This file is part of the following reference:


Access to this file is available from:


The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owner of any third party copyright material included in this document. If you believe that this is not the case, please contact ResearchOnline@jcu.edu.au and quote [http://eprints.jcu.edu.au/29749/](http://eprints.jcu.edu.au/29749/).
Tafenoquine in the Prophylaxis and Treatment of Malaria in Australian Defence Force Personnel

Peter Edwin NASVELD
BScMed (Hons) MB BS NSW, GradDipPH Qld, FACTM, FACRRM

Faculty of Medicine, Health and Molecular Sciences
James Cook University
Townsville, Australia

Date: July 2011

A thesis submitted in fulfilment of the requirements of the degree of Doctor of Philosophy within the School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University
STATEMENT OF ACCESS

I, the undersigned, author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

Reproduction of publications in the thesis is subject to the copyright restrictions of the respective publishers of the journal articles.

____________________________  14 July 2011
Signature          Date
STATEMENT OF SOURCES

DECLARATION

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 Information derived from published or unpublished works of others has been acknowledged in the text and a list of references is given.

_________________________________       14 July 2011
Signature       Date
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 consistencty 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.

_________________________________
Signature       Date

14 July 2011
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).

_________________________________       14 July 2011
Signature                                Date
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.
# TABLE OF CONTENTS

STATEMENT OF ACCESS........................................................................................................ii
STATEMENT OF SOURCES................................................................................................... iii
STATEMENT ON THE CONTRIBUTION OF OTHERS.......................................................... iv
DECLARATION ON ETHICS .................................................................................................. vi
ABSTRACT.......................................................................................................................... vii
ACKNOWLEDGEMENTS ...................................................................................................... x
TABLE OF CONTENTS....................................................................................................... xii
LIST OF TABLES ................................................................................................................ xv
LIST OF FIGURES ............................................................................................................. xvii
LIST OF ABBREVIATIONS ............................................................................................... xviii

CHAPTER 1 .................................................................
- Introduction......................................................................................................................1
  1.1 Background.................................................................................................................1
  1.2 Presentation of the research and the thesis ......................................................... 4
  1.3 Context.....................................................................................................................10
  1.4 References.................................................................................................................10

CHAPTER 2 .................................................................
- Field Settings for Tafenoquine Studies: Malaria Considerations ....................... 12
- List of peer reviewed and published papers presented in this chapter................. 12
  2.1 Introduction.................................................................................................................14
  2.2 Study sites.................................................................................................................16
  2.3 Evidence of Malaria Endemicity .......................................................................... 17
  2.4 Current issues with primaquine eradication....................................................... 20
  2.5 Key messages from this chapter........................................................................... 22
  2.6 References.................................................................................................................22

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 .................... 44
- List of Peer-reviewed and published papers presented in this chapter................. 44
  3.1 Introduction.................................................................................................................46
  3.2 Objectives..................................................................................................................46
  3.3 Ethics.........................................................................................................................47
  3.4 Methods....................................................................................................................49
  3.5 Results......................................................................................................................56
  3.6 Discussion.................................................................................................................70
  3.7 Key messages from this chapter........................................................................... 76
  3.8 References.................................................................................................................77

CHAPTER 4 .................................................................

---

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

4.1 Introduction
4.2 Objectives
4.3 Ethics
4.4 Methods
4.5 Results
4.6 Protocol Violation
4.7 Discussion
4.8 Key messages from this chapter
4.9 References

CHAPTER 5
Treatment of acute vivax malaria with tafenoquine

5.1 Introduction
5.2 Objectives
5.3 Ethics
5.4 Methods
5.5 Results
5.6 Discussion
5.7 Key messages from this chapter
5.8 References

CHAPTER 6
General Discussion

6.1 Overview
6.2 Contributions to the understanding of malaria within our immediate area of strategic defence interest
6.3 Longer term chemoprophylaxis with tafenoquine
6.4 Short-term post exposure prophylaxis with tafenoquine
6.5 The treatment of recurrent vivax malaria with tafenoquine
6.6 Specific issues about G6PD deficiency and tafenoquine
6.7 Further directions in research
6.8 References

Appendix 1
Instructions for Authors

Appendix 2
Ethics Approvals

Instructions for Authors
ADF Health Journal
Military Medicine
Medical Journal of Australia
Annals of Tropical Medicine and Parasitology
Antimicrobial Agents and Chemotherapy
European Journal of Clinical Pharmacology
Transactions of the Royal; Society of Tropical Medicine and Hygiene
American Journal of Tropical Medicine and Hygiene

Ethics Approvals
ADMEC 216/00
ADMEC 165/98
LIST OF TABLES

Table 1.1. Bibliographic data for chapters and papers presented in thesis .................... 6
Table 2.1 CMR Reporting Timor Leste and Bougainville till study completion ...... 19
Table 3-1 Outline of study procedures and assessments .............................................. 50
Table 3-2 Demographic characteristics: intent-to-treat and per protocol populations 52
Table 3-3 Prophylactic outcome based on clinical malaria (all species) during prophylactic treatment phase: per protocol population and intent-to-treat population 59
Table 3-4 Prophylactic outcome based on clinical malaria (all species) at any time during the study: per protocol and intent-to-treat populations................................. 60
Table 3-4 Number of subjects with the most frequently reported adverse events during prophylactic phase: safety population .............................................................. 62
Table 3-5 Number of subjects with serious adverse events during the prophylactic and relapse follow-up phase ...................................................................................... 64
Table 3-6 Number of subjects with corneal deposits during the follow-up period .... 66
Table 3-7 Clinical chemistry changes from baseline for bilirubin and creatinine and haematology changes from baseline for haematocrit and platelets: prophylactic phase .................................................................................. 68
Table 4-1 Subject disposition: all study subjects AMI 001, AMI 002, AMI 003 (number of subjects) ............................................................................................................. 115
Table 4-2 Demographic characteristics AMI 001, AMI 002, AMI 003: intention-to-treat population ...................................................................................................................... 116
Table 4-3 Outline of Study Assessments ..................................................................... 118
Table 4-4 Number (%) of subjects with confirmed parasitaemia during the 12 month follow-up period. AMI 001: intention-to-treat population ........................................ 122
Table 4-5 Number (%) of subjects with confirmed parasitaemia during the 12 month follow-up period. AMI 002: intention-to-treat population ........................................ 123
Table 4-6 Number (%) of subjects with confirmed parasitaemia during the 12 month follow-up period. AMI 003: intention-to-treat population ........................................ 124
Table 4-7 Number (%) of subjects with the most frequently reported adverse events related to study treatment: intention-to-treat population ........................................ 126
Table 4-8 Number (%) of subjects with the most frequently reported (>5% in any group) adverse events. Intention-to-treat population ......................................................... 128
Table 4-9 Number (%) of subjects with adverse events by severity AMI 001, AMI 00 and AMI 003: intention-to-treat population ................................................................. 130
Table 4-10 Summary of creatinine changes from baseline AMI 001, AMI 002, AMI 003: intention-to-treat population ..................................................................................... 132
Table 4-11 Number (%) of subjects with significant flagged laboratory values AMI 001: intention-to-treat population ................................................................................. 133
Table 4-12 Number (%) of subjects with significant flagged laboratory values AMI 002: intention-to-treat population ................................................................. 134

Table 4-13 Number (%) of subjects with significant flagged laboratory values AMI 003: intention-to-treat population ................................................................. 135

Table 4-14 Tafenoquine levels in participants experiencing adverse events and parasitaemia ..................................................................................................... 136

Table 4-15 Gender based tafenoquine levels in participants experiencing gastrointestinal adverse events ............................................................................. 137

Table 5-1 Study schedule ...................................................................................... 172

Table 6.1 Creatinine measurements in long term followup ................................ 191
LIST OF FIGURES

