91.7%) subjects in the mefloquine group. Overall, 10 subjects were referred for follow-up with a renal consultant for the reasons described in **Table 6-1** below:

Table 6.1 Creatinine measurements in long term follow-up

	Treatment Group	
	Tafenoquine N=147	Mefloquine N=36
Referred for renal follow-up	7 (4.8%)	3 (8.3%)
Creatinine above upper limit of normal	0	1 (2.8%)
Creatinine ≥0.03 mmol/L above baseline	2 (1.4%)	1 (2.8%)
Clinically significant urinalysis result	5 (3.4%)	2 (5.6%)

All 10 subjects were confirmed by the renal physician as having no clinical evidence of chronic renal injury. This follow-up did not demonstrate any evidence of long-term renal damage in healthy subjects who had received tafenoquine or mefloquine for 6 months.

6.4 Short-term post exposure prophylaxis with tafenoquine

Chapter 4 described a series of linked studies designed to evaluate the utility of tafenoquine as a replacement for primaquine in the post exposure prophylaxis of malaria, again in a relatively young Caucasian predominantly male population [Nasveld et al, 2002; Elmes et al, 2008]. The population sizes involved within the three cohorts of this study allow for a comprehensive evaluation of the effectiveness of tafenoquine in preventing malaria after a period of exposure to be made. A 3-day dosing regimen of tafenoquine (400 mg od, 200 mg bd or 200 mg od) was as effective as a post-exposure prophylaxis agent in this study, demonstrating similar efficacy to 14-day primaquine. In this study, tafenoquine was well tolerated, with no subjects being withdrawn due to adverse events. The most common adverse events were gastrointestinal events. Compliance with post exposure malaria prophylaxis with 14 days of primaquine within the Defence population has been long thought of as problematic. It takes little imagination to identify that returning soldiers after an extended (6 months) deployment may be more focussed on leave and recreation than on adequately completing a14 day primaquine eradication course with associated cautions on alcohol consumption. Tafenoquine, with a shorter dosing regimen (3 days compared to 14 days primaquine), could be used as a more convenient, yet effective, post-exposure prophylaxis agent.

Within this study population however, the overall incidence of malaria after returning to Australia was somewhat reduced over that which had been seen in other deployments to the same areas [Kitchener et al, 2003] possibly indicating an improved compliance overall, secondary to the increased awareness of the disease and its significance as a direct result of study education / information and the collocation of the AMI research teams. Such an effect should reinforce command commitment to ensuring disease awareness education and adequate allocation of preventative health resources to an operational mission. Subsequent wind down of the number of troops allocated to these areas and the more urban base of continuing commitments make this difficult however to evaluate. This series of studies also contributed to the understanding of gender differences associated with the pharmacokinetics and adverse event profiles of tafenoquine between the genders [Edstein et al, 2007].

Perhaps one of the most useful but unexpected outcomes was a result of a protocol violation described within this Chapter. In a treatment related error, two participants in the AMI 001 cohort received tafenoquine alone instead of the planned reduction of parasitaemia using a loading course of chloroquine prior to tafenoquine administration. This represents the first time in man tafenoquine had been used alone in the treatment of vivax malaria. The information obtained from these two cases, and in particular the ability to match tafenoquine levels to a clinical response (resolution of symptoms and parasite clearance) has contributed significantly to the interest in tafenoquine as a treatment agent for malaria rather than just as a chemoprophylaxis agent[Nasveld and Kitchener, 2005]. There remains an obvious requirement to further investigate this potential use but the information gathered from these two cases will do much to inform the debate on adequate dose ranges and expected parasite clearance times.

6.5 The treatment of recurrent vivax malaria with tafenoquine

Chapter 5 describes a pilot study designed to look at the treatment of recurrent vivax malaria with a combination of chloroquine and primaquine [Kitchener et al, 2007]. Recurrences of vivax malaria in ADF personnel who have been previously “adequately” treated accounts for approximately 20 % of all reported cases of malaria in the ADF. The study was undertaken under the Section 19 Authorised Prescriber provision of the *Therapeutic Goods Act 1989* and was the first time the provisions of this section of the Act were applied to the clinical management of cases of malaria in the ADF. The provisions of the Act have been utilised within Defence in the past but only in application to vaccination procedures against Anthrax for vulnerable ADF personnel deploying to risk areas.

The use of these provisions is not without challenges and in retrospect it may have proved simpler and more informative if the study had been conducted under the strict requirements of an approved protocol with the compilation of study specific records rather than a reliance on standard clinical note taking and management. The ADMEC did approve the study and the Authorised Prescribers; however under this arrangement the obligations of the Authorised Prescriber are not as onerous in terms of completeness of record keeping as might be expected of formally obliged clinical study investigators. That said the study again provided valuable insight into the problems of treating recurring malaria and clearly established that tafenoquine / chloroquine in combination appears to be more effective than primaquine / chloroquine in eradicating the hepatic stages of viva malaria.

Primary issues with this study revolve around the adequacy of the clinical records to maximise the information available to the research teams and ensure the proposed study schedules for follow up study bleeds are rigorously adhered to. This can best be served by delivering such studies under the full review and approval processes of a Human Research Ethics Committee. Such an approach would also have allowed the ready availability of study medication avoiding the variance in tafenoquine start times seen with a laborious approval process before study drug could be made available, resulting in extended exposure to chloroquine over that originally envisaged. There remains, however, a need to more comprehensively investigate this combination or tafenoquine alone in the treatment of vivax malaria. Additionally, optimisation of dosing for tafenoquine needs to be further investigated.

This study also explored the use of a defacto comparison group drawn from the general reporting to the Central Malaria Register. While direct matching is not possible with this model, and compliance with primaquine treatment cannot be formally documented, the size of the comparison population gives validity to their use for comparison purposes. Such an approach simplifies study design while maximising the utilisation of data available to the ADF.

6.6 Specific issues about G6PD deficiency and tafenoquine

Tafenoquine, like its analogue primaquine produces significant haemolysis in those with a deficiency of Glucose-6-phosphate dehydrogenase (G6PD) [Shanks et al, 2001]. Wider utilisation of this promising antimalarial will in some ways be limited to populations where the assessment of G6PD deficiency can be made. While this is possible and readily achievable in a military population, as all recruits to the ADF have entry screening for G6PD undertaken, it will be a challenge in the wider travel market or in regions of relatively underdeveloped medical infrastructure. A study to expolore he extent of haemolysis following tafenoquine and primaquine administration is currently recruiting in Thailand [NCT 01205178, 2010].

Current assessment techniques are relatively simple to perform but require some storage and quality assurance steps that make the testing currently laboratory based. Wider utilisation may well depend of the development of a point of sale testing solution if the potential of tafenoquine in prophylaxis and treatment is to be realised.

6.7 Further directions in research

Tafenoquine is subject to an agreed co development plan between GSK and the US Army. The plan outlines the priorities for the development of tafenoquine and has undergone some modification since the outcomes of this study have been formally reported.

Initially the development priority for tafenoquine was centred on its use as a chemoprophylaxis agent to be taken while an individual was in an area of risk. The major study described in Chapter 3 was designed to validate this use and was considered as pivotal to subsequent registration of the compound with the Regulatory authorities in Australia, Europe and the United States. The findings of this study however raised additional concerns which needed to be resolved before further pursuit of this use could be reasonably contemplated. Specifically the issues were the development of vortex keratopathy and the potential for renal changes identified through both animal and later human studies.

Consequently, it was decided to return the compound back to Phase 1 status and more comprehensively investigate any renal or ophthalmological issues with tafenoquine. Study SB 252263: 057 was subsequently devised with the original intent that it be conducted by the Australian Army Malaria Institute. This study would look at renal clearance times of key markers as well as include a broad ophthalmology component including vision testing, time to onset and incidence of vortex keratopathy, and, as it is of concern to the military, any impact of tafenoquine of the operation of night vision equipment.

Discussion with the Australian Defence Human Research Ethics Committee indicated some reservation about conducting Phase 1 studies in a military operational population and it was agreed that the Phase 1 study could, and should be conducted in a volunteer civilian population. The study was consequently conducted in the United States and has now been reported in the peer reviewed literature [Leary et al, 2011]. Interestingly, there was no impact on night vision a lower rate of vortex keratopathy (which was reversible) was observed, and no renal concerns were confirmed. This now opens the way for future studies in the area of chemoprophylaxis, but while mefloquine remains effective in many places in the world, and Malarone™ is now widely available as a competitor chemoprophylaxis agent (also a GSK product) with good efficacy, development of tafenoquine for this role has been allocated a lower priority.

The focus has therefore shifted to the use of tafenoquine in the treatment of malaria. Recently a study has been completed in Thailand [NTC 01290601, 2005] in anadult population investigating further the utilisation of tafenoquine in this role; however, no results of this trial have yet been published. The information gathered in the studies conducted at AMI and elsewhere have been instrumental in the development of the study treatment schedules and in providing the background information for dose optimisation studies. Particularly, the results of the pilot study on longer term treatment of recurrent vivax malaria, pharmacodynamic studies undertaken as components of the current series, and the success of tafenoquine alone in the treatment of two subjects within our studies have provided the background information on which study planning could be developed.

Additionally, and as a very different application, there has been considerable interest in the development of tafenoquine as an adjunct to geographical eradication. While the eradication of malaria as a significant human disease has long been touted as possible the history of attempts has been less glorious. Wide scale eradication programs in earlier years have been disappointing with DDT programs, bed net programs and other vector control initiatives having displayed considerable promise, but ultimately failing. The lack of an effective vaccine against malaria, which would have been a valuable adjunct to physical control programs currently makes the eradication of malaria an unlikely short term outcome.

However, there is developing interest, including from the Gates Foundation, on the more immediate use of antimalarial drugs as an interim measure until vaccine development may be able to fill the gap. Tafenoquine in this role may prove to be suitable candidate given it can be given over three days as a treatment / eradicant and is generally well tolerated. Obviously much needs to done to investigate this potential use of tafenoquine. It is pleasing to see that AMI is now engaged in activities in the island nation of Vanuatu to establish potential island sites and conduct entomological and disease incidence surveys in preparation for the potential investigation of tafenoquine, in support of bed nets and vector control, in eradicating malaria from these islands.

While there is significant information available on the use of tafenoquine and its safety in Thai [Walsh et al, 1999] and Kenyan [Shanks et al, 2001] populations, the information from the studies described in this thesis provide the most comprehensive information on safety, efficacy and the pharmacokinetics available in an essentially non immune Caucasian population. It is therefore also essential that the potential uses of tafenoquine as developed in this thesis are further explored in other populations more reflective of the ultimate end user. Wider population studies designed to investigate treatment and prophylaxis need to be conducted. Specific investigations into two vulnerable populations in particular need to be explored; that is in paediatric populations and in the pregnant, where the burden of malaria morbidity and mortality are most pronounced.

The final development that is required to fully support the wider utilisation or investigation of tafenoquine is the issue of G6PD testing. As previously mentioned, the development of a robust and cost effective point of sale (pre prescribing) test for G6PD is essential if wider applications for this compound are to be fully explored.

6.8 References

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Appendix 1

- **Instructions for Authors**

ADMINISTRATIVE DOCUMENTATION HAS BEEN REMOVED

Antimicrobial Agents and Chemotherapy

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

2011 INSTRUCTIONS TO AUTHORS*

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ASM publishes a number of different journals covering various aspects of the field of microbiology. Each journal has a prescribed scope that must be considered in determining the most appropriate journal for each manuscript. The following guidelines may be of assistance.

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(vi) Biochemical analyses for β-lactamases that determine kinetic parameters (e.g., K_m, k_{cat}) must be performed on purified enzyme preparations. The enzyme must be in its native form, without any leader sequences or fusions used for purification (e.g., His tag). The determination of relative rates of hydrolysis may be performed on crude extracts.

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Correspondent footnote. The complete mailing address, a single telephone number, a single fax number, and a single e-mail address for the corresponding author should be included on the title page of the manuscript. This information will be published in the article as a footnote to facilitate communication, and the e-mail address will be used to notify the corresponding author of the availability of proofs and, later, of the PDF file of the published article. No more than two authors may be designated corresponding authors.

Abstract. Limit the abstract to 250 words or fewer and concisely summarize the basic content of the paper without presenting extensive experimental details. Avoid abbreviations and references, and do not include diagrams. When it is essential to include a reference, use the same format as shown for the References section but omit the article title. Conclude the abstract with a summary statement. Because the abstract will be published separately by abstracting services, it must be complete and understandable without reference to the text.

Introduction. The introduction should supply sufficient background information to allow the reader to understand and evaluate the results of the present study without referring to previous publications on the topic. The introduction should also provide the hypothesis that was addressed or the rationale for the study. References should be chosen carefully to provide the most salient background rather than an exhaustive review of the topic.

Case Report. The Case Report section, placed after the introduction and before Materials and Methods, is optional and gives relevant clinical information about one or more patients.

Materials and Methods. The Materials and Methods section should include sufficient technical information to allow the experiments to be repeated. When centrifugation conditions are critical, give enough information to enable another investigator to repeat the procedure: make of centrifuge, model of rotor, temperature, time at maximum speed, and centrifugal force ($\times g$ rather than revolutions per minute). For commonly used materials and methods (e.g., media and protein concentration determinations), a simple reference is sufficient. If several alternative methods are commonly used, it is helpful to identify the method briefly as well as to cite the reference. For example, it is preferable to state "cells were broken by ultrasonic treatment as previously described (9)" rather than "cells were broken as previously described (9)." This allows the reader to assess the method without constant reference to previous publications. Describe new methods completely, and give sources of unusual chemicals, equipment, or microbial strains. When large numbers of microbial strains or mutants are used in a study, include tables identifying the immediate sources (i.e., sources from whom the strains were obtained) and properties of the strains, mutants, bacteriophages, and plasmids, etc.

A method or strain, etc., used in only one of several experiments reported in the paper may be described in the Results section or very briefly (one or two sentences) in a table footnote or figure legend. It is expected that the sources from whom the strains were obtained will be identified.

Results. In the Results section, include the rationale or design of the experiments as well as the results; reserve extensive interpretation of the results for the Discussion section. Present the results as concisely as possible in one of the following: text, table(s), or figure(s). Avoid extensive use of graphs to present data that might be more concisely or more quantitatively presented in the text or tables. Limit photographs (particularly photomicrographs and electron micrographs) to those that are absolutely necessary to show the experimental findings. Number figures and tables in the order in which they are cited in the text, and be sure that all figures and tables are cited.

Discussion. The Discussion should provide an interpretation of the results in relation to previously published work and to the experimental system at hand and should not contain extensive repetition of the Results section or reiteration of the introduction. In short papers, the Results and Discussion sections may be combined.

Acknowledgments. The source of any financial support received for the work being published must be indicated in the Acknowledgments section. (It will be assumed that the absence of such an acknowledgment is a statement by the authors that no support was received.) The usual format is as follows: "This work was supported by Public Health Service grant CA-01234 from the National Cancer Institute."

Recognition of personal assistance should be given as a separate paragraph, as should any statements disclaiming endorsement or approval of the views reflected in the paper or of a product mentioned therein.

Appendixes. Appendixes that contain additional material to aid the reader are permitted. Titles, authors, and reference sections that are distinct from those of the primary article are not allowed. If it is not feasible to list the author(s) of the appendix in the byline or the Acknowledgments section of the primary article, rewrite the appendix so that it can be considered for publication as an independent article, either full-length or short-form style. Equations, tables, and figures should be labeled with the letter "A" preceding the numeral to distinguish them from those cited in the main body of the text.

References. (i) References listed in the References section. The References section must include all journal articles (both print and online), books and book chapters (both print and online), patents, theses and dissertations, published conference proceedings, meeting abstracts from published abstract books or journal supplements, letters (to the editor), and company publications, as well as in-press journal articles, book chapters, and books (publication title must be given). As we use the citation-name reference style, arrange the citations in **alphabetical order** (letter by letter, ignoring spaces and punctuation) by first-author surname and **number consecutively**. Provide the names of all the authors for each reference. All listed references must be cited parenthetically by number in the text. Since title and byline information that is downloaded from PubMed does not always show accents, italics, or special characters, authors should refer to the PDF files or hardcopy versions of the articles and incorporate the necessary corrections in the submitted manuscript. Abbreviate journal names according to the PubMed Journals Database (National Library of Medicine, National Institutes of Health; available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=journals), the primary source for ASM style, but use periods on abbreviated words.

Follow the styles shown in the examples below for print references.

1. **Alexander, T. W., et al.** 2008. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. Appl. Environ. Microbiol. **74:**4405–4416.
2. **Cox, C. S., B. R. Brown, and J. C. Smith.** J. Gen. Genet., in press.* {*Article title is optional; journal title is mandatory*.}
3. **da Costa, M. S., M. F. Nobre, and F. A. Rainey.** 2001. Genus I. Thermus Brock and Freeze 1969, 295,[AL] emend. Nobre, Trüper and da Costa 1996b, 605, p. 404–414. *In* D. R. Boone, R. W. Castenholz, and G. M. Garrity (ed.), Bergey's manual of systematic bacteriology, 2nd ed., vol. 1. Springer, New York, NY.
4. **Elder, B. L., and S. E. Sharp.** 2003. Cumitech 39, Competency assessment in the clinical laboratory. Coordinating ed., S. E. Sharp. ASM Press, Washington, DC.
5. **Falagas, M. E., and S. K. Kasiakou.** 2006. Use of international units when dosing colistin will help decrease confusion related to various formulations of the drug around the world. Antimicrob. Agents Chemother. **50:**2274–2275. (Letter.) {*"Letter" or "Letter to the editor" is allowed but not required at the end of such an entry*.}
6. **Fitzgerald, G., and D. Shaw.** *In* A. E. Waters (ed.), Clinical microbiology, in press. EFH Publishing Co., Boston, MA.* {*Chapter title is optional*.}
7. **Forman, M. S., and A. Valsamakis.** 2003. Specimen collection, transport, and processing: virology, p. 1227–1241. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, J. H. Jorgensen, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. ASM Press, Washington, DC.
8. **Garcia, C. O., et al.** 1996. Detection of salmonella DNA in synovial membrane and synovial fluid from Latin American patients. Arthritis Rheum. **39**(Suppl.)**:** S185. {*Meeting abstract published in journal supplement*.}
9. **Green, P. N., D. Hood, and C. S. Dow.** 1984. Taxonomic status of some methylotrophic bacteria, p. 251–254. *In* R. L. Crawford and R. S. Hanson (ed.), Microbial growth on C_1 compounds. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, DC.
10. **Odell, J. C.** April 1970. Process for batch culturing. U.S. patent 484,363,770. {*Include the name of the patented item/process if possible; the patent number is mandatory*.}
11. **O'Malley, D. R.** 1998. Ph.D. thesis. University of California, Los Angeles, CA. {*Title is optional*.}
12. **Rotimi, V. O., N. O. Salako, E. M. Mohaddas, and L. P. Philip.** 2005. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-1658. {*Abstract title is optional*.}
13. **Smith, D., C. Johnson, M. Maier, and J. J. Maurer.** 2005. Distribution of fimbrial, phage and plasmid associated virulence genes among poultry *Salmonella enterica* serovars, abstr. P-038, p. 445. Abstr. 105th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC. {*Abstract title is optional*.}
14. **Stratagene.** 2006. Yeast DNA isolation system: instruction manual. Stratagene, La Jolla, CA. {*Use the company name as the author if none is provided for a company publication*.}

*A reference to an in-press ASM publication should state the control number (e.g., AAC00577-11) if it is a journal article or the name of the publication if it is a book.

Online references must provide essentially the same information that print references do. For online journal articles, posting or revision dates may replace the year of publication, and a DOI or URL may be provided in addition to or in lieu of volume and page numbers. Some examples follow.

1. **Charlier, D., and N. Glansdorff.** September 2004, posting date. Chapter 3.6.1.10, Biosynthesis of arginine and polyamines. *In* R. Curtiss III et al. (ed.), EcoSal—*Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC. http://www.ecosal.org/. {*Note that each chapter has its own posting date*.}
2. **Dionne, M. S., and D. S. Schneider.** 2002. Screening the fruitfly immune system. Genome Biol. **3:** REVIEWS1010. http://genomebiology.com/2002/3/4/reviews/1010.
3. **Smith, F. X., H. J. Merianos, A. T. Brunger, and D. M. Engelman.** 2001. Polar residues drive association of polyleucine transmembrane helices. Proc. Natl. Acad. Sci. U. S. A. **98:**2250–2255. doi:10.1073/pnas.041593698.
4. **Winnick, S., D. O. Lucas, A. L. Hartman, and D. Toll.** 2005. How do you improve compliance? Pediatrics **115:**e718–e724.

Note: a posting or accession date is required for any online reference that is periodically updated or changed.

(ii) References cited in the text. References to unpublished data, manuscripts submitted for publication, unpublished conference presentations (e.g., a report or poster that has not appeared in published conference proceedings), personal communications, patent applications and patents pending, computer software, databases, and websites should be made parenthetically in the text as follows.

. . . similar results (R. B. Layton and C. C. Weathers, unpublished data).
. . . system was used (J. L. McInerney, A. F. Holden, and P. N. Brighton, submitted for publication).
. . . as described previously (M. G. Gordon and F. L. Rattner, presented at the Fourth Symposium on Food Microbiology, Overton, IL, 13 to 15 June 1989). {*For nonpublished abstracts and posters, etc.*}
. . . this new process (V. R. Smoll, 20 June 1999, Australian Patent Office). {*For non-U.S. patent applications, give the date of publication of the application.*}
. . . available in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/index.html).
. . . using ABC software (version 2.2; Department of Microbiology, State University [http://www.state.micro.edu]).

URLs for companies that produce any of the products mentioned in your study or for products being sold may not be included in the article. However, company URLs that permit access to scientific data related to the study or to shareware used in the study are permitted.

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(iv) Referencing ASM Accepts (publish-ahead-of-print) manuscripts. Citations of ASM Accepts manuscripts should look like the following example.

Wang, G. G., M. P. Pasillas, and M. P. Kamps. 15 May 2006. Persistent transactivation by Meis1 replaces Hox function in myeloid leukemogenesis models: evidence for co-occupancy of Meis1-Pbx and Hox-Pbx complexes on promoters of leukemia-associated genes. Mol. Cell. Biol. doi:10.1128/MCB.00586-06.

Other journals may use different styles for their publish-ahead-of-print manuscripts, but citation entries must include the following information: author name(s), posting date, title, journal title, and volume and page numbers and/or DOI. The following is an example:

Zhou, F. X., H. J. Merianos, A. T. Brunger, and D. M. Engelman. 13 February 2001, posting date. Polar residues drive association of polyleucine transmembrane helices. Proc. Natl. Acad. Sci. U. S. A. doi:10.1073/pnas.041593698.

Short-Form Papers

The short-form format is intended for the presentation of brief observations that do not warrant full-length papers. Submit short-form papers in the same way as full-length papers. They receive the same review, they are not published more rapidly than full-length papers, and they are not considered preliminary communications.

The title, running title (not to exceed 54 characters and spaces), byline, and correspondent footnote should be prepared as for a full-length paper. Each short-form paper must have an abstract of no more than 75 words. Do not use section headings in the body of the paper; combine methods, results, and discussion in a single section. Paragraph lead-ins are permissible. The text should be kept to a minimum and if possible should not exceed 1,000 words; the number of figures and tables should also be kept to a minimum. Materials and methods should be described in the text, not in figure legends or table footnotes. Present acknowledgments as in full-length papers, but do not use a heading. The References section is identical to that of full-length papers.

Minireviews

Minireviews are brief (**limit of six printed pages exclusive of references**) biographical profiles, historical perspectives, or summaries of developments in fast-moving areas of chemotherapy. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of AAC. For example, subject matter may range from structure-activity correlates among a group of semisynthetic cephalosporins to the comparative efficacies of new and old drugs in the prevention or treatment of diseases of microbial origin in humans.

Minireviews may be either solicited or proffered by authors responding to a recognized need. Irrespective of origin, Minireviews are subject to review and should be submitted via the online manuscript submission and peer review system. The cover letter should state whether the article was solicited and by whom.

Minireviews must have abstracts. Limit the abstract to 250 words or fewer. The body of the Minireview may have section headings and/or paragraph lead-ins.

Author biographies. At the editor's invitation, corresponding authors of Minireviews may submit brief biographical sketches (limit, 150 words) of each contributing author, to be published at the end of the article. If the editor asks you to submit a modified manuscript, you should submit biographical text and photos with your modification.

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Commentaries are invited communications concerning topics relevant to the readership of AAC and are intended to engender discussion. Reviews of the literature, methods and other how-to papers, and responses targeted at a specific published paper are not appropriate. Commentaries are subject to review.

The length may not exceed four printed pages, and the format is like that of a Minireview (see above) except that Commentaries do not have abstracts. (In the submission form, use "NA" or "Not Applicable" in the space provided for the abstract.)

Letters to the Editor

Two types of Letters to the Editor may be submitted. The first type (Comment Letter) is intended for comments on final, typeset articles published in the journal (not on publish-ahead-of-print manuscripts) and must cite published references to support the writer's argument. The second type (New-Data Letter) may report new, concise findings that are not appropriate for publication as full-length or short-form papers.

Letters may be **no more than 500 words long and must be typed double-spaced**. Refer to a recently published Letter for correct formatting. Note that authors and affiliations are listed at the foot of the Letter. Provide only the primary affiliation for each author.

All Letters to the Editor must be submitted electronically, and the type of Letter (New Data or Comment) must be selected from the drop-down list in the submission form. For Letters commenting on published articles, the cover letter should state the volume and issue in which the article was published, the title of the article, and the last name of the first author. In the Abstract section of the submission form, put "Not Applicable." Letters to the Editor do not have abstracts. Both types of Letter must have a title, which must appear on the manuscript and on the submission form. Figures and tables should be kept to a minimum.

If the Letter is related to a published article, it will be sent to the editor who handled the article in question. If the editor believes that publication is warranted, he/she will solicit a reply from the corresponding author of the article and give approval for publication.

New-Data Letters will be assigned to an editor according to subject matter and will be reviewed by that editor and/or a reviewer.

Please note that some indexing/abstracting services do not include Letters to the Editor in their databases.

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The Erratum section provides a means of correcting errors that occurred during the writing, typing, editing, or publication (e.g., a misspelling, a dropped word or line, or mislabeling in a figure) of a published article. Submit Errata via the online manuscript submission and peer review system (see "Submission, Review, and Publication Processes"). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Erratum as a Microsoft Word file. Please see a recent issue for correct formatting.

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ILLUSTRATIONS AND TABLES

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Image manipulation. Computer-generated images may be processed only minimally. Processing (e.g., changing contrast, brightness, or color balance) is acceptable only if applied to all parts of the image, as well as to the controls, equally, and descriptions of all such adjustments and the tools used (both hardware and software) must be provided in the manuscript. Unprocessed data and files must be retained by the authors and be provided to the editor on request.

File types and formats. Illustrations may be continuous-tone images, line drawings, or composites. Color graphics may be submitted, but the cost of printing in color must be borne by the author. Suggestions about how to reduce costs and ensure accurate color reproduction are given below.

On initial submission, illustrations should be supplied as PDF files, with the legend on the same page, to assist review. At the modification stage, production quality digital files must be provided, along with text files for the legends. The legends are copyedited and typeset for final publication, not included as part of the figure itself. All graphics submitted with modified manuscripts must be bitmap, grayscale, or in the RGB (preferred) or CMYK color mode. See "Color illustrations." Halftone images (those with various densities or shades) must be grayscale, not bitmap. AAC accepts TIFF or EPS files but discourages PowerPoint for either black-and-white or color images.

For instructions on creating acceptable EPS and TIFF files, refer to the Cadmus digital art website, http://art.cadmus.com/da/index.jsp. PowerPoint requires users to pay close attention to the fonts used in their images (see the section on fonts below). If instructions for fonts are not followed exactly, images prepared for publication are subject to missing characters, improperly converted characters, or shifting/obscuring of elements or text in the figure. For proper font use in PowerPoint images, refer to the Cadmus digital art website, http://art.cadmus.com/da/instructions/ppt_disclaimer.jsp.