Figure 1.1 – Life cycle of the malaria parasite in vector (mosquito) and humans .......2
Figure 1.2 – The structural relationship between primaquine and tafenoquine ...........4
Figure 2.1 – Orientation map of Bougainville ..........................................................16
Figure 2.2 – Orientation map of Timor Leste ............................................................17
Figure 4.1 Case 1 .........................................................................................................138
Figure 4.2 Case 2 .........................................................................................................138
Figure 6.1 Tafenoquine concentrations in subjects receiving all 8 weeks of dosing 179
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Unabridged Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADF</td>
<td>Australian Defence Force</td>
</tr>
<tr>
<td>ADMEC</td>
<td>Australian Defence Medical Ethics Committee</td>
</tr>
<tr>
<td>ADHREC</td>
<td>Australian Defence Human Research Ethics Committee</td>
</tr>
<tr>
<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>ALT (SGPT)</td>
<td>alanine transaminase (serum glutamic-pyruvic transaminase)</td>
</tr>
<tr>
<td>AMI</td>
<td>Australian Army Malaria Institute</td>
</tr>
<tr>
<td>ART</td>
<td>adverse event terminology</td>
</tr>
<tr>
<td>AST (SGOT)</td>
<td>Aspartate transaminase (serum glutamic-oxaloacetic transaminase)</td>
</tr>
<tr>
<td>ATC</td>
<td>Anatomical Therapeutic Chemical</td>
</tr>
<tr>
<td>Bid</td>
<td>twice a day</td>
</tr>
<tr>
<td>B/l</td>
<td>Baseline</td>
</tr>
<tr>
<td>Bd/bid</td>
<td>Twice daily (bis in die)</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CL/F</td>
<td>oral clearance</td>
</tr>
<tr>
<td>Cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CRF</td>
<td>case report form</td>
</tr>
<tr>
<td>CRO</td>
<td>contract research organisation</td>
</tr>
<tr>
<td>D</td>
<td>Day</td>
</tr>
<tr>
<td>dL</td>
<td>decilitre</td>
</tr>
<tr>
<td>DDW</td>
<td>Diseases of the Developing World</td>
</tr>
<tr>
<td>DLCO</td>
<td>diffusion capacity of the lungs to carbon monoxide</td>
</tr>
<tr>
<td>DMPK</td>
<td>Drug Metabolism and Pharmacokinetics</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EU CPMP</td>
<td>European Union Committee for Proprietary Medicinal Products</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>forced expired volume in 1 minute</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose-6-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GCSP</td>
<td>Global Clinical Safety and Pharmacovigilance</td>
</tr>
<tr>
<td>GGT</td>
<td>gamma-glutamyl transferase</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HCG</td>
<td>human chorionic gonadotrophin</td>
</tr>
<tr>
<td>HSRRB</td>
<td>Human Subjects Research Review Board</td>
</tr>
<tr>
<td>ICH</td>
<td>International Committee on Harmonisation</td>
</tr>
<tr>
<td>IDMC</td>
<td>Independent Data Monitoring Committee</td>
</tr>
<tr>
<td>ITT</td>
<td>intent-to-treat</td>
</tr>
<tr>
<td>ka</td>
<td>first-order absorption rate constant</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>Max</td>
<td>Maximum</td>
</tr>
</tbody>
</table>
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.
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 (IC50) 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:
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 prophylactic 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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Indexing</th>
<th>Impact Factor (IF)*</th>
<th>#Other information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 2. Background</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Source</th>
<th>Impact Factor</th>
<th>ERA Category</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>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. (PI; I.F.=2.2; Prov ERA Cat. A)</td>
<td>PubMed</td>
<td>2.2</td>
<td>ERA Category A</td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 4. Evaluation of Tafenoquine for the post-exposure prophylaxis of vivax malaria (Southwest Pacific Type) in non-immune Australian soldiers

<table>
<thead>
<tr>
<th>Section</th>
<th>Authors</th>
<th>Title</th>
<th>Source</th>
<th>PubMed</th>
<th>ERA</th>
<th>Citation</th>
</tr>
</thead>
</table>
**Chapter 5. Treatment of acute vivax malaria with tafenoquine**


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


CHAPTER 2

- Field Settings for Tafenoquine Studies: Malaria Considerations

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


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.


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.


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.

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. vivax 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](image-url)
2.2.2 Timor 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](image.png)

2.3 Evidence 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 (\textit{P. falciparum} and \textit{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 \textit{P. vivax} being most prevalent followed by \textit{P. falciparum}. No cases of \textit{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, \textit{P. vivax} was the most prevalent followed by \textit{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

<table>
<thead>
<tr>
<th>Species</th>
<th>1 January – 30 June 2001</th>
<th>Total at 30 June 2001 – Timor Leste</th>
<th>Total at 30 June 2001 - Bougainville</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum</td>
<td>3</td>
<td>51</td>
<td>3</td>
</tr>
<tr>
<td>P. vivax</td>
<td>7</td>
<td>335</td>
<td>47</td>
</tr>
<tr>
<td>Mixed</td>
<td>-</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Uncertain</td>
<td>-</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>P. malariae</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>409</td>
<td>51</td>
</tr>
</tbody>
</table>

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


Centres for Disease Control and Prevention, Division of Parasitic Diseases and Malaria (DPDM). (2010) Diagnostic Images Database. URL


World Health Organisation. (2011) URL. 

THIS ARTICLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS
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


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.


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.


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.

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

<table>
<thead>
<tr>
<th>Prophylactic Phase</th>
<th>Days</th>
<th>Weeks</th>
<th>Relapse Follow-up Phase</th>
<th>Weeks (after end of prophylactic phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-14</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Eligibility</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical exam</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical history</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECG †</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood smear¶</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematology / Biochemistry</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma drug concentration</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy test</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline signs and symptoms</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concomitant medication</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipidosis assessments †</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methaemoglobin †</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Demographic Characteristic</th>
<th>Intent-to-treat population</th>
<th>Per Protocol population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tafenoquine 200 mg N=492</td>
<td>Mefloquine 250 mg N=162</td>
</tr>
<tr>
<td></td>
<td>Tafenoquine 200 mg N=462</td>
<td>Mefloquine 250 mg N=153</td>
</tr>
<tr>
<td>Gender %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>478 (97.2%)</td>
<td>154 (95.1%)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (2.8%)</td>
<td>8 (4.9%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>286 (58.1%)</td>
<td>97 (59.9%)</td>
</tr>
<tr>
<td>26-35</td>
<td>178 (36.2%)</td>
<td>48 (29.6%)</td>
</tr>
<tr>
<td>36-45</td>
<td>27 (5.5%)</td>
<td>16 (9.9%)</td>
</tr>
<tr>
<td>46-55</td>
<td>1 (0.2%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>mean (SD)</td>
<td>25.4 (5.3)</td>
<td>26.0 (6.5)</td>
</tr>
<tr>
<td>Range</td>
<td>18 – 47</td>
<td>18 – 51</td>
</tr>
<tr>
<td>Race %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>484 (98.4%)</td>
<td>160 (98.8%)</td>
</tr>
<tr>
<td>ATSI*</td>
<td>4 (0.8%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (0.8%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (sd)</td>
<td>80.9 (11.9)</td>
<td>81.3 (12.2)</td>
</tr>
<tr>
<td>Range</td>
<td>50 – 135</td>
<td>53 – 135</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (sd)</td>
<td>177.8 (7.0)</td>
<td>177.1 (6.7)</td>
</tr>
<tr>
<td>Range</td>
<td>155 – 198</td>
<td>157 – 192</td>
</tr>
<tr>
<td>Company</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (Rifle Company)</td>
<td>65 (13.2%)</td>
<td>22 (13.6%)</td>
</tr>
<tr>
<td>B (Rifle Company)</td>
<td>90 (18.3%)</td>
<td>25 (15.4%)</td>
</tr>
<tr>
<td>C (Rifle Company)</td>
<td>67 (13.6%)</td>
<td>21 (13.0%)</td>
</tr>
<tr>
<td>D (Rifle Company)</td>
<td>80 (16.3%)</td>
<td>27 (16.7%)</td>
</tr>
<tr>
<td>E (HQ)</td>
<td>92 (18.7%)</td>
<td>32 (19.8%)</td>
</tr>
<tr>
<td>F (Others)</td>
<td>98 (19.9%)</td>
<td>35 (21.6%)</td>
</tr>
</tbody>
</table>

*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
(\textit{P. falciparum} only and \textit{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).