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300 dpi for grayscale and color
600 dpi for combination art (lettering and images)
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Maximum width for a 1-column figure: 3 5/16 inches (ca. 8.4 cm)
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Fonts. To avoid font problems, set all type in one of the following fonts: Arial, Helvetica, Times Roman, European PI, Mathematical PI, or Symbol. Courier may be used but should be limited to nucleotide or amino acid sequences in which a nonproportional (monospace) font is required. All fonts other than these must be converted to paths (or outlines) in the application with which they were created.

Compression. When figure files are uploaded to the manuscript submission and peer review system, they may be compressed with WinZip.

Color illustrations. Color costs must be borne by the author. See "Publication Fees." **All figures submitted in color will be processed as color.** Adherence to the following guidelines will help to minimize costs and to ensure color reproduction that is as accurate as possible.

The online version is considered the version of record for AAC and all other ASM journals. To maximize online reproduction, color illustrations should be supplied in the RGB color mode, as either (i) RGB TIFF images with a resolution of at least 300 pixels per inch (raster files, consisting of pixels) or (ii) Illustrator-compatible EPS files with RGB color elements (vector files, consisting of lines, fonts, fills, and images). CMYK files are also accepted. Other than in color space, CMYK files must meet the same production criteria as RGB files. The RGB color space is the native color space of computer monitors and of most of the equipment and software used to capture scientific data, and it can display a wider range of colors (especially bright fluorescent hues) than the CMYK (cyan, magenta, yellow, black) color space used by print devices that put ink (or toner) on paper. For the print version (and reprints), ASM's print provider will automatically create CMYK versions of color illustrations from the supplied RGB versions. Color in the print journal may not match that in the online journal of record because of the smaller range of colors capable of being reproduced by CMYK inks on a printing press. For additional information on RGB versus CMYK color, refer to the Cadmus digital art site, http://art.cadmus.com/da/guidelines_rgb.jsp.

Drawings

Submit graphs, charts, complicated chemical or mathematical formulas, diagrams, and other drawings as finished products not requiring additional artwork or typesetting. All elements, including letters, numbers, and symbols, must be easily readable, and both axes of a graph must be labeled. Keep in mind that the journal is published both in print and online and that the same electronic files submitted by the authors are used to produce both.

When creating line art, please use the following guidelines:

(i) **All art must be submitted at its intended publication size.** For acceptable dimensions, see "Size," above.

(ii) **Avoid using screens (i.e., shading) in line art.** It can be difficult and time-consuming to reproduce these images without moiré patterns. Various pattern backgrounds are preferable to screens as long as the patterns are not imported from another application. If you must use images containing screens,

(a) Generate the image at line screens of 85 lines per inch or less.

(b) When applying multiple shades of gray, differentiate the gray levels by at least 20%.

(c) Never use levels of gray below 5% or above 95% as they are likely to fade out or become totally black when output.

(iii) Use thick, solid lines that are no finer than 1 point in thickness.

(iv) No type should be smaller than 6 points at the final publication size.

(v) Avoid layering type directly over shaded or textured areas.

(vi) Avoid the use of reversed type (white lettering on a black background).

(vii) Avoid heavy letters, which tend to close up, and unusual symbols, which the printer may not be able to reproduce in the legend.

(viii) If colors are used, avoid using similar shades of the same color and avoid very light colors.

In figure ordinate and abscissa scales (as well as table column headings), avoid the ambiguous use of numbers with exponents. Usually, it is preferable to use the Système International d'Unités (SI) symbols (μ for 10^{-6}, m for 10^{-3}, k for 10^{3}, and M for 10^{6}, etc.). A complete listing of SI symbols can be found in the International Union of Pure and Applied Chemistry (IUPAC) publication *Quantities, Units and Symbols in Physical Chemistry* (RSC Publishing, Cambridge, United Kingdom, 2007); an abbreviated list is available at http://old.iupac.org/reports/1993/homann/index.html. Thus, a representation of 20,000 cpm on a figure ordinate should be made by the number 20 accompanied by the label kcpm.

When powers of 10 must be used, the journal requires that the exponent power be associated with the number shown. In representing 20,000 cells per ml, the numeral on the ordinate should be "2" and the label should be "10^4 cells per ml" (not "cells per ml $\times$ 10^{-4}"). Likewise, an enzyme activity of 0.06 U/ml might be shown as 6 accompanied by the label "10^{-2} U/ml." The preferred designation is 60 mU/ml (milliunits per milliliter).

Presentation of Nucleic Acid Sequences

Long nucleic acid sequences must be presented as figures in the following format to conserve space. Print the sequence in lines of approximately 100 to 120 nucleotides in a nonproportional (monospace) font that is easily legible when published with a line length of 6 inches (ca. 15.2 cm). If possible, lines of nucleic acid sequence should be further subdivided into blocks of 10 or 20 nucleotides by spaces within the sequence or by marks above it. Uppercase and lowercase letters may be used to designate the exon-intron structure or transcribed regions, etc., if the lowercase letters remain legible at a 6-inch (ca. 15.2-cm) line length. Number the sequence line by line; place numerals representing the first base of each line to the left of the lines. Minimize spacing between lines of sequence, leaving room only for annotation of the sequence. Annotation may include boldface, underlining, brackets, and boxes, etc. Encoded amino acid sequences may be presented, if necessary, immediately above or below the first nucleotide of each codon, by using the single-letter amino acid symbols. Comparisons of multiple nucleic acid sequences should conform as nearly as possible to the same format.

Figure Legends

On initial submission, to assist review, the legend should be incorporated in the image file and appear beneath the figure. At the modification stage, figure legends must be provided as text files separate from the image file.

Legends should provide enough information so that the figure is understandable without frequent reference to the text. However, detailed experimental methods must be described in the Materials and Methods section, not in a figure legend. A method that is unique to one of several experiments may be set forth in a legend only if the description is very brief (one or two sentences). Define all symbols used in the figure and define all abbreviations that are not used in the text.

TABLE 1. Distribution of protein and ATPase in fractions of dialyzed membranes[a]

Membrane	Fraction	ATPase	
		U/mg of protein	Total U
Control	Depleted membrane	0.036	2.3
	Concentrated supernatant	0.134	4.82
E1 treated	Depleted membrane	0.034	1.98
	Concentrated supernatant	0.11	4.6

[a] Specific activities of ATPase of nondepleted membranes from control and treated bacteria were 0.21 and 0.20, respectively.

Tables

Tables that contain artwork, chemical structures, or shading must be submitted as illustrations in an acceptable format at the modification stage. The preferred format for regular tables is Microsoft Word; however, WordPerfect and Acrobat PDF are also acceptable. Note that a straight Excel file is not currently an acceptable format. Excel files must be either embedded in a Word or WordPerfect document or converted to PDF before being uploaded. **If your modified manuscript contains PDF tables and is being submitted in Rapid Review, select "for reviewing purposes only" at the beginning of the file upload process.**

Tables should be formatted as follows. Arrange the data so that **columns of like material read down, not across.** The headings should be sufficiently clear so that the meaning of the data is understandable without reference to the text. See the "Abbreviations" section of these Instructions for those that should be used in tables. Explanatory footnotes are acceptable, but more-extensive table "legends" are not. Footnotes should not include detailed descriptions of the experiment. Tables must include enough information to warrant table format; those with fewer than six pieces of data will be incorporated into the text by the copy editor. Table 1 is an example of a well-constructed table.

Avoid tables (or figures) of raw data on drug susceptibility, therapeutic activity, or toxicity. Such data should be analyzed by an approved procedure, and the results should be presented in tabular form.

NOMENCLATURE

Chemical and Biochemical Nomenclature

The recognized authority for the names of chemical compounds is *Chemical Abstracts* (CAS; http://www.cas.org/) and its indexes. *The Merck Index*, 14th ed. (Merck & Co., Inc., Whitehouse Station, NJ, 2006), is also an excellent source. For guidelines to the use of biochemical terminology, consult *Biochemical Nomenclature and Related Documents* (Portland Press, London, United Kingdom, 1992), available at http://www.chem.qmul.ac.uk/iupac/bibliog/white.html, and the instructions to authors of the *Journal of Biological Chemistry* and the *Archives of Biochemistry and Biophysics* (first issues of each year).

Molecular weight should not be expressed in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name as assigned by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in *Enzyme Nomenclature* (Academic Press, Inc., New York, NY, 1992) and its supplements and at http://www.chem.qmul.ac.uk/iubmb/enzyme/. If a nonrecommended name is used, place the proper (trivial) name in parentheses at first use in the abstract and text. Use the EC number when one has been assigned. Authors of papers describing enzymological studies should review the standards of the STRENDA Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (http://www.beilstein-institut.de/en/projekte/strenda/guidelines/).

Nomenclature of Microorganisms

Binary names, consisting of a generic name and a specific epithet (e.g., *Escherichia coli*), must be used for all microorganisms. Names of categories at or above the genus level may be used alone, but specific and subspecific epithets may not. A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Names of all taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be italicized in the manuscript; strain designations and numbers are not. Vernacular (common) names should be in lowercase roman type (e.g., streptococcus, brucella). For *Salmonella*, genus, species, and subspecies names should be rendered in standard form: *Salmonella enterica* at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. *arizonae* at first use, *S. enterica* subsp. *arizonae* thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar Typhimurium. After the first use, the serovar may also be given without a species name: *Salmonella* Typhimurium, *S.* Typhimurium, or *Salmonella* serovar Typhimurium. For other information regarding serovar designations, see *Antigenic Formulae of the Salmonella Serovars*, 9th ed. (P. A. D. Grimont and F.-X. Weill, WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France, 2007; see http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089). For a summary of the current standards for *Salmonella* nomenclature and the Kaufmann-White criteria, see the article by Brenner et al. (J. Clin. Microbiol. **38:**2465–2467, 2000), the opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes (Int. J. Syst. Evol. Microbiol. **55:** 519–520, 2005), and the article by Tindall et al. (Int. J. Syst. Evol. Microbiol. **55:**521–524, 2005).

The spelling of bacterial names should follow the *Approved Lists of Bacterial Names (Amended) & Index of the Bacterial and Yeast Nomenclatural Changes* (V. B. D. Skerman et al., ed., American Society for Microbiology, Washington, DC, 1989) and the validation lists and notification lists published in the *International Journal of Systematic and Evolutionary Microbiology* (formerly the *International Journal of Systematic Bacteriology*) since January 1989. In addition, two sites on the World Wide Web list current approved bacterial names: Bacterial Nomenclature Up-to-Date (http://www.dsmz.de/microorganisms/main.php?contentleft_id=14) and List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.cict.fr/). If there is reason to use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title and at its first use in the abstract and the text and an appropriate statement concerning the nomenclatural status of the name should be made in the text. "*Candidatus*" species should always be set in quotation marks.

Since the classification of fungi is not complete, it is the responsibility of the author to determine the accepted binomial for a given organism. Sources for these names include *The Yeasts: a Taxonomic Study*, 5th ed. (C. P. Kurtzman, J. W. Fell, and T. Boekhout, ed., Elsevier Science, Amsterdam, Netherlands, 2010), and *Dictionary of the Fungi*, 10th ed. (P. M. Kirk, P. F. Cannon, and J. A. Stalpers, ed., CABI Publishing, Wallingford, Oxfordshire, United Kingdom, 2008); see also http://www.speciesfungorum.org/Names/Fundic.asp.

Names used for viruses should be those approved by the International Committee on Taxonomy of Viruses (ICTV) and reported on the ICTV Virus Taxonomy website (http://www.ictvonline.org/index.asp). In addition, the recommendations of the ICTV regarding the use of species names should generally be followed: when the entire species is discussed as a taxonomic entity, the species name, as with other taxa, is italic and has the first letter and any proper nouns capitalized (e.g., *Tobacco mosaic virus*, *Murray Valley encephalitis virus*). When the behavior or manipulation of individual viruses is discussed, the vernacular (e.g., tobacco mosaic virus, Murray Valley encephalitis virus) should be used. If desired, synonyms may be added parenthetically when the name is first mentioned. Approved generic (or group) and family names may also be used.

Microorganisms, viruses, and plasmids should be given designations consisting of letters and serial numbers. It is generally advisable to include a worker's initials or a descriptive symbol of locale or laboratory, etc., in the designation. Each new strain, mutant, isolate, or derivative should be given a new (serial) designation. This designation should be distinct from those of the genotype and phenotype, and genotypic and phenotypic symbols should not be included. Plasmids are named with a lowercase "p" followed by the designation in uppercase letters and numbers. To avoid the use of the same designation as that of a widely used strain or plasmid, check the designation against a publication database such as Medline.

Genetic Nomenclature

To facilitate accurate communication, **it is important that standard genetic nomenclature be used whenever possible and that deviations or proposals for new naming systems be endorsed by an appropriate authoritative body**. Review and/or publication of submitted manuscripts that contain new or nonstandard nomenclature may be delayed by the editor or the Journals Department so that they may be reviewed by the Genetics and Genomics Committee of the ASM Publications Board.

Before submission of manuscripts, authors may direct questions on genetic nomenclature to the committee's chairperson: Maria Costanzo (maria@genome.stanford.edu). Such a consultation should be mentioned in the manuscript submission letter.

Bacteria. The genetic properties of bacteria are described in terms of phenotypes and genotypes. The phenotype describes the observable properties of an organism. The genotype refers to the genetic constitution of an organism, usually in reference to some standard wild type. The guidelines that follow are based on the recommendations of Demerec et al. (Genetics **54:**61–76, 1966).

(i) Phenotype designations must be used when mutant loci have not been identified or mapped. They can also be used to identify the protein product of a gene, e.g., the OmpA protein. Phenotype designations generally consist of three-letter symbols; these are not italicized, and the first letter of the symbol is capitalized. It is preferable to use Roman or Arabic numerals (instead of letters) to identify a series of related phenotypes. Thus, a series of nucleic acid polymerase mutants might be designated Pol1, Pol2, and Pol3, etc. Wild-type characteristics can be designated with a superscript plus (Pol$^+$), and, when necessary for clarity, negative superscripts (Pol$^-$) can be used to designate mutant characteristics. Lowercase superscript letters may be used to further delineate phenotypes (e.g., Str^r for streptomycin resistance). Phenotype designations should be defined.

(ii) Genotype designations are also indicated by three-letter locus symbols. In contrast to phenotype designations, these are lowercase italic (e.g., *ara his rps*). If several loci govern related functions, these are distinguished by italicized capital letters following the locus symbol (e.g., *araA araB araC*). Promoter, terminator, and operator sites should be indicated as described by Bachmann and Low (Microbiol. Rev. **44:**1–56, 1980): e.g., *lacZp*, *lacAt*, and *lacZo*.