\textbf{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 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

<table>
<thead>
<tr>
<th>Population</th>
<th>Per protocol population</th>
<th>Intent to treat population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tafenoquine</td>
<td>Mefloquine</td>
</tr>
<tr>
<td>N</td>
<td>462</td>
<td>153</td>
</tr>
<tr>
<td>Prophylactic success (total)</td>
<td>462 (100%)</td>
<td>153 (100%)</td>
</tr>
<tr>
<td>Prophylactic success (known)</td>
<td>462 (100%)</td>
<td>153 (100%)</td>
</tr>
<tr>
<td>Prophylactic success (assumed)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prophylactic failure</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Population</th>
<th>Per protocol population</th>
<th>Intent to treat population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tafenoquine + Placebo</td>
<td>Mefloquine + Primaquine</td>
</tr>
<tr>
<td>Prophylactic success (total)</td>
<td>N=462 (99.1%)</td>
<td>N=153 (99.3%)</td>
</tr>
<tr>
<td>Prophylactic success (known)</td>
<td>458 (99.1%)</td>
<td>152 (99.3%)</td>
</tr>
<tr>
<td>Prophylactic failure</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treatment difference</td>
<td>4 (0.9%)</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td>95% CI</td>
<td>-1.32, 1.74</td>
<td>-1.26, 1.65</td>
</tr>
</tbody>
</table>

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

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

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

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

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.
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 fluorescein 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 fluorescein 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.

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

<table>
<thead>
<tr>
<th></th>
<th>End of Prophylaxis</th>
<th>3 Months*</th>
<th>6 Months*</th>
<th>1 year*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects with vortex keratopathy</td>
<td>69/74 (93.2%)</td>
<td>32/74 (43.2%)</td>
<td>6/74 (8.1%)</td>
<td>0</td>
</tr>
<tr>
<td>No. subjects with vortex keratopathy resolved</td>
<td>37/69 (53.6%)</td>
<td>63/69 (91.3%)</td>
<td>69/69 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

* Timings are approximate
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

<table>
<thead>
<tr>
<th>Bilirubin</th>
<th>Baseline</th>
<th>Days 0-10</th>
<th>2-6 weeks</th>
<th>7-12 weeks</th>
<th>13-21 weeks</th>
<th>22-30 weeks</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tafenoquine (N)</td>
<td>9.0</td>
<td>413</td>
<td>0</td>
<td>3.8</td>
<td>3.0</td>
<td>3.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Mefloquine (N)</td>
<td>9.5</td>
<td>135</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
<td>1.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Creatinine</th>
<th>Mean change from baseline (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tafenoquine (N)</td>
<td>88.7</td>
</tr>
<tr>
<td>Mefloquine (N)</td>
<td>88.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>Baseline</th>
<th>Days 0-10</th>
<th>2-6 weeks</th>
<th>7-12 weeks</th>
<th>13-21 weeks</th>
<th>22-30 weeks</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tafenoquine (N)</td>
<td>48.1</td>
<td>492</td>
<td>-0.6</td>
<td>-4.4</td>
<td>-3.5</td>
<td>-4.1</td>
<td>-2.8</td>
</tr>
<tr>
<td>Mefloquine (N)</td>
<td>47.8</td>
<td>162</td>
<td>0.1</td>
<td>-2.8</td>
<td>-2.4</td>
<td>-4.0</td>
<td>-2.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Baseline</th>
<th>Days 0-10</th>
<th>2-6 weeks</th>
<th>7-12 weeks</th>
<th>13-21 weeks</th>
<th>22-30 weeks</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tafenoquine (N)</td>
<td>263.8</td>
<td>492</td>
<td>5.3</td>
<td>-3.0</td>
<td>-2.9</td>
<td>-7.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Mefloquine (N)</td>
<td>264.5</td>
<td>162</td>
<td>15.4</td>
<td>19.0</td>
<td>19.8</td>
<td>15.3</td>
<td>30.8</td>
</tr>
</tbody>
</table>
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 subpopulation 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-occasion variability (IOV) for clearance. Typical values of the absorption rate constant (ka), oral clearance (CL/F), and central volume of distribution (V/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/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 fluorescein 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 fluorescein 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 \textit{P. falciparum} and \textit{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 \textit{P. vivax} relapse. Subjects in the tafenoquine group received matching placebo, as tafenoquine is proposed to be active against the liver hypnozoites that cause \textit{P. vivax} relapse. The rate of \textit{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 \textit{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


Chapter 3 Paper 3.1

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

Peter E. Nasveld,1,5* Michael D. Edstein,1 Mark Reid,1 Leonard Brennan,1,5 Ivor E. Harris,1 Scott J. Kitchener,1,5 Peter A. Leggat,1 Philip Pickford,2 Caron Kerr,2 Colin Ohrt,3 William Prescott,4 and the Tafenoquine Study Team†

Australian Army Malaria Institute, Brisbane, Queensland, Australia; GlaxoSmithKline Research & Development Limited, Harlow, Essex, United Kingdom; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, Maryland; U.S. Army Medical Material Development Activity, Frederick, Maryland; and Centre for Military and Veterans’ Health, University of Queensland, Herston, Queensland, Australia

Received 16 March 2009/Returned for modification 3 June 2009/Accepted 3 August 2009

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).

* Corresponding author. Mailing address: Centre for Military and Veterans’ Health, Mayne Medical School, University of Queensland, Herston, QLD, Australia 4006. Phone: 61-7-33464870. Fax: 61-7-33464878. E-mail: P.Nasveld@uq.edu.au.
† For a list of Tafenoquine Study Team members, see the Acknowledgments.
‡ Published ahead of print on 7 December 2009.

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

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

\section*{MATERIALS AND METHODS}

\subsection*{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, GrpPD 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.

\subsection*{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 day (for the mefloquine group) or matched placebo twice a day (for the tafenoquine group). 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.

\subsection*{RESULTS}

\subsection*{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.

\subsection*{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 antiplasmodial regimen).
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.

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 3. Adverse events occurring in >5% of subjects on tafenoquine or mefloquine (prophylactic phase)*