(iii) Wild-type alleles are indicated with a superscript plus (ara^+ his^+). A superscript minus is not used to indicate a mutant locus; thus, one refers to an *ara* mutant rather than an ara^- strain.

(iv) Mutation sites are designated by placing serial isolation numbers (allele numbers) after the locus symbol (e.g., *araA1 araA2*). If only a single such locus exists or if it is not known in which of several related loci the mutation has occurred, a hyphen is used instead of the capital letter (e.g., *ara-23*). It is essential in papers reporting the isolation of new mutants that allele numbers be given to the mutations. For *Escherichia coli*, there is a registry of such numbers: *E. coli* Genetic Stock Center (http://cgsc.biology.yale.edu/). For the genus *Salmonella*, the registry is *Salmonella* Genetic Stock Center (http://people.ucalgary.ca/~kesander/). For the genus *Bacillus*, the registry is *Bacillus* Genetic Stock Center (http://www.bgsc.org/).

(v) The use of superscripts with genotypes (other than + to indicate wild-type alleles) should be avoided. Designations indicating amber mutations (Am), temperature-sensitive mutations (Ts), constitutive mutations (Con), cold-sensitive mutations (Cs), production of a hybrid protein (Hyb), and other important phenotypic properties should follow the allele number [e.g., *araA230*(Am) *hisD21*(Ts)]. All other such designations of phenotype must be defined at the first occurrence. If superscripts must be used, they must be approved by the editor and defined at the first occurrence in the text.

Subscripts may be used in two situations. Subscripts may be used to distinguish between genes (having the same name) from different organisms or strains; e.g., $his_{E.\ coli}$ or $his_{\text{K-12}}$ for the *his* gene of *E. coli* or strain K-12, respectively, may be used to distinguish this gene from the *his* gene in another species or strain. An abbreviation may also be used if it is explained. Similarly, a subscript is also used to distinguish between genetic elements that have the same name. For example, the promoters of the *gln* operon can be designated $glnAp_1$ and $glnAp_2$. This form departs slightly from that recommended by Bachmann and Low (e.g., *desC1p*).

(vi) Deletions are indicated by the symbol Δ placed before the deleted gene or region, e.g., Δ*trpA432*, Δ(*aroP-aceE*)*419*, or Δ(*hisQ-hisJo*)*1256*. Similarly, other symbols can be used (with appropriate definition). Thus, a fusion of the *ara* and *lac* operons can be shown as Φ(*ara-lac*)*95*. Likewise, Φ($araB'$-$lacZ^+$)*96* indicates that the fusion results in a truncated *araB* gene fused to an intact *lacZ* gene, and Φ(*malE-lacZ*)*97*(Hyb) shows that a hybrid protein is synthesized. An inversion is shown as IN(*rrnD-rrnE*)*1*. An insertion of an *E. coli his* gene into plasmid pSC101 at zero kilobases (0 kb) is shown as pSC101 Ω(0kb::K-12*hisB*)*4*. An alternative designation of an insertion can be used in simple cases, e.g., *galT236*::Tn*5*. The number *236* refers to the locus of the insertion, and if the strain carries an additional *gal* mutation, it is listed separately. Additional examples, which utilize a slightly different format, can be found in the papers by Campbell et al. and Novick et al. cited below. It is important in reporting the construction of strains in which a mobile element was inserted and subsequently deleted that this fact be noted in the strain table. This can be done by listing the genotype of the strain used as an intermediate in a table footnote or by making a direct or parenthetical remark in the genotype, e.g., (F$^-$), ΔMu *c*ts, or *mal*::ΔMu *c*ts::*lac*. In setting parenthetical remarks within the genotype or dividing the genotype into constituent elements, parentheses and brackets are used without special meaning; brackets are used outside parentheses. To indicate the presence of an episome, parentheses (or brackets) are used (λ, F^+). Reference to an integrated episome is indicated as described above for inserted elements, and an exogenote is shown as, for example, W3110/F′8(*gal*$^+$).

For information about genetic maps of locus symbols in current use, consult Berlyn (Microbiol. Mol. Biol. Rev. **62:**814–984, 1998) for *E. coli* K-12, Sanderson and Roth (Microbiol. Rev. **52:**485–532, 1988) for *Salmonella* serovar Typhimurium, Holloway et al. (Microbiol. Rev. **43:**73–102, 1979) for the genus *Pseudomonas*, Piggot and Hoch (Microbiol. Rev. **49:**158–179, 1985) for *Bacillus subtilis*, Perkins et al. (Microbiol. Rev. **46:**426–570, 1982) for *Neurospora crassa*, and Mortimer and Schild (Microbiol. Rev. **49:**181–213, 1985) for *Saccharomyces cerevisiae*. For yeasts, *Chlamydomonas* spp., and several fungal species, symbols such as those given in the *Handbook of Microbiology*, 2nd ed. (A. I. Laskin and H. A. Lechevalier, ed., CRC Press, Inc., Cleveland, OH, 1988) should be used.

Conventions for naming genes. It is recommended that (entirely) new genes be given names that are mnemonics of their function, avoiding names that are already assigned and earlier or alternative gene names, irrespective of the bacterium for which such assignments have been made. Similarly, it is recommended that, whenever possible, orthologous genes present in different organisms receive the same name. When homology is not apparent or the function of a new gene has not been established, a provisional name may be given by one of the following methods. (i) The gene may be named on the basis of its map location in the style *yaaA*, analogous to the style used for recording transposon insertions (*zef*) as discussed below. A list of such names in use for *E. coli* has been published by Rudd (Microbiol. Mol. Biol. Rev. **62:**985–1019, 1998). (ii) A provisional name may be given in the style described by Demerec et al. (e.g., *usg*, gene upstream of *folC*). Such names should be unique, and names such as *orf* or *genX* should not be used. For reference, the *E. coli* Genetic Stock Center's database includes an updated listing of *E. coli* gene names and gene products. It is accessible on the Internet (http://cgsc.biology.yale.edu/index.php). A list can also be found in the work of Riley (Microbiol. Rev. **57:**862–952, 1993). For the genes of other bacteria, consult the references given above.

For prokaryotes, gene names should not begin with prefixes indicating the genus and species from which the gene is derived. (However, subscripts may be used where necessary to distinguish between genes from different organisms or strains as described in section v of "Bacteria" above.) For eukaryotes, such prefixes may be used for clarity when discussing genes with the same name from two different organisms (e.g., Sc*URA3* versus Ca*URA3*); the prefixes are not considered part of the gene name proper and are not italicized.

Locus tags. Locus tags are systematic, unique identifiers that are assigned to each gene in GenBank. All genes mentioned in a manuscript should be traceable to their sequences by the reader, and locus tags may be used for this purpose in manuscripts to identify uncharacterized genes. In addition, authors should check GenBank to make sure that they are using the correct, up-to-date format for locus tags (e.g., uppercase versus lowercase letters and the presence or absence of an underscore, etc.). Locus tag formats vary between different organisms and also may be updated for a given organism, so it is important to check GenBank at the time of manuscript preparation.

"Mutant" versus "mutation." Keep in mind the distinction between a mutation (an alteration of the primary sequence of the genetic material) and a mutant (a strain carrying one or more mutations). One may speak about the mapping of a mutation, but one cannot map a mutant. Likewise, a mutant has no genetic locus, only a phenotype.

"Homology" versus "similarity." For use of terms that describe relationships between genes, consult the articles by Theissen (Nature **415:**741, 2002) and Fitch (Trends Genet. **16:**227–231, 2000). "Homology" implies a relationship between genes that have a common evolutionary origin; partial homology is not recognized. When sequence comparisons are discussed, it is more appropriate to use the term "percent sequence similarity" or "percent sequence identity," as appropriate.

Strain designations. Do not use a genotype as a name (e.g., ". . . subsequent use of *leuC6* for transduction . . ."). If a strain designation has not been chosen, select an appropriate word combination (e.g., "another strain containing the *leuC6* mutation").

Viruses. The genetic nomenclature for viruses differs from that for bacteria. In most instances, viruses have no phenotype, since they have no metabolism outside host cells. Therefore, distinctions between phenotype and genotype cannot be made. Superscripts are used to indicate hybrid genomes. Genetic symbols may be one, two, or three letters. For example, a mutant strain of λ might be designated λ *A*am11 *int*2 *red*114 *c*I857; this strain carries mutations in genes *c*I, *int*, and *red* and an amber-suppressible (am) mutation in gene *A*. A strain designated λ att^{434} imm^{21} would represent a hybrid of phage λ that carries the immunity region (*imm*) of phage 21 and the attachment (*att*) region of phage 434. Host DNA insertions into viruses should be delineated by square brackets, and the genetic symbols and designations for such inserted DNA should conform to those used for the host genome. Genetic symbols for phage λ can be found in reports by Szybalski and Szybalski (Gene **7:**217–270, 1979) and Echols and Murialdo (Microbiol. Rev. **42:**577–591, 1978).

Eukaryotes. FlyBase (http://flybase.org/) is the genetic nomenclature authority for *Drosophila melanogaster*. WormBase (http://wormbase.org/) is the genetic nomenclature authority for *Caenorhabditis elegans*. When naming genes for *Aspergillus* species, the nomenclature guidelines posted at http://www.aspergillus.org.uk/indexhome.htm?secure/sequence_info/nomenclature.htm~main should be followed, and the *Aspergillus* Genome Database (http://www.aspgd.org) should be searched to ensure that any new name is not already in use. For information about the genetic nomenclature of other eukaryotes, see the Instructions to Authors for *Eukaryotic Cell* and *Molecular and Cellular Biology.*

Transposable elements, plasmids, and restriction enzymes. Nomenclature of transposable elements (insertion sequences, transposons, and phage Mu, etc.) should follow the recommendations of Campbell et al. (Gene **5:**197–206, 1979), with the modifications given in section vi of "Bacteria," above. The Internet site where insertion sequences of eubacteria and archaea are described and new sequences can be recorded is http://www-is.biotoul.fr/is.html.

The system of designating transposon insertions at sites where there are no known loci, e.g., *zef-123*::Tn*5*, has been described by Chumley et al. (Genetics **91:**639–655, 1979). The nomenclature recommendations of Novick et al. (Bacteriol. Rev. **40:**168–189, 1976) for plasmids and plasmid-specified activities, of Low (Bacteriol. Rev. **36:**587–607, 1972) for F′ factors, and of Roberts et al. (Nucleic Acids Res. **31:**1805–1812, 2003) for restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes should be used whenever possible. The nomenclature for recombinant DNA molecules, constructed *in vitro*, follows the nomenclature for insertions in general. DNA inserted into recombinant DNA molecules should be described by using the gene symbols and conventions for the organism from which the DNA was obtained.

Tetracycline resistance determinants. The nomenclature for tetracycline resistance determinants is based on the proposal of Levy et al. (Antimicrob. Agents Chemother. **43:**1523–1524, 1999). The style for such determinants is, e.g., Tet B; the space helps distinguish the determinant designation from that for phenotypes and proteins (TetB). The above-referenced article also gives the correct format for genes, proteins, and determinants in this family.

ABBREVIATIONS AND CONVENTIONS

Verb Tense

ASM strongly recommends that for clarity you use the **past** tense to narrate particular events in the past, including the procedures, observations, and data of the study that you are reporting. Use the present tense for your own general conclusions, the conclusions of previous researchers, and generally accepted facts. Thus, most of the abstract, Materials and Methods, and Results will be in the past tense, and most of the introduction and some of the Discussion will be in the present tense.

Be aware that it may be necessary to vary the tense in a single sentence. For example, it is correct to say "White (30) demonstrat*ed* that XYZ cells *grow* at pH 6.8," "Figure 2 show*s* that ABC cells fail*ed* to grow at room temperature," and "Air *was* removed from the chamber and the mice *died*, which *proves* that mice *require* air." In reporting statistics and calculations, it is correct to say "The values for the ABC cells *are* statistically significant, indicating that the drug inhibit*ed* . . . ."

For an in-depth discussion of tense in scientific writing, see p. 191–193 in *How To Write and Publish a Scientific Paper*, 6th ed.

Abbreviations

General. Abbreviations should be used as an aid to the reader, rather than as a convenience to the author, and therefore their **use should be limited**. Abbreviations other than those recommended by the IUPAC-IUB (*Biochemical Nomenclature and Related Documents*, 1992) should be used only when a case can be made for necessity, such as in tables and figures.

It is often possible to use pronouns or to paraphrase a long word after its first use (e.g., "the drug" or "the substrate"). Standard chemical symbols and trivial names or their symbols (folate, Ala, and Leu, etc.) may also be used.

Define each abbreviation and introduce it in parentheses the first time it is used; e.g., "cultures were grown in Eagle minimal essential medium (MEM)." Generally, eliminate abbreviations that are not used at least three times in the text (including tables and figure legends).

Not requiring introduction. In addition to abbreviations for Système International d'Unités (SI) units of measurement, other common units (e.g., bp, kb, and Da), and chemical symbols for the elements, the following should be used without definition in the title, abstract, text, figure legends, and tables: DNA (deoxyribonucleic acid); cDNA (complementary DNA); RNA (ribonucleic acid); cRNA (complementary RNA); RNase (ribonuclease); DNase (deoxyribonuclease); rRNA (ribosomal RNA); mRNA (messenger RNA); tRNA (transfer RNA); AMP, ADP, ATP, dAMP, ddATP, and GTP, etc. (for the respective 5′ phosphates of adenosine and other nucleosides) (add 2′-, 3′-, or 5′- when needed for contrast); ATPase and dGTPase, etc. (adenosine triphosphatase and deoxyguanosine triphosphatase, etc.); NAD (nicotinamide adenine dinucleotide); NAD^+ (nicotinamide adenine dinucleotide, oxidized); NADH (nicotinamide adenine dinucleotide, reduced); NADP (nicotinamide adenine dinucleotide phosphate); NADPH (nicotinamide adenine dinucleotide phosphate, reduced); $NADP^+$ (nicotinamide adenine dinucleotide phosphate, oxidized); poly(A) and poly(dT), etc. (polyadenylic acid and polydeoxythymidylic acid, etc.); oligo(dT), etc. (oligodeoxythymidylic acid, etc.); UV (ultraviolet); PFU (plaque-forming units); CFU (colony-forming units); MIC (minimal inhibitory concentration); Tris [tris(hydroxymethyl)aminomethane]; DEAE (diethylaminoethyl); EDTA (ethylenediaminetetraacetic acid); EGTA [ethylene glycolbis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid]; HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid); PCR (polymerase chain reaction); and AIDS (acquired immunodeficiency syndrome). Abbreviations for cell lines (e.g., HeLa) also need not be defined.

The following abbreviations should be used without definition in tables:

amt (amount)	SE (standard error)
approx (approximately)	SEM (standard error of the mean)
avg (average)	sp act (specific activity)
concn (concentration)	sp gr (specific gravity)
diam (diameter)	temp (temperature)
expt (experiment)	tr (trace)
exptl (experimental)	vol (volume)
ht (height)	vs (versus)
mo (month)	wk (week)
mol wt (molecular weight)	wt (weight)
no. (number)	yr (year)
prepn (preparation)	
SD (standard deviation)	

Drugs and pharmaceutical agents. Should an author decide to abbreviate the names of antimicrobial agents in a manuscript, the following standard abbreviations are strongly recommended.

(i) Antibacterial agents. Amikacin, AMK; amoxicillin, AMX; amoxicillin-clavulanic acid, AMC; ampicillin, AMP; ampicillin-sulbactam, SAM; azithromycin, AZM; azlocillin, AZL; aztreonam, ATM; carbenicillin, CAR; cefaclor, CEC; cefadroxil, CFR; cefamandole, FAM; cefazolin, CFZ; cefdinir, CDR; cefditoren, CDN; cefepime, FEP; cefetamet, FET; cefixime, CFM; cefmetazole, CMZ; cefonicid, CID; cefoperazone, CFP; cefotaxime, CTX; cefotetan, CTT; cefoxitin, FOX; cefpodoxime, CPD; cefprozil, CPR; ceftazidime, CAZ; ceftibuten, CTB; ceftizoxime, ZOX; ceftriaxone, CRO; cefuroxime (axetil or sodium), CXM; cephalexin, LEX; cephalothin, CEF; cephapirin, HAP; cephradine, RAD; chloramphenicol, CHL; cinoxacin, CIN; ciprofloxacin, CIP; clarithromycin, CLR; clinafloxacin, CLX; clindamycin, CLI; colistin, CST; daptomycin, DAP; dicloxacillin, DCX; dirithromycin, DTM; doxycycline, DOX; enoxacin, ENX; erythromycin, ERY; fleroxacin, FLE; fosfomycin, FOF; gatifloxacin, GAT; gentamicin, GEN; grepafloxacin, GRX; imipenem, IPM; kanamycin, KAN; levofloxacin, LVX; linezolid, LZD; lomefloxacin, LOM; loracarbef, LOR; meropenem, MEM; methicillin, MET; mezlocillin, MEZ; minocycline, MIN; moxalactam, MOX; moxifloxacin, MXF; nafcillin, NAF; nalidixic acid, NAL; netilmicin, NET; nitrofurantoin, NIT; norfloxacin, NOR; ofloxacin, OFX; oxacillin, OXA; penicillin, PEN; piperacillin, PIP; piperacillin-tazobactam, TZP; polymyxin B, PMB; quinupristin-dalfopristin (Synercid), Q-D; rifabutin, RFB; rifampin, RIF; rifapentine, RFP; sparfloxacin, SPX; spectinomycin, SPT; streptomycin, STR; teicoplanin, TEC; telithromycin, TEL; tetracycline, TET; ticarcillin, TIC; ticarcillin-clavulanic acid, TIM; tigecycline, TGC; tobramycin, TOB; trimethoprim, TMP; trimethoprim-sulfamethoxazole, SXT; trovafloxacin, TVA; and vancomycin, VAN.

(ii) β-Lactamase inhibitors. Clavulanic acid, CLA; sulbactam, SUL; and tazobactam, TZB.

(iii) Antifungal agents. Amphotericin B, AMB; clotrimazole, CLT; flucytosine, 5FC; fluconazole, FLC; itraconazole, ITC; ketoconazole, KTC; nystatin, NYT; terbinafine, TRB; and voriconazole, VRC.

(iv) Antiviral agents. Acyclovir, ACV; cidofovir, CDV; famciclovir, FCV; foscarnet, FOS; ganciclovir, GCV; penciclovir, PCV; valacyclovir, VCV; and zidovudine, AZT.

The use of "nonstandard" abbreviations to designate names of antibiotics and other pharmaceutical agents generally will not be accepted, because the use of different abbreviations for a single agent has often caused confusion. If, on occasion, a nonstandardized abbreviation for a drug or pharmaceutical substance is used, it will be accepted under the following conditions: (i) it must be defined at the first use in the text, (ii) it must be unambiguous in meaning, and (iii) it must contribute to ease of assimilation by readers.

Chemical or generic names of drugs should be used; the use of trade names is not permitted. Avoid the ambiguous term "generation" when classes of drugs are described. When code names or corporate proprietary numbers are to be used, either the chemical structure of the compound or a published literature reference illustrating the chemical structure, if known, must be provided at the first occurrence of the code name or number. For compounds not identified by generic nomenclature, all previous or concurrent identification numbers or appellations should be listed in the manuscript.

Pharmacodynamic terminology. Pharmacodynamic indices (PDIs) must be introduced at their first occurrence in the text and follow guidelines set forth by Mouton et al. (J. Antimicrob. Chemother. **55:**601–607, 2005). In Materials and Methods, it should be clearly stated how the PDIs were derived. The most common indices used are the following: AUC/MIC ratio (the area under the concentration-time curve over 24 h in steady state divided by the MIC), AUIC (the area under the inhibitory curve; note that the AUC/MIC ratio is not equal to the AUIC), $\%T_{\mathrm{MIC}}$ (the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions), C_{max}/MIC ratio (the peak level divided by the MIC), PTA (probability of target attainment), and CFR (cumulative fraction of response). Clear distinction should be made between $\%T_{\mathrm{MIC}}$, which is expressed as a percentage of the dosing interval, and T_{MIC}, expressed in hours. It is strongly recommended that the prefix *f* be used with an index (e.g., *f*AUC) if the free, unbound fraction of the drug is meant.

β-Lactamases

Studies performed to characterize a β-lactamase or the interaction of a compound with a β-lactamase (i.e., as a substrate, inhibitor, or inducer) should follow the guidelines set forth by Bush and Sykes (Antimicrob. Agents Chemother. **30:**6–10, 1986). Assays that measure the hydrolysis of β-lactam antibiotics must be appropriate for the substrate examined (e.g., iodometric methods are not appropriate quantitative assays for substrates whose products are unknown). Reproducibility of results must be shown. When referring to β-lactamases, please use the functional designations defined by Bush et al. (Antimicrob. Agents Chemother. **39:**1211–1233, 1995). Alternatively, if the amino acid sequence for the enzyme is known, the β-lactamases may be described by molecular class as initiated by Ambler (Philos. Trans. R. Soc. Lond. B Biol. Sci. **289:**321–331, 1980).

A database of defining amino acid alterations for many β-lactamases is maintained at the Internet address http://www.lahey.org/studies/. The managers of that site should be consulted about the name of a potentially novel β-lactamase sequence before a new designation or number is proposed for publication.

In Vitro Susceptibility Tests

Tabulate results of determinations of minimal inhibitory and bactericidal concentrations according to the range of concentrations of each antimicrobial agent required to inhibit or kill the members of a species or of each group of microorganisms tested, as well as the corresponding concentrations required to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively) and those required to kill 50 and 90% of the strains (MBC_{50} and MBC_{90}, respectively). The MIC_{50} and MIC_{90} reported should be the actual concentrations tested that inhibited 50 and 90%, respectively, of the strains. They should not be values calculated from the actual data obtained. When only six to nine isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested.

If more than a single drug is studied, insert a column labeled "Test agent" between the columns listing the organisms and the columns containing the numerical data and record data for each agent in the same isolate order. Cumulative displays of MICs or MBCs in tables or figures are acceptable only under unusual circumstances.

The percentage of strains susceptible and/or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by the Clinical and Laboratory Standards Institute, 940 W. Valley Rd., Suite 1400, Wayne, PA 19087-1898. In the absence of approved breakpoints, authors cannot assign breakpoints or use breakpoints from related antibiotics. An exploratory analysis tabulating the percentage of strains inhibited over a range of concentrations is acceptable.

Bactericidal tests must be performed with a sufficient inoculum ($>5 \times 10^5$ CFU/ml) and subculture volume (0.01 ml) to ensure accurate determination of the 99.9% killing endpoint, as described by Pearson et al. (Antimicrob. Agents Chemother. **18:**699–708, 1980) and Taylor et al. (Antimicrob. Agents Chemother. **23:**142–150, 1983). Inoculum size and subculture volume are also critical to studies of combinations of antimicrobial agents.

Synergy is defined in two-dimensional or checkerboard tests when the fractional inhibitory concentration (FIC) or fractional bactericidal concentration (FBC) index (Σ) is ≤ 0.5. In killing curves, synergy is defined as a ≥ 2-$\log_{10}$ decrease in CFU per milliliter between the combination and its most active constituent after 24 h, and the number of surviving organisms in the presence of the combination must be $\geq 2 \log_{10}$ CFU/ml below the starting inoculum. At least one of the drugs must be present in a concentration which does not affect the growth curve of the test organism when used alone. Antagonism is defined by a ΣFIC or ΣFBC of >4.0.

When standard twofold-dilution schemes are used to determine checkerboard interactions, the inherent variability of the method casts doubt on the significance of interactions represented by ΣFICs or ΣFBCs of >0.5 but ≤ 4. Therefore, such interactions, if labeled at all, should be termed "indifferent." Alternatively, indices in this range may be described as "nonsynergistic" or "nonantagonistic," as appropriate. The technically imprecise term "additive" should be avoided as it is too easily misunderstood. See reports by W. R. Greco et al. (Pharmacol. Rev. **47:**331–385, 1995), F. C. Odds (J. Antimicrob. Chemother. **52:**1, 2003), and M. D. Johnson et al. (Antimicrob. Agents Chemother. **48:**693–715, 2004) for further discussion of these issues.

For killing curve tests, the minimal, accurately countable number of CFU per milliliter must be stated and the method used for determining this number must be described. In the absence of any drug and with a sample size of 1 ml, this number is 30 (1.5 in $\log_{10}$) CFU. If procedures for drug inactivation or removal have not been performed, the author must state how drug carryover effects were eliminated or quantified. For drugs showing an inoculum effect, mere dilution below the MIC obtained in standard tests is not sufficient.

Clinical Trials

(i) Criteria for enrollment. The methods used to find and enroll patients and the criteria for enrollment in a clinical trial should be stated. In addition, the time period (month/year to month/year) of the enrollment should be specified. It should be indicated, if appropriate, that written informed consent was obtained and that the trial was approved by the pertinent committee on human subjects.

(ii) Method of randomization. Randomized, double-blind studies are preferred. Comparisons using historical controls are usually regarded as questionable unless the differences in outcome between the groups are dramatic and almost certainly the result of the new intervention. The rationale for the choice of the control group should be explained. The sample size should be justified, and the method of randomization should be stated.

(iii) Criteria for determining whether a case is evaluable. The minimum criteria for evaluability should be stated explicitly. For example, it should be stated that the minimum criterion for evaluability was *a* or the combination of *b* and *c* rather than *a*, *b*, and *c* without designating which were the minimum criteria. The criteria for evaluability are usually different from those for enrollment.

(iv) Reasons for nonevaluability. State the number of patients in each group who were excluded from evaluation and the reason(s) for each exclusion.

(v) Criteria for assessment. Define each outcome for each category of assessment (e.g., "clinical outcomes were classified as cure, improvement, and failure; microbiological outcomes were classified as eradication, persistence, and relapse"). The frequency and timing of such assessments in relation to treatment should be stated. Specify any changes made in the study regimen(s) during the trial; the results for regimens with and without such modification generally should be stated separately. The criteria (questionnaires, results of specific laboratory tests) for evaluation of adverse effects should be stated, as should the period encompassed in the assessment and the time of assessment in relation to the time of treatment (e.g., daily during treatment). Some authors prefer to consider superinfections as failures of treatment, whereas others prefer to consider them separately or even as adverse effects. In any event, the manuscript should state the number of superinfections with each regimen and should differentiate between superinfections and colonization. The duration of follow-up should be mentioned.

(vi) Statistical analyses. The type of statistical test should be stated and, when appropriate, the reason for the choice of test should be given. References should be given for statistical procedures other than the *t* test, chi-square test, and Wilcoxon rank sum test. The comparability of the treatment groups at the baseline should be evaluated statistically.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the article by Olsen (Infect. Immun. **71:**6689–6692, 2003).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J. Virol. **79:**669–676, 2005).

(vii) Beta error. For trials which show no statistically significant difference between regimens, the authors should calculate the probability (β) of a type II error and the power of the study ($1 - \beta$) to detect a specified clinically meaningful difference in efficacy between the regimens. For further details, see the article by Freiman et al. (N. Engl. J. Med. **299:**690–694, 1978). Alternatively, or in addition, the authors should indicate the magnitude of difference between the regimens that could have been detected at a statistically significant level with the number of evaluable patients studied.

For further details, see the editorial on guidelines for clinical trials (Antimicrob. Agents Chemother. **33:**1829–1830, 1989).

Reporting Numerical Data

Standard metric units are used for reporting length, weight, and volume. For these units and for molarity, use the prefixes m, μ, n, and p for 10^{-3}, 10^{-6}, 10^{-9}, and 10^{-12}, respectively. Likewise, use the prefix k for 10^3. Avoid compound prefixes such as mμ or μμ. Use μg/ml or μg/g in place of the ambiguous ppm. Units of temperature are presented as follows: 37°C or 324 K.

When fractions are used to express units such as enzymatic activities, it is preferable to use whole units, such as g or min, in the denominator instead of fractional or multiple units, such as μg or 10 min. For example, "pmol/min" is preferable to "nmol/10 min," and "μmol/g" is preferable to "nmol/μg." It is also preferable that an unambiguous form such as exponential notation be used; for example, "μmol g^{-1} min^{-1}" is preferable to "μmol/g/min." Always report numerical data in the appropriate SI units.