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>109 (22)</td>
<td>36 (22)</td>
<td>80 (16)</td>
<td>17 (11)</td>
<td>6 (1)</td>
<td>0</td>
<td>182 (37)</td>
<td>51 (32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>77 (16)</td>
<td>28 (17)</td>
<td>0</td>
<td>2 (1)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>77 (16)</td>
<td>30 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>27 (6)</td>
<td>13 (8)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>28 (6)</td>
<td>13 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>19 (4)</td>
<td>11 (7)</td>
<td>5 (1)</td>
<td>3 (2)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>24 (5)</td>
<td>13 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>19 (4)</td>
<td>8 (5)</td>
<td>2 (&lt;1)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>21 (4)</td>
<td>8 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury</td>
<td>149 (30)</td>
<td>46 (28)</td>
<td>45 (9)</td>
<td>4 (3)</td>
<td>3 (&lt;1)</td>
<td>2 (1)</td>
<td>178 (36)</td>
<td>49 (30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td>65 (13)</td>
<td>24 (15)</td>
<td>12 (2)</td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
<td>74 (15)</td>
<td>26 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>52 (11)</td>
<td>17 (11)</td>
<td>9 (2)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>55 (11)</td>
<td>18 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>URTI</td>
<td>97 (20)</td>
<td>30 (19)</td>
<td>6 (1)</td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
<td>101 (21)</td>
<td>32 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>24 (5)</td>
<td>2 (1)</td>
<td>2 (&lt;1)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>25 (5)</td>
<td>3 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatological</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>70 (14)</td>
<td>20 (12)</td>
<td>1 (&lt;1)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>70 (14)</td>
<td>21 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal dermatitis</td>
<td>43 (9)</td>
<td>8 (5)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>44 (9)</td>
<td>8 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache (constitutional AE)</td>
<td>59 (12)</td>
<td>18 (11)</td>
<td>2 (&lt;1)</td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
<td>61 (12)</td>
<td>20 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral infection</td>
<td>23 (5)</td>
<td>7 (4)</td>
<td>16 (3)</td>
<td>6 (4)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>39 (8)</td>
<td>13 (8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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 fluorescein angiograms were performed on 14 tafenoquine subjects and 4 (23.5%) of 17 mefloquine subjects. Retinal fluorescein 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%)
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 3 [1.9%] 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 4. Neuropsychiatric events in subjects on tafenoquine or mefloquine (prophylactic phase)^a^**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
<th>Tafenoquine</th>
<th>Mefloquine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Mild</td>
<td>Moderate</td>
<td>Total</td>
<td>Total</td>
<td>Total</td>
<td>Total</td>
</tr>
<tr>
<td>Nausea</td>
<td>22 (5)</td>
<td>7 (4)</td>
<td>0</td>
<td>0</td>
<td>22 (5)</td>
<td>8 (5)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>12 (2)</td>
<td>6 (4)</td>
<td>0</td>
<td>0</td>
<td>12 (2)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Abnormal dreams</td>
<td>7 (1)</td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
<td>7 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>5 (1)</td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
<td>5 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>4 (&lt;1)</td>
<td>3 (2)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>5 (1)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Abnormal coordination</td>
<td>2 (&lt;1)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>2 (&lt;1)</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>2 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (&lt;1)</td>
<td>0</td>
</tr>
<tr>
<td>Agitation</td>
<td>2 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (&lt;1)</td>
<td>0</td>
</tr>
<tr>
<td>Euphoria</td>
<td>2 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (&lt;1)</td>
<td>0</td>
</tr>
<tr>
<td>Tremor</td>
<td>2 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (&lt;1)</td>
<td>0</td>
</tr>
<tr>
<td>Depression</td>
<td>0</td>
<td>0</td>
<td>1 (&lt;1)</td>
<td>1 (&lt;1)</td>
<td>1 (&lt;1)</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Paromoria</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (&lt;1)</td>
<td>0</td>
</tr>
<tr>
<td>Amnesia</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (&lt;1)</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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^**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Tafenoquine (n = 492)</th>
<th>Mefloquine (n = 162)</th>
<th>Tafenoquine (n = 492)</th>
<th>Mefloquine (n = 162)</th>
<th>Tafenoquine (n = 492)</th>
<th>Mefloquine (n = 162)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one AE</td>
<td>66 (13.4)</td>
<td>19 (11.7)</td>
<td>22 (5)</td>
<td>8 (5)</td>
<td>40 (11.1)</td>
<td>3 (1.8)</td>
</tr>
<tr>
<td>Nausea</td>
<td>14 (2.8)</td>
<td>4 (2.5)</td>
<td>11 (1.4)</td>
<td>3 (1.9)</td>
<td>10 (1.8)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>Vertigo</td>
<td>10 (2.0)</td>
<td>2 (1.2)</td>
<td>12 (2.4)</td>
<td>4 (2.5)</td>
<td>23 (3.7)</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>9 (1.8)</td>
<td>3 (1.9)</td>
<td>10 (1.4)</td>
<td>2 (1.2)</td>
<td>15 (2.0)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>7 (1.4)</td>
<td>2 (1.2)</td>
<td>10 (1.4)</td>
<td>2 (1.2)</td>
<td>13 (2.0)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>Abnormal dreaming</td>
<td>6 (1.2)</td>
<td>1 (0.6)</td>
<td>14 (2.8)</td>
<td>4 (2.5)</td>
<td>12 (1.8)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>6 (1.2)</td>
<td>1 (0.6)</td>
<td>12 (2.4)</td>
<td>4 (2.5)</td>
<td>12 (1.8)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>Headache</td>
<td>3 (0.6)</td>
<td>2 (1.2)</td>
<td>3 (0.6)</td>
<td>2 (1.2)</td>
<td>6 (0.9)</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>3 (0.6)</td>
<td>2 (1.2)</td>
<td>3 (0.6)</td>
<td>2 (1.2)</td>
<td>6 (0.9)</td>
<td>4 (2.5)</td>
</tr>
</tbody>
</table>

^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 16% (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.

ACKNOWLEDGMENTS

Tafenoquine Study Team members included Karl Rieckmann, Bob Cooper, Stephen Frances, Michael Reid, Alyson Auliff, Bruce Russell, Stephen McLeod-Robertson, John Staley, Kerryn Rowcliffe, John Ross, and Brian Potter from the Australian Army Malaria Institute, Keith Barker and Dominic Galvin from GlaxoSmithKline Research & Development Limited, and Ann Aultman from the U.S. Army Medical Material Development Activity.

We thank John Calagari, Stephen Ferndale, Damien Wood, and officers and soldiers of the 1st Battalion Group, Royal Australian Regiment, East Timor, who participated in the study for their support and cooperation. We are grateful to G. Dennis Shanks and Bob Cooper for commenting on the manuscript.

Financial support was from the U.S. Army Medical Material Development Activity, GlaxoSmithKline Research & Development Limited, and the Australian Defence Force.

P.P. and C.K. are employees of GlaxoSmithKline Research & Development Limited. For all other authors, there are no conflicts.

The opinions expressed are ours and do not necessarily reflect those of the Joint Health Command, Australian Defence Force, the U.S. Army, or any extant defense force policy.

REFERENCES

12. Kolaczinski, J., and J. Webster. 2003. Malaria control in complex emergen-
Chapter 3 Paper 3.2

Population Pharmacokinetics of Tafenoquine during Malaria Prophylaxis in Healthy Subjects

Bruce G. Charles, Ann K. Miller, Peter E. Nasveld, Mark G. Reid, Ivor E. Harris, and Michael D. Edstein.

School of Pharmacy, The University of Queensland, Brisbane, Queensland, Australia; GlaxoSmithKline, Clinical Pharmacokinetics/Modeling and Simulation, King of Prussia, Pennsylvania; and Australian Army Malaria Institute, Brisbane, Queensland, Australia

Received 21 September 2006/Returned for modification 19 February 2007/Accepted 8 May 2007

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/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
were to determine whether individual pharmacokinetic estimates for tafenofluoro would predict prolactinetic outcomes and to investigate if there was any relationship between tafenofluoro 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 tafenofluoro 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.

Tafenofluoro dosing regimen. Following a loading dose regimen of 200 mg tafenofluoro base daily for three consecutive days, the subjects then received an oral weekly maintenance dose of 200 mg tafenofluoro over approximately 6 months. An opaque Swedish Orange size 1 hard gelatin capsule (Capsugel) containing tafenofluoro at 200 mg (pure free base) was used as the dosage form. Subjects were directed to take their tafenofluoro 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 predetermined times after the last loading dose and then at predetermined times at weeks 4, 8, and 16. Samples were collected on predeterimined 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 (precise; trough phase). For example, on week 4, one group of soldiers (about 12 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-loading dose) was collected from the subject body (blood 7 ml) was drawn by venipuncture into EDTA tubes and transported on ice blocks to the field laboratory within 3 h of collection. Whole-blood samples were centrifuged at 1,200 x g for 15 min (Sigma, Quantum, Australia), and plasma was 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. Tafenofluoro was stable under these handling and storage conditions.

Measurement of tafenofluoro. Plasma tafenofluoro concentrations were determined using a validated HPLC method with a triple-quadrupole mass spectrometer. Briefly, plasma (0.05 ml) was spiked with [14H]tafenofluoro 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; Genesil C18 column; 50 mm x 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 1:4 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 tafenofluoro and 469 to 379 m/z for stable-isotope-labeled tafenofluoro. Linear responses in analyte/internal standard peak area ratios were observed for tafenofluoro concentrations ranging from 5 to 500 ng/ml, using a weighted (1/C2) 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 1.0 to 4.4%. The lower level of quantification of the method was 5 ng/ml.

Population pharmacokinetic modeling. The population pharmacokinetics of tafenofluoro 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., to covariables). The influence of mean-centered continuous variables, i.e., age, current weight, and estimated creatinine clearance (CLCr [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 (ΔOFV = 6.0).