Representation of data as accurate to more than two significant figures must be justified by presentation of appropriate statistical analyses.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the article by Olsen (Infect. Immun. **71:**6689–6692, 2003).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J. Virol. **79:**669–676, 2005).

Isotopically Labeled Compounds

For simple molecules, labeling is indicated in the chemical formula (e.g., $^{14}CO_2$, ^3H_2O, and $H_2{}^{35}SO_4$). Brackets are not used when the isotopic symbol is attached to the name of a compound that in its natural state does not contain the element (e.g., ^{32}S-ATP) or to a word that is not a specific chemical name (e.g., ^{131}I-labeled protein, ^{14}C-amino acids, and ^{3}H-ligands).

For specific chemicals, the symbol for the isotope introduced is placed in square brackets directly preceding the part of the name that describes the labeled entity. Note that configuration symbols and modifiers precede the isotopic symbol. The following examples illustrate correct usage:

[^{14}C]urea	[γ-^{32}P]ATP
L-[*methyl*-^{14}C]methionine	UDP-[U-^{14}C]glucose
[2,3-^{3}H]serine	*E. coli* [^{32}P]DNA
[α-^{14}C]lysine	fructose 1,6-[1-^{32}P]bisphosphate

AAC follows the same conventions for isotopic labeling as the *Journal of Biological Chemistry*, and more-detailed information can be found in the instructions to authors of that journal (first issue of each year).

European Journal of Clinical Pharmacology

Instructions for Authors
Editorials (without abstract)
Letter to the Editor
A commentary or a case report or otherwise a brief communication on a specific topic should have no more than 600 words main text (excepting the reference list) and contain no more than one table or one figure. The letter should not contain an abstract and should not be subdivided into sections.
Review articles
Review articles on various topics are welcome. Both invited and unsolicited submissions are published. The submitted review will be peer-reviewed as other submissions. A word limit is no specified for reviews. The Journal welcomes “full-sized” reviews of up to 4,000 words (main text) as well as “condensed” reviews or “occasional updates” of around 1,000 words.
Reporting Clinical Trials
The Editors believe that it is important to foster a comprehensive, publicly available database of clinical trials. In compliance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), EJCP therefore requires that authors must register clinical trials before the first subject is enrolled. This policy goes into effect on June 1, 2007. Trials that were under way before that date and not registered and that are submitted to EJCP no later than June 1, 2008 will not be forced under the new guideline. For EJCP, clinical trial is defined as any research project that prospectively assigns human subjects to a pharmacological intervention or concurrent comparison or control groups to study the cause-and-effect relationship between this intervention and a health outcome. The ICMJE policies on registration of clinical trials can be found at: http://www.icmje.org/clin_trialup.htm.
EJCP does not advocate one particular registry. Appropriate registries (such as www.clinicaltrials.gov) must be (1) accessible to the public at no charge, (2) open to all prospective registrants, and (3) managed by a not-for-profit organization. There must be a mechanism to ensure the validity of the registration data, and the registry should be electronically searchable. An acceptable registry must include at minimum the data elements available at the ICMJE website listed above. The title page of a manuscript describing the results of a clinical trial must contain the name of the clinical trial registry and registration number of the trial. Any report of a clinical trial not containing such information will be returned to the corresponding author without review.
Reports of randomized, controlled trials should follow the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) statement. See for the current CONSORT guidelines and checklist
http://www.consort-statement.org/statement/revisedstatement.htm.
• Contributions that are part of a Special Issue must include the following footnote on the title page:
"This article is published as part of the Special Issue on [title of the Special Issue]"
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Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.
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Online Submission
Authors should submit their manuscripts online. Electronic submission substantially reduces the editorial processing and reviewing times and shortens overall publication times. Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.
Title Page
The title page should include:
The name(s) of the author(s)

A concise and informative title
The affiliation(s) and address(es) of the author(s)
The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

Purpose (stating the main purposes and research question)
Methods
Results
Conclusions

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes

Text Formatting

Manuscripts should be submitted in Word.
Use a normal, plain font (e.g., 10-point Times Roman) for text.
Use italics for emphasis.
Use the automatic page numbering function to number the pages.
Do not use field functions.
Use tab stops or other commands for indents, not the space bar.
Use the table function, not spreadsheets, to make tables.
Use the equation editor or MathType for equations.
Note: If you use Word 2007, do not create the equations with the default equation editor but use the Microsoft equation editor or MathType instead.
Save your file in doc format. Do not submit docx files.
Word template (zip, 154 kB)
Manuscripts with mathematical content can also be submitted in LaTeX.
LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.
Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data).
Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

Specific remarks

Introduction

This section can be brief and should state the relevant background for and the main purposes of the study reported. Avoid review type introductions.

Terminology

Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention. The proprietary name, chemical composition, and manufacturer should be stated in full in Materials and Methods. If a generic name has not been created or otherwise is not available, the chemical name should be given. Use of an industry code name alone is not sufficient.

SI units

Please always use internationally accepted signs and symbols for units, SI units.

Statistics

Sample size consideration must be given for any clinical study and power calculations are needed for negative results of pivotal variables. This can be done post-hoc if insufficient information was available a priori. Bioequivalence/bioavailability and drug-drug interaction studies should include tests/reference ratios and the respective 90% or 95% confidence intervals.

Analytical methods

Any method used to quantify drug or metabolite concentrations in body fluids should be characterised at least by the following information:
- Range of quantification (defined by an acceptable accuracy/precision and not by a factor above baseline noise),
- accuracy and precision over the entire range of quantification,
- recovery (if applicable) and stability information for the period of measurement.
This information is needed either in the manuscript or must be available in a reference the author provides. Normally the methods should be described in such a detailed way that other researchers will be able to repeat it.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:
1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.
The entries in the list should be numbered consecutively.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal’s name according to the ISSN List of Title Word Abbreviations, see
www.issn.org/2-22661-LTWA-online.php
For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.
EndNote style (zip, 2 kB)
Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer’s LaTeX macro package.

Tables

All tables are to be numbered using Arabic numerals.
Tables should always be cited in text in consecutive numerical order.
For each table, please supply a table caption (title) explaining the components of the table.
Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork

For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

Electronic Figure Submission

Supply all figures electronically.

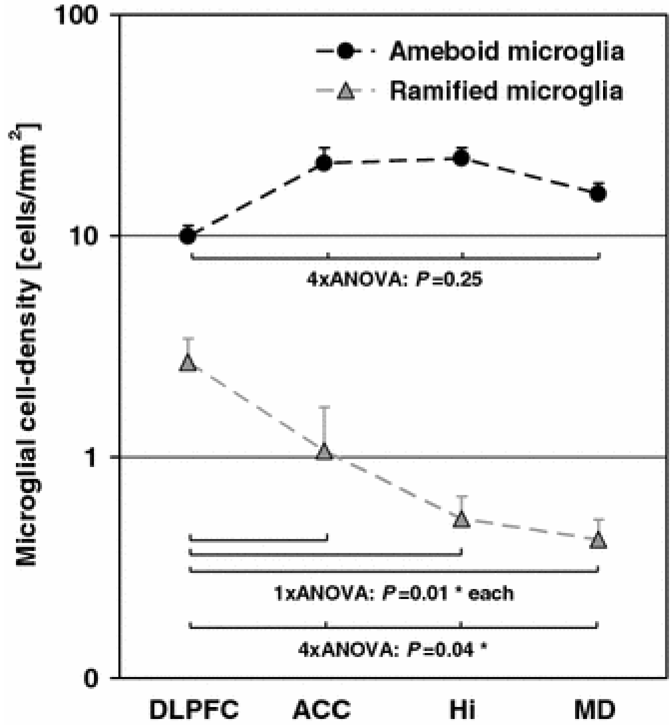
Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MS Office files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



Definition: Black and white graphic with no shading.

Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

Halftones should have a minimum resolution of 300 dpi.

Combination Art

Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color art is free of charge for online publication.

If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.

If the figures will be printed in black and white, do not refer to color in the captions.

Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
Avoid effects such as shading, outline letters, etc.
Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.
Figures should always be cited in text in consecutive numerical order.
Figure parts should be denoted by lowercase letters (a, b, c, etc.).
If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts.
Include the captions in the text file of the manuscript, not in the figure file.
Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

When preparing your figures, size figures to fit in the column width.
For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.
For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

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In order to give people of all abilities and disabilities access to the content of your figures, please make sure that
All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
Patterns are used instead of or in addition to colors for conveying information (color-blind users would then be able to distinguish the visual elements)
Any figure lettering has a contrast ratio of at least 4.5:1

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Supply all supplementary material in standard file formats.
Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
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Audio, Video, and Animations

Always use MPEG-1 (.mpg) format.

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Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
A collection of figures may also be combined in a PDF file.

Spreadsheets

Spreadsheets should be converted to PDF if no interaction with the data is intended.

If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

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Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

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It is possible to collect multiple files in a .zip or .gz file.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.

Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.

Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Transactions of the Royal; Society of Tropical Medicine and Hygiene

General

Authors are advised to consult the **Submission checklist** (below)to guide their writing and submission.
Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its submission and potential publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the copyright-holder.

Authors are advised to consult Appendix 1 **(Special Subject Repositories)** if their funding agency has a public access policy.

Authors are required to consult Appendix 2 **(Proofs)** to ensure that they understand their role in the processing of their manuscript after acceptance.

The detailed requirements for **Manuscripts** are set out in sections 1-6 below.

Submission checklist

It is hoped that this list will be useful during the final checking of an article prior to sending it to the journal's editor for review.

Ensure that the following items are present:

• One author designated as corresponding author
• e-mail address
• Full postal address
• Telephone and fax numbers
• One or more contributors designated as guarantors of the paper

Statements on the following are included:

• Authors' contributions
• Acknowledgements
• Funding
• Conflicts of interest
• Ethical clearance

All necessary files have been uploaded in the following order:

• Manuscript file (containing title page, summary, keywords, the main text of the manuscript, references, tables and figure legends; tables can be attached as separate files if necessary)
• Figure file(s)
• Cover letter
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Manuscript has been 'spellchecked'
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All references mentioned in the Reference list are cited in the text, and vice versa Permission has been obtained for use of copyrighted material from other sources (including the Web)
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Should authors be requested by the Editor to revise the text, the revised version should be submitted within the proscribed period. After this period, the article will be regarded as a new submission.

1. Article content

Original Articles and Short Communications
These provide accounts of original investigations in all aspects of tropical medicine and international health including:
• Chemotherapy and chemoprophylaxis
• Clinical tropical medicine
• Epidemiology
• Infectious diseases
• Immunology and vaccines
• Laboratory studies
• Microbiology and virology
• Noncommunicable and chronic disease
• Parasitology and entomology
• Public health and social medicine
• Qualitative and quantitative studies
Animal studies and in vitro studies will be considered only in so far as the results are directly relevant to human health.

Short Communications
These are similar to original articles but do not include sufficient new information to warrant a full-length article. The Results and Discussion sections can be combined if appropriate.

Leading Articles
These set in context and illustrate the significance of articles published in the Transactions and are usually written as a result of a specific invitation. The Editor may invite Leading Articles on other topics that highlight developments in tropical medicine and international health.

Reviews
These give an authoritative account of an aspect of tropical medicine and international health. The intention is that these reviews will provide the readers with an insight into topics of current interest and to widen the scope of the journal to bring to the attention of readers emerging diseases and other developing aspects of International Health. Reviews do not recapitulate material found in postgraduate textbooks.

Mini-reviews
These do **not** reiterate accepted ideas and information but rather challenge the reader with new thoughts that will stimulate debate; lead to the emergence of new ideas and approaches that may change policy in International Health and Tropical Medicine The purpose of a mini-review may be: -
• To highlight and set in context a recent discovery
• To critically appraise and cast in a new light established information and ideas
• To illustrate how information and ideas established in one place or at one time can be relevant in a new context
• To suggest ways in which insights from other disciplines may be of value in the understanding of International Health and Tropical Medicine
• To show how established policy in International Health and Tropical Medicine may have unintended consequences.

Correspondence
The Transactions accepts correspondence from readers related to published papers and from Society Fellows on other matters of current concern. Authors will be asked to respond to comments on their papers and letters will be published together.

Images
The Transactions will publish images that illustrate all aspects of tropical medicine and international health. An author submitting an image will provide an explanation of its significance. Copyright of the image will become the property of the Transactions and it may be used by the Society as it sees fit. A detailed guide on electronic artwork is available on the website http://www.elsevier.com/artworkinstructions

2. Presentation of manuscript

2.1. General

We expect documents to be prepared in Microsoft (MS) Word. Always keep a backup copy of the electronic file for reference and safety. Save your files using the default extension of Word. Files should not be saved as 'read-only'.

Manuscripts must be written in good English and the spelling should follow that in the Oxford English Dictionaries. Italics should not be used for expressions of Latin origin, for example, in vivo, et al., per se. A single 12-point font should be used for the whole of the manuscript, preferably Arial. The text should be in single-column format and the pages should be numbered consecutively. Double spacing should be used throughout including the references, tables and legends to figures. Punctuation should be consistent and only a single space should be inserted between words and after punctuation. Each new paragraph should be clearly indicated (use two hard returns at the end of each paragraph). The whole text, including headings and references, should be aligned left and ragged right. Formatting should be kept to an absolute minimum as most formatting codes will be removed and replaced on processing the article, in particular, do not use the Word options to hyphenate words. However, do use bold face, italics, subscripts, superscripts, etc. where appropriate. Do not embed 'graphically designed' equations or tables.

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2.2. Title page (should include the following in the order given)

Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

Author names and affiliations. The forename(s) or initial(s) and surname(s) should be included for all the authors. Where the family name may be ambiguous (e.g. a double name), please indicate this clearly. The authors' affiliation addresses (where the actual work was done) should be listed below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name.

Corresponding author. The post-publication corresponding author should be indicated by an asterisk after the author's name and before 'Corresponding author' in the footnote. In the footnote, include the full postal address if it is different from that in the affiliation or the author has more than affiliation, telephone and fax numbers (with country and area code) and e-mail address.

Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Running title. A short informative running title of no more than 50 characters.

2.3. Summary

A concise and factual summary is required (maximum length 200 words). Summaries for short communications, mini-reviews and leading articles are limited to 100 words. Do not use subheadings. The summary should state briefly the purpose of the research, the principal results and major conclusions. A summary is often presented separate from the article, so it must be able to stand alone.