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

$$CL_{F} = CL/F = e^{\log CL_{F} + K_{e}}$$

$$V/F = V/F_{0} + K_{e}$$

$$K_{e} = K_{e0} + K_{e1}$$

where $CL_{F}$, $V/F$, and $K_{e}$ represent the true but unknown values of the parameters for the ith subject on the jth occasion about the typical respective population values $CL/F$, $V/F$, and $K_{e}$. The parameters $K_{e0}$, $K_{e1}$, and $K_{e2}$ are random variables distributed with means of 0 and respective variances of $\sigma^{2}_{e}$, $\sigma^{2}_{e2}$, and $\sigma^{2}_{e3}$ (kappa) is a random variable representing the variability of a given pharmacokinetic parameter value on different occasions, with an occasion being defined as a prior 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 $\tau^{2}$. In modeling the IOV, it was assumed that the variance of each parameter was sampled from the same distribution. The residual unexplained variability (RUV) among observed plasma tafenofluoro concentrations and those predicted by the final population model were estimated by a combined proportional plus additive error model, as follows: $C_{p} = C_{obs} + (1 + e_{1})e_{2} + e_{3}$, where $C_{p}$ is the ith observed concentration in the jth subject, $C_{obs}$ is the plasma tafenofluoro concentration predicted by the pharmacokinetic model, and $e_{1}$, $e_{2}$, and $e_{3}$ are randomly distributed variables having mean values of 0 and variances of $\sigma^{2}_{e}$ and $\sigma^{2}_{e2}$, respectively.

Model assessment. The final model was assessed by an inspection of standard diagnostic plots of observed concentration versus population model predicted concentration. Separate plots of weighted residual versus model-predicted concentration, elapsed time, subject identification, and screened covariates (2). A degressive 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 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 tafenofluoro 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 tafenofluoro was judged by the physician to be 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

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameterization</th>
<th>ΔOFV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$CL/F = \theta_1; V/F = \theta_2; K_a = \theta_3$</td>
<td>-2</td>
</tr>
<tr>
<td>2</td>
<td>$CL/F = \theta_1 \cdot (1 + \omega_{CL,F} \cdot \text{age}/25.4); V/F = \theta_2; K_a = \theta_3$</td>
<td>-4</td>
</tr>
<tr>
<td>3</td>
<td>$CL/F = \theta_1; V/F = \theta_2 \cdot (1 + \omega_{CL,F} \cdot \text{age}/25.4); K_a = \theta_3$</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>$CL/F = \theta_1 \cdot (1 + \omega_{CL,F} \cdot CL_{cr}/121); V/F = \theta_2; K_a = \theta_3$</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>$CL/F = \theta_1 \cdot \text{PHOS} + \theta_2 \cdot (1 - \text{PHOS}); V/F = \theta_2; K_a = \theta_3$</td>
<td>-1</td>
</tr>
<tr>
<td>6</td>
<td>$CL/F = \theta_1; V/F = \theta_2 \cdot \text{PHOS} + \theta_3 \cdot (1 - \text{PHOS}); K_a = \theta_3$</td>
<td>-2</td>
</tr>
<tr>
<td>7</td>
<td>$CL/F = \theta_1 \cdot \text{sex} + \theta_2 \cdot (1 - \text{sex}); V/F = \theta_2; K_a = \theta_3$</td>
<td>-12</td>
</tr>
<tr>
<td>8</td>
<td>$CL/F = \theta_1 \cdot V/F = \theta_2 \cdot \text{sex} + \theta_3 \cdot (1 - \text{sex}); K_a = \theta_3$</td>
<td>-39</td>
</tr>
<tr>
<td>9</td>
<td>$CL/F = \theta_1 \cdot (WT/80.9); V/F = \theta_2 \cdot (1 + \omega_{WT,F} \cdot \text{WT}/80.9); K_a = \theta_3$</td>
<td>-39</td>
</tr>
<tr>
<td>10</td>
<td>$CL/F = \theta_1 \cdot (WT/70)^{0.75}; V/F = \theta_2 \cdot (WT/70)^{0.75}; K_a = \theta_3$</td>
<td>-39</td>
</tr>
</tbody>
</table>

* ΔOFV, change in OFV from that of model 1 (OFV = 122.177).
* Rounding errors occurred during fitting.
* Final model.
* aWT/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 = 123.77). 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 $\omega_{CL,F}$ and $\omega_{V/F}$ reduced the OFV from 22,265 to 22,248 compared with the corresponding model when $\omega_{CL,F}$ and $\omega_{V/F}$ 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 $\omega_{V/F}$ was suspiciously low and the correlation coefficient (r) calculated from the diagonal and off-diagonal elements of the variance matrix [r = $\omega_{CL,F,V/F}(\omega_{CL,F} - \omega_{CL,F})^{0.5}$] 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 $T_{max} = \ln(K_e/K_a)/(K_a - K_e)$ 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.](image-url)
TABLE 2. Comparison of parameter estimates for the population model with the results of 200 bootstrapped runs

<table>
<thead>
<tr>
<th>Parameter and model</th>
<th>Final model value</th>
<th>Bootstrap value (n = 200) median [95% CI]**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F (h⁻₁, liters)</td>
<td>0.032</td>
<td>0.242 -2.353</td>
</tr>
<tr>
<td>V/F (h⁻¹, liters)</td>
<td>1.110</td>
<td>0.245 (0.212-0.380)</td>
</tr>
<tr>
<td>Weight centered on CL/F</td>
<td>0.448</td>
<td>0.447 (0.249-0.810)</td>
</tr>
<tr>
<td>Weight centered on V/F</td>
<td>0.713</td>
<td>0.713 (0.371-1.20)</td>
</tr>
<tr>
<td>Variance model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVCL/F (CV%)</td>
<td>18</td>
<td>18 (16-20)</td>
</tr>
<tr>
<td>IVV/F (CV%)</td>
<td>22</td>
<td>22 (20-25)</td>
</tr>
<tr>
<td>IVV (CV%)</td>
<td>76</td>
<td>76 (64-85)</td>
</tr>
<tr>
<td>IOVCL/F (CV%)</td>
<td>18</td>
<td>18 (16-20)</td>
</tr>
<tr>
<td>RUV (CV%)</td>
<td>5.9</td>
<td>5.9 (4.7-7.4)</td>
</tr>
<tr>
<td>RUV (ng/ml)</td>
<td>22.9</td>
<td>23.1 (18.7-26.3)</td>
</tr>
</tbody>
</table>

* CL/F = h⁻¹, V/F, Kₚ, and Kᵦ were all 0.243 h⁻¹. The IV of CL/F, V/F, and Kᵦ 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/kg and 23.7 ± 5.4 liters/kg, respectively. The elimination half-life (t₁/₂), derived from the expression t₁/₂ = 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 tafenoxine 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 t₁/₂ 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 tafenoxine 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, paraesthesia, headache, anorexia, depression, coordination abnormality, appetite increase, and thirst. Tafenoxine 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 tafenoxine 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 tafenoxine 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 tafenoxine, while the single subject with

![FIG. 2. Degenerate visual predictive check of the final population model for tafenoxine. Plots are shown for plasma tafenoxine concentration versus postdose time in sampling windows of (a) week 1 (post-loading dose), (b) week 2, (c) week 4, 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.](image-url)
TABLE 3. Tafenoquine pharmacokinetic data for six subjects reporting at least one adverse effect classified as severe \((n = 1)\) or moderate \((n = 5)\)

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Treatment duration (days)*</th>
<th>Cumulative dose (mg)*</th>
<th>Dosing stopped</th>
<th>CL/F (liters/kg)</th>
<th>V/F (liters/kg)</th>
<th>(t_{1/2}) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe event</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea and/or abdominal pain</td>
<td>2</td>
<td>400</td>
<td>No</td>
<td>*</td>
<td>0.059</td>
<td>24.4</td>
</tr>
<tr>
<td>Moderate events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insomnia</td>
<td>1</td>
<td>200</td>
<td>No</td>
<td>*</td>
<td>0.059</td>
<td>23.2</td>
</tr>
<tr>
<td>Hyperesthesia</td>
<td>12</td>
<td>800</td>
<td>Yes</td>
<td>283</td>
<td>0.046</td>
<td>20.7</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>20</td>
<td>1,000</td>
<td>Yes</td>
<td>253</td>
<td>0.053</td>
<td>27.8</td>
</tr>
<tr>
<td>Depression</td>
<td>24</td>
<td>1,000</td>
<td>Yes</td>
<td>275</td>
<td>0.061</td>
<td>25.1</td>
</tr>
<tr>
<td>Vomiting and/or nausea</td>
<td>3</td>
<td>600</td>
<td>No</td>
<td>315</td>
<td>0.077</td>
<td>26.1</td>
</tr>
</tbody>
</table>

* Number of days from starting dosing until adverse event reported.
* Total amount of drug taken before adverse event reported.

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 \(K_{e}\) 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 \(T_{\text{max}}\) 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\%) \(T_{\text{max}}\) 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 \(T_{\text{max}}\) and its variability were increased by food. Nonetheless, it should be remembered that \(T_{\text{max}}\) 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 IV in the \(T_{\text{max}}\) reflecting the considerable variability in both \(K_{e}\) and \(K_{\text{a}}\), 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 \(K_{e}\). 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 were reasonable. Thus, 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, and 16 during maintenance 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 lipophobic, are slowly absorbed from the gastrointestinal tract, are extensively bound to tissues, and have elimination t1/2 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.

ACKNOWLEDGMENTS

We thank all the Australian soldiers who participated in the phase III study for providing blood samples, the Australian Army Malaria Institute personnel who conducted the clinical trial, and Kym Ward for giving coordination and monitoring support. We are grateful to William Prescott (U.S. Army Medical Materiel Development Activity [USAMMDA]) and Philip Pickford (GlaxoSmithKline) for being the project managers. We acknowledge the support of Thomas Travers in data assessment. We also thank Jurgen Bulitta (IBMP, Nürnberg, Germany) for assistance with the ActivePerl script used for the visual predictive checks.

Financial support for the study was provided by USAMMDA, GlaxoSmithKline, and the Australian Defence Force.

The opinions expressed are those of the authors and do not necessarily reflect those of the Australian Defence Health Service or any extant Australian Defence Force policy.

REFERENCES

sure prophylaxis of vivax malaria in Australian Defence Force personnel.
12. Schlagenhauf, P., R. Steffen, H. Lohel, R. Johnson, R. Letz, A. Tschopp, N.
Vranjes, Y. Bergqvist, O. Ericsson, U. Hellgren, L. Rombo, S. Mannino,
J. Handschin, and D. Sturchler. 1996. Mefloquine tolerability during che-
mphrophylaxis: focus on adverse event assessments, stereochemistry and
J. Horton, and R. Bruckner. 2001. A new primaquine analogue, tafeno-
quine (WR 338005), for prophylaxis against Plasmodium falciparum malaria.
Population pharmacokinetics of mefloquin in patients with acute falcipar-
15. Walsh, S. D., C. Ramthai, T. Sasiapha, S. Sangkharomya, P.
Khaewsathien, P. Suppakun, D. B. Tang, P. Jarasrumsichol, C.
Efficacy of monthly tafenoquine for prophylaxis of Plasmodium vivax
and multidrug-resistant P. falciparum malaria. J. Infect. Dis. 190:1455–
1463.
kineic/pharmacodynamic models using the posterior predictive check. J.
Pharma-
Chapter 3 Paper 3.3

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


   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.


   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.


   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.


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 5\textsuperscript{th} 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 27\textsuperscript{th} 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 19\textsuperscript{th} 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)

<table>
<thead>
<tr>
<th>AMI 001 Population</th>
<th>Treatment Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR</td>
<td>Primaquine 7.5 mg tid</td>
</tr>
<tr>
<td>Screened</td>
<td>3</td>
<td>213</td>
</tr>
<tr>
<td>Met inclusion criteria</td>
<td>0</td>
<td>213</td>
</tr>
<tr>
<td>ITT population</td>
<td>0</td>
<td>210</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>AMI 002 Population</th>
<th>Treatment Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR</td>
<td>Primaquine 7.5 mg tid</td>
</tr>
<tr>
<td>Screened</td>
<td>18</td>
<td>144</td>
</tr>
<tr>
<td>Met inclusion criteria</td>
<td>0</td>
<td>144</td>
</tr>
<tr>
<td>ITT population</td>
<td>0</td>
<td>131</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>AMI 003 Population</th>
<th>Treatment Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR</td>
<td>Primaquine 7.5 mg tid</td>
</tr>
<tr>
<td>Screened</td>
<td>4</td>
<td>158</td>
</tr>
<tr>
<td>Met inclusion criteria</td>
<td>1</td>
<td>158</td>
</tr>
<tr>
<td>ITT population</td>
<td>0</td>
<td>158</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>AMI 001 Demographic Characteristic</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primaquine 7.5 mg tid N=210</td>
</tr>
<tr>
<td>Gender n(%)</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>186 (88.6%)</td>
</tr>
<tr>
<td>female</td>
<td>24 (11.4%)</td>
</tr>
<tr>
<td>Age (years) mean (sd)</td>
<td>30.9 (7.2)</td>
</tr>
<tr>
<td>range</td>
<td>19 – 56</td>
</tr>
<tr>
<td>Weight (kg) mean (sd)</td>
<td>78.8 (11.4)</td>
</tr>
<tr>
<td>range</td>
<td>52.0 – 105.0</td>
</tr>
<tr>
<td>Height (cm) mean (sd)</td>
<td>178.0 (8.3)</td>
</tr>
<tr>
<td>range</td>
<td>152.0 – 198.0</td>
</tr>
<tr>
<td>Body Mass Index mean (sd)</td>
<td>24.8 (2.6)</td>
</tr>
<tr>
<td>range</td>
<td>18.5 – 34.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMI 002 Demographic Characteristic</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primaquine 7.5 mg tid N=131</td>
</tr>
<tr>
<td>Gender n(%)</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>131 (100%)</td>
</tr>
<tr>
<td>Age (years) mean (sd)</td>
<td>25.4 (6.0)</td>
</tr>
<tr>
<td>range</td>
<td>18 – 47</td>
</tr>
<tr>
<td>Weight (kg) mean (sd)</td>
<td>80.2 (10.0)</td>
</tr>
<tr>
<td>range</td>
<td>57.0 – 110.0</td>
</tr>
<tr>
<td>Height (cm) mean (sd)</td>
<td>180.0 (7.2)</td>
</tr>
<tr>
<td>range</td>
<td>156.0 – 196.0</td>
</tr>
<tr>
<td>Body Mass Index mean (sd)</td>
<td>24.7 (2.5)</td>
</tr>
<tr>
<td>range</td>
<td>17.4 – 30.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMI 003 Demographic Characteristic</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primaquine 7.5 mg tid N=158</td>
</tr>
<tr>
<td>Gender n(%)</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>158 (100%)</td>
</tr>
<tr>
<td>female</td>
<td>0</td>
</tr>
<tr>
<td>Age (years) mean (sd)</td>
<td>25.8 (5.7)</td>
</tr>
<tr>
<td>range</td>
<td>18 – 44</td>
</tr>
<tr>
<td>Weight (kg) mean (sd)</td>
<td>79.8 (10.0)</td>
</tr>
<tr>
<td>range</td>
<td>54.0 – 105.0</td>
</tr>
<tr>
<td>Height (cm) mean (sd)</td>
<td>179.8 (7.4)</td>
</tr>
<tr>
<td>range</td>
<td>154.0 – 200.0</td>
</tr>
<tr>
<td>Body Mass Index mean (sd)</td>
<td>24.7 (2.5)</td>
</tr>
<tr>
<td>range</td>
<td>17.4 – 31.4</td>
</tr>
</tbody>
</table>
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

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

(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

<table>
<thead>
<tr>
<th>Parasitaemia</th>
<th>Treatment Group</th>
<th>Primaquine 7.5 mg tid N=210</th>
<th>Tafenoquine 200 mg bid N=86</th>
<th>Tafenoquine 400 mg od N=288</th>
</tr>
</thead>
<tbody>
<tr>
<td>No confirmed parasitaemia</td>
<td></td>
<td>205 (97.6%)</td>
<td>85 (98.8%)</td>
<td>282 (97.9%)</td>
</tr>
<tr>
<td>Confirmed parasitaemia</td>
<td></td>
<td>5 (2.4%)</td>
<td>1 (1.2%)</td>
<td>6 (2.1%)</td>
</tr>
</tbody>
</table>