No references should be included in the summary.

Non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the summary.

Keywords
Immediately after the summary, provide six keywords, avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. Authors are recommended to use keywords from the National Library of Medicine's Medical Subject List, wherever possible. The suitability of keywords can be checked on the NLM MeSH Browser at http://www.nlm.nih.gov/mesh/

Choosing keywords in this manner may help increase citation of your paper by making it more readily searchable.

2.4. Arrangement of the article

When appropriate divide your article into clearly defined sections. Mini-reviews and letters are not subdivided. Each subsection should be given a brief heading. Each heading should appear on its own separate line in bold type. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'. The subdivisions set out below relate to original articles, the subdivision of reviews is dictated by the subject matter and will be suggested by the author.

Introduction. State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Experimental/Materials and methods. Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory and/or calculation. A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

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Discussion. This should explore the significance of the results, not repeat them. The limitations of the study should be highlighted where relevant.

The main conclusions of the study should be presented in a short concluding paragraph at the end of the Discussion section.

Declarations. Statements on the authors' contributions, acknowledgements, funding, conflicts of interest and ethical approval must be placed after the Discussion section (see paragraphs below for more detail).

If you have no declaration to make for funding, conflicts of interest and ethical approval please insert the following statements:

Funding: None.
Conflicts of interest: None declared.
Ethical approval: Not required.

Please note the statement that ethical approval is not required, should not reflect the authors' opinion but indicate that advice has been properly sought and that the approval has been deemed unnecessary.

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Responsibility for the accuracy of bibliographic citations lies entirely with the authors. The style of citation and referencing was changed in June 2008. Authors may find it helpful to refer to a copy of the Lancet to familiarize themselves with the new style. This will be similarly helpful to those using a reference manager system.

Citations in the text. Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Unpublished results and personal communications should not be in the reference list, but may be mentioned in the text. Citation of a reference as 'in press' implies that the item has been accepted for publication.

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Examples:

Reference to a journal publication:
1.Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. *J Sci Commun* 2000, **163**: 51-9.

Reference to a book:
2.Strunk Jr W, White EB. *The Elements of Style*. 3rd ed. New York: Macmillan; 1979.

Reference to a chapter in an edited book:
3.Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ. *Introduction to the Electronic Age*. New York: E-Publishing Inc; 1999, p 281-304.

Please note the shortened form of the last page number e.g., 51-9 and that for more than 6 authors the first 6 should be listed followed by 'et al'. For further details you are referred to "Uniform Requirements for Manuscripts submitted to Biomedical Journals" (*J Am Med Assoc* 1997, **277**: 927-34). See also http://www.nlm.nih.gov/tsd/serials/terms_cond.html

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Number tables consecutively in accordance with their appearance in the text. Each table must have a self-explanatory title and abbreviations that are not standard in this field must be defined. Place footnotes to tables below the table body and indicate them with lowercase superscript letters. Avoid vertical and horizontal rules apart from the horizontal rules above and below the Table, and one below the column headings extending over the full width of the Table. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. Tables must be prepared using a spreadsheet or the Tables function of Microsoft Word, i.e. they must be cell based [tabs and hard returns must not be used to separate columns and rows].

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Number figures consecutively in the order in which they are referred to in the text. Subdivided figures should be marked A, B, C, etc. and referred to in the text as 1A, 1B, 1C, etc. Each figure must have a self-explanatory legend which should comprise a brief title and description of the figure. Keep text in the figures themselves to a minimum but explain all symbols and abbreviations used. Figure legends should be listed on a separate page of the end of the manuscript file, not attached to the figure(s).

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See detailed note below.

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Abbreviations. Define abbreviations that are not standard in this field at their first occurrence in both the summary and the main text. Ensure consistency of abbreviations throughout the article.

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Organisms should be referred to by their scientific names according to the Linnaean binomial system.

Italics must be used for generic and specific names and for genes.

Generic names should be given in full and in italics when first used and subsequently abbreviated to a single letter in italics followed by a full stop and a space, e.g. *Plasmodium vivax* and *P. vivax*. The full generic name should always be used at the beginning of a sentence or in a heading or subheading. Use one letter for genus abbreviation except when a two letter abbreviation is needed to avoid confusion, e.g. when *Aedes* and *Anopheles*, are mentioned in same paper. However, when several unusual genera are being discussed with only a few references to each spread throughout the manuscript it is better to use the whole generic name.

Numbers one to nine are spelt unless they are measurements, e.g. 5 mg. Numbers (and units if appropriate) are spelled out if they begin a sentence, e.g. Five microlitres. Large numbers should be set without commas, i.e. 10 000 not 10,000. Decimal points must be indicated by a full point on the line (not commas). Decimal fractions should always be preceded by a zero, e.g. 0.05.

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• Number the illustrations according to their sequence in the text
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The above represents a very brief outline of online submission. It can be advantageous to print this 'Guide for Authors' section from the site for reference in the subsequent stages of article preparation.

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Authors' contributions. The contributions of each author to the study and its publication must be listed (see detailed note above). We suggest the following format (please use initials to refer to each author's contribution): BJA and CJ designed the study protocol; BJA and HGM carried out the clinical assessment; CJ and FT carried out the immunoassays and cytokine determination, and analysis and interpretation of these data. BJA and CJ drafted the manuscript. All authors read and approved the final manuscript. BJA and CJ are guarantors of the paper.

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A competing interest arises when a professional judgment concerning a primary interest (such as the conduct of a trial, a patient's welfare or the validity and interpretation of the research) tends to be unduly influenced by financial gain or other self-interested motive which may be at odds with professional obligations. Authors should disclose at the time of submission information on financial competing interests that may influence the manuscript and summarise these interests under the competing interests declaration in the final manuscript. Authors must declare other interests that could influence the results of the study or the conclusions of the manuscript (e.g. employment, academic links, family relationships, political or social interest group membership, deep personal conviction, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding). For further information, see the web site of the International Committee of Medical Journal Editors at http://www.icmje.org/sponsor.htm.

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Work on human beings that is submitted to Transactions should comply with the principles laid down in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989. The manuscript should contain a statement that the work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and that subjects gave informed consent to the work. Studies involving experiments with animals must state that their care was in accordance with institution guidelines.

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Which trial registries are acceptable to the Transactions?
Acceptable registries must:
• be accessible to the public at no charge
• open to all prospective registrants, i.e. investigators are able to register without restriction by geographic location, academic affiliation, patient demographics, or clinical condition
• managed by a not-for-profit organization
• have a mechanism to ensure the validity of the registration data
• be electronically searchable
• include the required data elements

The following registries have been reviewed by the International Committee of Medical Journal Editors (ICMJE) and met their criteria as of January 2006. These are currently the registries which are acceptable to the Editor of the Transactions. This list will be updated when the ICMJE revises its list of registries in April 2007.
1. http://www.actr.org.au
2. http://www.clinicaltrials.gov
3. http://www.ISRCTN.org
4. http://www.umin.ac.jp/ctr/index/htm
5. http://www.trialregister.nl

The World Health Organization is also working towards the implementation of an international trials registration process. Its most recent statement (May 2006) can be accessed at http://www.who.int/mediacentre/news/releases/2006/pr25/en/index.html

Registration of clinical trials
Publication of the results of trials beginning on or after 1 July 2005 will only be considered if registration occurred before the first patient was enrolled.
What do we do about trials that began before 1 July 2005?

Investigators wishing to publish their work in the Transactions should register trials that began enrolling patients before 1 July 2005 as soon as possible. We will accept retrospective registration of trials that began before 1 July 2005, i.e. registration occurred after patient enrolment began.

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A statement that the material is original and has not already been published
First and last names of all contributing authors (middle names and initials are optional), accompanied by a statement indicating that they have participated in the study and concur with the submission and subsequent revisions submitted by the corresponding author

Each contributing author must sign a copy of the cover letter and send a copy of the signed cover letter to the journal office in one of the following ways:

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The journal allows up to 15 authors per manuscript. Other contributors may be mentioned in the acknowledgments section or in a footnote to the author list. Only those persons listed as authors on the manuscript must submit their signature to the journal.

Manuscript types
Original research papers, clinical case reports, technical reports, comprehensive and authoritative reviews, diagnostic exercises, short reports, and Letters to the Editor written in English will be considered. There is no word limit for original research papers, but all efforts should be made to make the paper as succinct as possible. As with all journals, the editors can recommend that certain parts of the paper be eliminated or incorporated into supplementary information.

The journal is now accepting review articles. Review articles should be 750-1000 words in length, with no more than 15 references. There are no author page charges for review articles.

Images in clinical tropical medicine
The American Journal of Tropical Medicine and Hygiene will publish Images in Clinical Tropical Medicine focused on typical and unusual presentations of tropical diseases--infectious and non- infectious--that occur anywhere in the world, whether in developing countries or in immigrants or returning travelers in industrialized countries. This venue is intended to focus on clinical cases that have visual immediacy and are of clinical interest and importance to our readership.
We will consider original, high-quality images for publication in this special feature, for which page charges will be waived. Material must not have been published or be under consideration for publication elsewhere. Images may appear in the print version of the Journal, the electronic version, or both. Images will be published in black and white in the print version of the journal (unless the author wishes to pay color charges) and in color in the on-line version of the journal. A compendium of Images in Tropical Medicine will be put together on the ASTMH website for members.
Text should be double-spaced in MS Word format, and include a brief title. Up to three authors may be listed. A case may be submitted as an exemplar of a particular aspect of a tropical disease or as an unknown. Submissions should include name, highest academic degree, address, e-mail address, telephone number, and fax number of each author. Text should be no more than 200 words and will be subject to editing and shortening. There may be up to 2 references.
Submissions for this section may be submitted at http://mc.manuscriptcentral.com/ajtmh or may be sent to the managing editor for consideration.

File types
Prepare your manuscript using a word processing program and save it as a .doc or .rtf file. You may also upload .xls or .ppt supplemental files as part of the manuscript submission process. All of these files will be converted to .pdf format. Image files such as .gif, .jpg, .eps, .png, and .tif may be uploaded. These will be converted to small .jpg files.

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A Short Report is preferred for the submission of important preliminary observations, technique modifications or data that simply do not warrant publication as a full paper. Short reports should be approximately 500-1500 words, but must provide adequate information to allow for the same stringent peer review given other submissions. The number of authors, references, and number and size of figures and tables should be limited to the minimum necessary. A brief abstract is required for indexing, but delineated sections, such as Material and Methods, should not be used. Preliminary data published as a short report will not preclude subsequent publication of more complete results if the work is significantly expanded.

Letters to the Editor
Letters to the Editor should not contain unpublished data or material which is being submitted for publication and will not be cited in this Journal, nor should they be cited elsewhere.

Requested and excluded reviewers
The journal's submission site allows authors the opportunity to suggest up to six potential reviewers for their manuscript and up to six reviewers to be excluded from reviewing the manuscript. The Editors strongly suggest that authors use this feature when submitting a manuscript to the journal, as it will serve to expedite the review process.

Spacing
The text should be in 12 point type, fully double-spaced (1? spacing is not acceptable), leaving a margin of 1 inch on all sides. Also double-space table and figure legends, tabular material and references. Number all pages consecutively, starting with the title page.

Illustrations
Graphs, drawings and photographs (please include patients' permission to use their photographs or "blinders" to protect identity) should be numbered and cited in the text. Color illustrations are costly and can be reproduced only at the expense of the author.

Tables
Tables should be on a separate page, serially numbered in Arabic numerals, and cited in the text. Tables should be designed for printing in one-column width, if possible, and should never require more than full-page width.

Equations
While the journal office accepts manuscript submissions in Microsoft Word 2007, we have determined that there are problems associated with equations created by this program's equation builder. Authors using Microsoft Word 2007 must use the original equation editor, which still exists in Word 2007, or they may use the full MathType add-in to create equations. To access Equation Editor 3.0 in Word 2007, click the Insert tab from the ribbon, then click the Object button from the text group. Select Object from the drop-down menu. When the Object Dialog box appears, scroll down and select Microsoft Equation 3.0 and click OK.

Style
Proprietary names of drugs or chemicals may not appear in the title but may be used in conjunction with the generic name where the substance is first mentioned in the abstract, and again where first mentioned in the body of the article. Thereafter, use only the generic name.

The basis for decisions on viral nomenclature is Stedman's ICTV Virus Words. Symbols and common abbreviations should be spelled out the first time they appear in the abstract, the text, figures legends, and tables. Such abbreviations should conform to the AMA Style Manual. The inventing of abbreviations is not encouraged. No sentence may begin with an abbreviation.

Show superscripts, references and subscripts using numbers directly following any punctuation marks. Indicate italics by using italic type, or use the character palette on the Manuscript Central website.

See formatting and style glossary below for more complete information and specific examples of usage.

Ethical guidelines
Experimental investigation papers must state in the Material and Methods section that 1) informed consent was obtained from all human adult participants and from parents or legal guardians of minors, with the name of the appropriate institutional review board having approved the project, and/or 2) the maintenance and care of experimental animals complies with the National Institutes of Health guidelines for the humane use of laboratory animals, or equivalent country authority or agency.

Page charges
Page charges for manuscripts submitted by a Corresponding Author who is not a current member of the American Society of Tropical Medicine and Hygiene are $125 per printed page, or portion thereof. Society members are entitled to a discount and will pay $100 per page. The Journal publishes articles on a pre-paid basis and does not accept institutional or

governmental purchase orders. Page charges are calculated and paid upon receipt of galley proofs.There are no page charges for Letters to the Editor or Book and CD Reviews.

Manuscripts that are clinical in nature may qualify for a partial or full waiver of publication charges. Authors who would like their papers to be considered for a waiver should include this request in their cover letter or in the comments to the Editor upon submission. We do not grant any waivers until a paper is accepted. These funds are limited and are provided by the American Committee on Clinical Tropical Medicine and Travelers' Health (ACCTMTH), the clinical branch of the ASTMH.

Journal policy on open access
Effective July 1, 2008, the journal is allowing authors the option of making their manuscripts freely available online immediately upon publication for an additional fee. Authors may elect to pay a flat fee of $2,500 instead of the usual page charges. This fee does not include the additional charges for printed color figures, which must be paid regardless of whether authors choose the Open Access option. However, authors who elect to pay the Open Access fee may publish their figures in color online at no additional charge. Authors who choose to pay the usual page charges instead of the Open Access fee must honor the journal's twelve-month embargo policy on current content and not deposit their paper in a public repository without permission from the journal office. This policy applies only to papers published in the July 2008 issue or later. Authors may elect this option on the page charge form when they receive their galley proofs, or they may notify the journal office of their decision.