Treatment Comparison (Tafenoquine – Primaquine)

- 95% CI for comparison: -4.3%, 1.85% to -2.9%, 2.34%
- P-value (chi-square): 0.49955 to 0.82341
- P-value (Fisher’s exact): 0.67581 to 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%) in 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

<table>
<thead>
<tr>
<th>Parasitaemia</th>
<th>Treatment Group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primaquine</td>
<td>Tafenoquine</td>
<td>Tafenoquine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5 mg tid</td>
<td>200 mg bid</td>
<td>400 mg od</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=131</td>
<td>N=75</td>
<td>N=158</td>
<td></td>
</tr>
<tr>
<td>No confirmed parasitaemia</td>
<td>113 (86.3%)</td>
<td>71 (94.7%)</td>
<td>141 (89.2%)</td>
<td></td>
</tr>
<tr>
<td>Confirmed parasitaemia</td>
<td>18 (13.7%)</td>
<td>4 (5.3%)</td>
<td>17 (10.8%)</td>
<td></td>
</tr>
</tbody>
</table>

**Treatment Comparison (Tafenoquine – Primaquine)**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>-17%, -1.2%</th>
<th>-11%, 4.06%</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% CI for comparison</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value (chi-square)</td>
<td></td>
<td>0.04617</td>
<td>0.34993</td>
</tr>
<tr>
<td>P-value (Fisher’s exact)</td>
<td></td>
<td>0.06406</td>
<td>0.37544</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parasitaemia</th>
<th>Treatment Group</th>
<th>Primaquine 7.5 mg tid N=158</th>
<th>Tafenoquine 200 mg od N=406</th>
</tr>
</thead>
<tbody>
<tr>
<td>No confirmed parasitaemia</td>
<td></td>
<td>151 (95.6%)</td>
<td>386 (95.1%)</td>
</tr>
<tr>
<td>Confirmed parasitaemia</td>
<td></td>
<td>7 (4.4%)</td>
<td>20 (4.9%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>AMI 001</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse event (Verbatim Term)</td>
<td>Primaquine 7.5 mg tid N=210</td>
</tr>
<tr>
<td>Related to Study Treatment</td>
<td></td>
</tr>
<tr>
<td>Suspected Relationship to Treatment</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>27 (12.6%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5 (2.4%)</td>
</tr>
<tr>
<td>Headache*</td>
<td>7 (3.3%)</td>
</tr>
<tr>
<td>Oesophageal reflux</td>
<td>6 (2.9%)</td>
</tr>
<tr>
<td>Abdominal pain*</td>
<td>6 (2.9%)</td>
</tr>
</tbody>
</table>

| 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

<table>
<thead>
<tr>
<th>AMI 002</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse event (Verbatim Term)</td>
<td>Primaquine 7.5 mg tid N=131</td>
</tr>
<tr>
<td>Related to Study Treatment</td>
<td></td>
</tr>
<tr>
<td>Suspected Relationship to Treatment</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>7 (5.3%)</td>
</tr>
<tr>
<td>Abdominal pain*</td>
<td>3 (2.3%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3 (2.3%)</td>
</tr>
</tbody>
</table>

| Probable Relationship to Treatment | | | |
| None | | | |

* Abdominal pain consists of the verbatim term abdo pain.
* ≥5% of subjects in any treatment group

<table>
<thead>
<tr>
<th>AMI 003</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse event (Verbatim Term)</td>
<td>Primaquine 7.5 mg tid N=158</td>
</tr>
<tr>
<td>Related to Study Treatment</td>
<td></td>
</tr>
<tr>
<td>Suspected Relationship to Treatment</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>10 (6.3%)</td>
</tr>
<tr>
<td>Abdominal cramps*</td>
<td>1 (0.6%)</td>
</tr>
</tbody>
</table>

| 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

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

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

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

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

---

128
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

<table>
<thead>
<tr>
<th>AMI 001</th>
<th>Severity of Adverse Experiences</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primaquine 7.5 mg tid N=210</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>78 (37.1%)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>7 (3.3%)</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMI 002</th>
<th>Severity of Adverse Experiences</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primaquine 7.5 mg tid N=131</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>16 (12.2%)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMI 003</th>
<th>Severity of Adverse Experiences</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primaquine 7.5 mg tid N=158</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>23 (14.6%)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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

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

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

<table>
<thead>
<tr>
<th>AMI 003</th>
<th>Treatment Group</th>
<th>Creatinine change from baseline µmol/L</th>
<th>Primaquine 7.5 mg tid N=3</th>
<th>Tafenoquine 200 mg od N=395</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>-6.7</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>min</td>
<td>-30</td>
<td>-20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>max</td>
<td>30</td>
<td>50</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Laboratory Variable</th>
<th>Treatment Group</th>
<th>Primaquine 7.5 mg tid N=210</th>
<th>Tafenoquine 200 mg bid N=86</th>
<th>Tafenoquine 400 mg od N=242</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>B/l: low</td>
<td>0</td>
<td>149</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: low</td>
<td>1 (0.5%)</td>
<td>199</td>
<td>0</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>B/l: low</td>
<td>0</td>
<td>149</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: low</td>
<td>1 (0.5%)</td>
<td>199</td>
<td>0</td>
</tr>
<tr>
<td>WBC</td>
<td>B/l: high</td>
<td>0</td>
<td>147</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: high</td>
<td>2 (1.0%)</td>
<td>199</td>
<td>0</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>B/l: low</td>
<td>0</td>
<td>147</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: low</td>
<td>1 (0.5%)</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B/l: high</td>
<td>0</td>
<td>147</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: high</td>
<td>3 (1.5%)</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes + Monocytes</td>
<td>B/l: high</td>
<td>0</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: high</td>
<td>0</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td>MCHC</td>
<td>B/l: low</td>
<td>0</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: low</td>
<td>0</td>
<td>199</td>
<td>0</td>
</tr>
<tr>
<td>Platelets</td>
<td>B/l: low</td>
<td>0</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: low</td>
<td>0</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B/l: high</td>
<td>0</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: high</td>
<td>0</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td>Clinical Chemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>B/l: high</td>
<td>0</td>
<td>149</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: high</td>
<td>0</td>
<td>199</td>
<td>0</td>
</tr>
<tr>
<td>AST</td>
<td>B/l: high</td>
<td>1 (0.7%)</td>
<td>149</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PD: high</td>
<td>1 (0.5%)</td>
<td>198</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>B/l: high</td>
<td>1 (0.7%)</td>
<td>149</td>
<td>2 (2.4%)</td>
</tr>
<tr>
<td></td>
<td>PD: high</td>
<td>0</td>
<td>199</td>
<td>1 (1.2%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Laboratory Variable</th>
<th>Treatment Group</th>
<th>N=131</th>
<th>N=75</th>
<th>N=158</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primaquine 7.5 mg tid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tafenoquine 200 mg bid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tafenoquine 400 mg od</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>PD: high</td>
<td>0</td>
<td>13</td>
<td>1 (1.4%)</td>
<td>72</td>
</tr>
<tr>
<td>Haematocrit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>12</td>
<td>1 (1.4%)</td>
<td>72</td>
</tr>
<tr>
<td>PD: high</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td><strong>WBC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>12</td>
<td>1 (1.4%)</td>
<td>72</td>
</tr>
<tr>
<td>PD: high</td>
<td>0</td>
<td>13</td>
<td>1 (1.4%)</td>
<td>71</td>
</tr>
<tr>
<td><strong>Granulocytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>PD: high</td>
<td>0</td>
<td>8</td>
<td>1 (7.1%)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>PD: high</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td><strong>Clinical Chemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>PD: high</td>
<td>2 (1.8%)</td>
<td>109</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>PD: high</td>
<td>2 (1.8%)</td>
<td>109</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>GGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>PD: high</td>
<td>1 (0.9%)</td>
<td>109</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>PD: high</td>
<td>1 (0.9%)</td>
<td>109</td>
<td>0</td>
<td>71</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Laboratory Variable</th>
<th>Treatment Group</th>
<th>Primaquine 7.5 mg tid N=158</th>
<th>Tafenoquine 200 mg od N=406</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: low</td>
<td></td>
<td>1 (0.6%)</td>
<td>0 (0.2%)</td>
</tr>
<tr>
<td>PD: low</td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>B/l: high</td>
<td></td>
<td>0</td>
<td>158</td>
</tr>
<tr>
<td>PD: high</td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>WBC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td></td>
<td>0</td>
<td>158</td>
</tr>
<tr>
<td>PD: high</td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: low</td>
<td></td>
<td>0</td>
<td>158</td>
</tr>
<tr>
<td>PD: low</td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>B/l: high</td>
<td></td>
<td>0</td>
<td>158</td>
</tr>
<tr>
<td>PD: high</td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: low</td>
<td></td>
<td>0</td>
<td>158</td>
</tr>
<tr>
<td>PD: low</td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>B/l: high</td>
<td></td>
<td>0</td>
<td>158</td>
</tr>
<tr>
<td>PD: high</td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Clinical Chemistry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td></td>
<td>0</td>
<td>156</td>
</tr>
<tr>
<td>PD: high</td>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/l: high</td>
<td></td>
<td>1 (0.6%)</td>
<td>156</td>
</tr>
<tr>
<td>PD: high</td>
<td></td>
<td>3</td>
<td>4 (1.0%)</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single dose (n = 76)</td>
<td>Split dose (n = 73)</td>
</tr>
<tr>
<td>All participants</td>
<td>563 ± 110 (n = 67)</td>
<td>601 ± 114 (n = 58)</td>
</tr>
<tr>
<td>Without GI AE</td>
<td>542 ± 116 (n = 33)</td>
<td>589 ± 107 (n = 34)</td>
</tr>
<tr>
<td>With GI AE</td>
<td>584 ± 101 (n = 34)</td>
<td>619 ± 123 (n = 24)</td>
</tr>
</tbody>
</table>