Journal policy on depositing NIH-funded manuscripts in PubMedCentral and other repositories
On January 11, 2008, the National Institutes of Health ("NIH") adopted a revised Public Access Policy for peer-reviewed journal articles reporting research supported in whole or in part by NIH funds. Under the revised policy, the grantee shall ensure that a copy of the author's final manuscript, including any revisions made during the peer review process, is electronically submitted to the National Library of Medicine's PubMed Central ("PMC") archive and that the person submitting the manuscript will designate a time not later than 12 months after publication at which NIH may make the full text of the manuscript publicly accessible in PMC. For more information and to deposit your manuscript in this database, visit http://publicaccess.nih.gov.

All articles published in 2010 or later will be deposited in PubMedCentral by the journal office. Open access articles will be made freely available on PMC at the time of publication. All other articles will be deposited in PMC but will not be available until the 12-month embargo period has expired.

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If the author pays the open access fee, the journal office will deposit the final version directly in PubMedCentral and it will be made freely available at the time of publication. These articles will be licensed using the Creative Commons, Attribution, Non-commercial license which permits others to use, reproduce, disseminate, or display the open access version of this article for non-commercial purposes provided that the original authorship is properly and fully attributed. This policy is fully compliant with the requirements of funders such as the Wellcome Trust, Medical Research Council and HHMI.

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FIRST MANUSCRIPT PAGE

The running heads are flush left. The title, authors, and authors? affiliations are centered. The abstract is flush left, and immediately follows the authors? affiliations with one space between.

Left running head: All capital letters (All Caps); last name of first author plus "and Others" e.g.,
LRH: BOCKARIE AND OTHERS
Right running head: All Caps; this is the running head for your ~50-character short title
e.g.,
RRH: PCR-ELISA FOR THE DETECTION OF W. BANCROFTI

The Title is centered, Roman type (no bold, no italics except for Genus and species, which are italicized.) No punctuation at the end.
e.g.,
Application of a Polymerase Chain Reaction-ELISA to Detect *Wuchereria Bancrofti* in Pools of Wild-Caught *Anopheles Punctulatus* in a Filariasis Control Area in Papua New Guinea

Authors' names are centered. Commas are used throughout except at the end of a line. Do not split a name to the next line. Please be sure to use include the first and last name of each author. Middle names or initials are optional.
e.g.,
Moses J. Bockarie, Peter Fischer, Steven A. Williams, Peter A. Zimmerman, Lysaght Griffin, Michael P. Alpers, and James W. Kazura

Authors' locations/affiliations: departments, institutions, city, state, and/or country are spelled out in full, in italics using Title Case without any numbers. A semi-colon separates each address. Do not split a phrase to the next line. There is no punctuation after the last author?s location. Do not use any symbols such as asterisks after authors? names to refer to the specific affiliations of authors. Just list the institutions in the order that the author is listed. If two authors are at the same place, it is listed only once, in the order of the first author mentioned. If the country is the United States of America, it is not included in the address because AJTMH is published in the USA. Other countries are spelled out in full with no abbreviations.
e.g.,
Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea; Clark Science Center, Department of Biological Sciences, Smith College, Northampton, Massachusetts; Molecular and Cellular Biology Program, University of Massachusetts, Amherst, Massachusetts; Division of Geographic Medicine, Case Western Reserve University School of Medicine, University Hospitals of Cleveland, Cleveland, Ohio

(Note that the authors' addresses, complete with street and room numbers, postal codes, phone, FAX, and e-mail are found only at the end of the text in the Authors? addresses section of the paper, just above the References.)

TEXT

Format
Please provide the following (in order):

1. A concise abstract (150 words maximum)

2. An introductory paragraph
3. Separate sections for Materials and Methods
4. Results
5. Discussion
6. Separate paragraphs for acknowledgments, listing of financial support, all authors' detailed addresses including telephone and FAX numbers, a shipping address for reprints, if reprints are being ordered
7. A list of the references cited.

REFERENCES

References should be cited by consecutive numbers in the text. The numbers should appear in superscripts, not in parentheses, and should appear after any closing punctuation. Abbreviate journal names in the style used by the National Library of Medicine. References should be from peer-reviewed publications that are generally available to the readers of the Journal.

Abstracts, proceedings, works in progress, theses, dissertations, and manuscripts submitted but not yet accepted for publication are not acceptable to cite as references. If it is necessary to cite information from these sources, they should be cited in the text only, in parentheses as follows: (Jamestown JW and others, unpublished data).

Format. All authors must be listed; never use "et al." or the phrase "or others." Authors are indicated by their last names followed by a space and their initial(s) (with no period/full stop). Periods are not used after abbreviated words in journal titles. Authors? names are separated by commas only, and is not used. The year of publication follows the final name, preceded by a comma. Double check all information including the correct abbreviation of the journal cited. Note that the abbreviated journal, the volume number, and the colon that follows are in italics. There is a space after the colon, before the page numbers. The page numbers are written out completely: 472--476 (not 473--76).

See pages 28--51 in the American Medical Association (AMA) Manual of Style (9th Edition) for various types of reference sources so that you can incorporate them and then modify the formatting for AJTMH as follows:

Authors' names: Never use et al. in the references or text. See the following examples:

Examples of articles:
Michaels E, Bunyan DJP, Charlesworth JM Jr, Black JM III, 1997. Global mapping: lymphatic filariasis in perspective. Parasitol Today 11: 472--476.
Examples of books:
Olive EA, 1995. Lymphatic Filariasis Infection and Disease. London: Academic Press, 129-131.
Chapter in a book:
Gilles HM, 1993. Epidemiology of malaria. Gilles HM, Warrell DA, eds. Bruce-Chwatt's Essential Malariology. Third edition. Boston, MA: Edward Arnold, 124-163.
Web reference:
Centers for Disease Control and Prevention, 2008. Advisory Committee on Immunization Practices (ACIP) recommendations. Available at: http://www.cdc.gov/vaccines/pubs/ACIP-list.htm. Accessed May 1, 2008.

At AJTMH, contrary to AMA Style, the year of publication always follows the comma after the last author?s initial(s).

Consult Index Medicus for the correct abbreviation of the journal cited. The journal abbreviation, the volume number, and the colon are all italicized. The colon is followed by a space, then the page numbers. The page numbers are both written in their entirety, separated by an en dash which you represent in your manuscript by two hyphens.

FORMATTING AND STYLE GLOSSARY

Abbreviations and acronyms. The first time it appears, a word or phrase is spelled out in its entirety preceding the abbreviation or acronym which appears in parentheses. The first instance of the acronym is designated in both the abstract and in the text, the first time it appears and for each figure and table, using the acronym subsequently. Try not to use an abbreviation at the beginning of a sentence or as part of a heading.

Plurals of acronyms have no apostrophes. STDs. M & Ms.

Commonly used abbreviations:
Injections. IP = intraperitioneal, IV = intravenous, IM = intramuscular

Commas. Always insert a comma before the "and" at the end of a series of three or more items.

Ethical guidelines. Ethical considerations must be addressed in the materials and methods section. 1) Please state that informed consent was obtained from all human adult participants and from the parents or legal guardians of minors. Include the name of the appropriate institutional review board that approved the project. 2) Indicate in the text that the maintenance and care of experimental animals complies with National Institutes of Health guidelines for the humane use of laboratory animals, or those of your country?s equivalent authority or agency.

Formatting. Use Times New Roman with the font size of 12 throughout. The entire manuscript, including figure legends and tables, should be double spaced (not 1.5 spaces, not a variation such as "equivalent") with one inch margins all around, flush left with a ragged right margin even though when printed in the journal it will be single-spaced and justified?the printer sets all of that up for printing from the manuscript format. Insert only one space between words and sentences, including after a colon (except for the colon after the Acknowledgments, Financial support, Disclaimers, Authors? addresses, and Reprint request sections at the end of the text, just before the REFERENCES).

Headings.
A primary heading is used for the main sections and is centered alone in all capital letters, no bold or italics.
A secondary heading is indented, bold, sentence case, and ends with a period. The text follows on the same line after one space.
A tertiary heading is indented, in sentence case, italicized, and ends with a period.
Sentences and paragraphs. Indent the first sentence of each paragraph. Try not to use an abbreviation at the beginning of a sentence.

Hyphens and dashes.

Dashes. Insert 2 hyphens (--) between numbers or other cases whenever it means "to" as in 4 to 6 years (4--6 years, 1994--1999), The printer will translate -- into an "en dash" [?] which is longer than a hyphen. All you need to do is insert 2 hyphens. The proofreader?s mark for an en dash is - . On the rare occasion that you want to put a long dash between the phrases in a sentence for emphasis, use three hyphens (---) to indicate an "em dash" [?]. There are no spaces before or after the hyphen, the en dash, and the em dash. Do not use a -- for the "to" in ratios or mathematical formulas; they require a virgule or forward slash mark.

Hyphens. Insert a hyphen between words that together modify a noun (T-cell group, but group of T cells) or when an adverb and other word modify a noun.

half-life
antimalarial
Italics. Italicize the words and phrases in your text directly, do not underline. There is no need to both italicize and underline.

Italicize *in vitro, in vivo.*

Nomenclature. Genus and species. Genus is spelled out completely the first time an organism is mentioned in the abstract, the text, and in every figure and table. If you are discussing several different species within a genus, so that the genus is the same for each species mentioned, spell the genus + species out in full the first time each new species is mentioned, even if it seems redundant. After the first time, use the genus abbreviation with a period. Genus and species are always italicized. Do not italicize "spp." or "sensu stricto" or "sensu lato" that may follow genus and species. Genus is italicized when it appears alone (i.e., *Plasmodium* infections. Adjectives such as plasmodial are not italicized.). Species, when used as in the text is not italicized (i.e., falciparum malaria).

Numbers and symbols. Insert one space between the number and the units of measure, no space between the numeral and the % sign. Add a space before and after the≥, ≤, and = symbols.

Parentheses and brackets. Parentheses enclose brackets. In a sentence, the punctuation comes after the close-parentheses symbol.

Quotation Marks. Use quotation marks sparingly, only when absolutely necessary for clarity and to designate a particular, unusual use of a word. When redefining a word, use the quotes only in the first instance in both the text and abstract. Subsequent use does not require quotes, as you have already alerted the reader to make the mental adjustment.

References and citations

Note: Published abstracts; published or unpublished proceedings, works in progress, theses, and dissertations; and manuscripts submitted but not yet accepted for publication are not acceptable references to cite. If it is necessary to cite information from these sources, cite them in the text only, in parentheses as follows: (Jamestown JW and others, unpublished data).

See pp. 28--51 in the AMA Manual of Style [9th Edition] for expressing various sources of reference. Modify the formatting for AJTMH as follows:
Authors' names. Never use et al. in the references or text. All authors must be listed in the following format:
First author?s last name (no comma) single space, his or her initial(s) without periods (no full stops until after the year), comma, single space, next author(s) in the same format until all have been listed. After the final author?s initial(s), comma, single space, insert the year of publication, period (full stop).

Year of publication. At AJTMH, contrary to AMA Style, the year of publication always follows the comma after the last author?s initial(s).

Journal abbreviation. Consult Index Medicus for the correct abbreviation of the journal cited. The journal abbreviation, the volume number, and the colon are all italicized. The colon is followed by a space before the page numbers. The page numbers are both written in their entirety, separated by 2 hyphens (en dash).

Spacing between sentences. Use one space only.

Spelling. Use American spelling except in the references where spelling and punctuation follow the original citation.

Chagas disease. No apostrophe as per the current CDC standard usage. Bed net.

Possum is the correct word for opossum in Australia.

Superscripts and subscripts. Use your software to create a true superscript or subscript. Insert superscripts correctly, after the punctuation with no space in between.2,3,6--8 Here, there are no spaces between the reference number 2, the comma, the 3, or the 6--8 of the superscript. For serial references of 3 or more, insert 2 hyphens between numbers. For example: Other studies reported that opossums usually inspect triatomes both manually and visually before ingesting them.1, 6--8,11 For subscripts follow same procedure, check the Subscript box.

Symbols. A minus sign itself should be used, not a hyphen.

Time. Time of day. Use AM and PM.

Time. sec = second(s), msec = millisecond(s); hr = hour(s); yr = year(s); d = day(s). Plurals of years have no apostrophes. 1940s. 1800s.

Units of measure. Abbreviate in the Methods section, but not in the abstract, introduction and results (unless describing a procedure), and discussion.

Abbreviations.

Weights and measures.
g = gram and is always lower case, mg, µg. kilogram = kg.
L = liter. Use the word ?liter? for liter when it is mentioned alone in the text. Use a capital L for liter in the instance of g/L and in conjunction with m (milliliter = mL), µ (microliter = µL), and d (deciliter = dL).
Note that if designations are followed by numerals or letters, capitalize them: i.e., when particular day(s), week(s), site(s), lane(s), subject(s), group(s) and similar designations are followed by numbers or letters (Day 6; Weeks 1--7. Site 15, Lanes A--D; Subjects A2 and A5, Genotype A). When designating without a following number, use the third day, the fifth week, second subject, etc.

Appendix 2

- **Ethics Approvals**

Ethics letters obtained from the Executive Secretary in electronic format on 7th July 2011. Original signed copies are archived at the Australian Army Malaria Institute, Gallipoli Barrackes, Enoggera, QLD and are available on request.

ADMINISTRATIVE DOCUMENTATION HAS BEEN REMOVED

ADMEC 216/00

A randomised, double-blind, comparative study to evaluate the safety, tolerability and effectiveness of tafenoquine and mefloquine for the prophylaxis of malaria in non-immune Australian soldiers deployed to Timor Leste

ADMEC 165/98

Evaluation of Tafenoquine for the post-exposure prophylaxis of vivax malaria (Southwest Pacific Type) in non-immune Australian soldier

ADMINISTRATIVE DOCUMENTATION HAS BEEN REMOVED

ADMEC 267/01

Treatment of acute vivax malaria with tafenoquine

ADMINISTRATIVE DOCUMENTATION HAS BEEN REMOVED