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 4.1 Parasitaemia and tafenoquine drug levels for case 1](image)

**Figure 4.2** Parasitaemia and tafenoquine drug levels for case 2

![Figure 4.2 Parasitaemia and tafenoquine drug levels for case 2](image)

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.

4.9 References


Rieckmann KH, Yeo AET, Davis DR, Hutton DC, Wheatley PF, Simpson R. (1993). Recent military experience with malaria chemoprophylaxis. MJA. 158:446-449


Chapter 4 Paper 4.1

Chapter 4 Paper 4.2


THIS ARTICLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS
Chapter 4 Paper 4.4

CHAPTER 5

Treatment of acute vivax malaria with tafenoquine

List of peer reviewed and published papers presented in this chapter


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.
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

<table>
<thead>
<tr>
<th></th>
<th>Clinical assessment</th>
<th>Blood film</th>
<th>Full Blood Count*</th>
<th>GGT, AST, ALT</th>
<th>Blood sample for TQ levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>At diagnosis</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>24 hrs post last dose CQ</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>12 hours post third dose TQ</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2 hours prior to week 2 TQ dose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>12 hours after week 4 TQ dose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2 hours prior to week 6 TQ dose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>12 hours after week 8 TQ dose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* 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.

5.4.3.1 Compliance 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](image-url)
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


Chapter 5 Paper 5.1


THIS ARTICLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS
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 \( \geq 0.02 \text{ mmol/L} \) 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 urinalysis. 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

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

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 explore the 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 on 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 an adult 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 a suitable candidate given it can be given over three days as a treatment / eradicant and is generally well tolerated. Obviously much needs to be 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


Appendix 1

- Instructions for Authors
ADMINISTRATIVE DOCUMENTATION HAS BEEN REMOVED
Antimicrobial Agents and Chemotherapy
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

2011 INSTRUCTIONS TO AUTHORS

SCOPE

Antimicrobial Agents and Chemotherapy (AAC) is an interdisciplinary journal devoted to the dissemination of knowledge relating to all aspects of antimicrobial and antiparasitic agents and chemotherapy. Within the circumscriptions set forth below, any report involving studies of or with antimicrobial, antiviral (including antiretroviral), antifungal, or antiparasitic agents as these relate to human disease is within the purview of AAC. Studies involving animal models, pharmacological characterization, and clinical trials are appropriate for consideration.

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.

(i) Papers which describe the use of antimicrobial agents as tools for elucidating the basic biological processes of bacteria are considered more appropriate for the Journal of Bacteriology.

(ii) Manuscripts that (a) describe the use of antimicrobial or antiparasitic agents as tools in the isolation, identification, or epidemiology of microorganisms associated with disease; (b) are concerned with quality control procedures for diffusion, elution, or dilution tests for determining susceptibilities to antimicrobial agents in clinical laboratories; and (c) deal with applications of commercially prepared tests or kits to assays performed in clinical laboratories to measure the activities of established antimicrobial agents or their concentrations in body fluids are considered more appropriate for the Journal of Clinical Microbiology. Manuscripts concerned with the development or modification of assay methods (e.g., plasma antimicrobial concentrations and high-throughput screening techniques, etc.) and validation of their sensitivity and specificity with a sufficiently large number of determinations or compounds are considered appropriate for AAC.

(iii) Manuscripts describing new or novel methods or improvements in media and culture conditions will not be considered for publication in AAC unless these methods are applied to the study of problems related to the production or activity of antimicrobial agents. Such manuscripts are more appropriate for Applied and Environmental Microbiology or the Journal of Clinical Microbiology.

(iv) Manuscripts dealing with properties of unpurified natural products, with entities that are primarily antitumor agents, or with immunomodulatory agents that are not antimicrobial agents are not appropriate for AAC.

(v) Manuscripts dealing with novel small molecular antimicrobials must provide at least some data showing that the proposed new agents or scaffolds have the potential to become therapeutic agents. Appropriate demonstrations will vary but generally should be some combination of data on physical properties (solubility, protein binding, log P [logarithm of the ratio of the concentrations of un-ionized solute in solvents]), pharmacological properties (Caco2 predictions of bioavailability, pharmacokinetics in an animal species), or tolerability (mammalian cell toxicity, likelihood of hepatic metabolism, potential for receptor interactions, potential for human ERG liability). Initial presentations of compounds are not expected to address all these areas but rather to show an appropriate initial subset. For example, the first publication of a novel compound or compound series might address selected physical properties plus mammalian cell toxicity. Subsequent publications are expected to add progressively to the proof of the agent’s therapeutic potential.

(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.

(vii) 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/).

(viii) A manuscript limited to the nucleic acid sequence of a gene encoding an antibiotic target, receptor, or resistance mechanism may be submitted as a short-form paper (see “Short-Form Papers”) or a New-Data Letter to the Editor (see “Letters to the Editor”), depending on its length. Formatting instructions for nucleic acid sequences are given below (see “Presentation of Nucleic Acid Sequences”). Repetition of sequences already in a database should be avoided.

Questions about these guidelines may be directed to the editor in chief of the journal being considered.

If transfer to another ASM journal is recommended by an editor, the corresponding author will be contacted.

Note that a manuscript rejected by one ASM journal on scientific grounds or on the basis of its general suitability for publication is considered rejected by all other ASM journals.

*Instructions to Authors are published annually in the January issue. A separate html version, which is updated throughout the year, is at http://aac.asm.org/misc/ifora.dtl.
EDITORIAL POLICY

Use of Microbiological Information

The Council Policy Committee (CPC) of the American Society for Microbiology affirms the long-standing position of the Society that microbiologists will work for the proper and beneficial application of science and will call to the attention of the public or the appropriate authorities misuses of microbiology or of information derived from microbiology. ASM members are obligated to discourage any use of microbiology contrary to the welfare of humankind, including the use of microbes as biological weapons. Bioterrorism violates the fundamental principles expressed in the Code of Ethics of the Society and is abhorrent to ASM and its members.

ASM recognizes that there are valid concerns regarding the publication of information in scientific journals that could be put to inappropriate use as described in the CPC resolution mentioned above. Members of the ASM Publications Board will evaluate the rare manuscript that might raise such issues during the review process. However, as indicated elsewhere in these Instructions, research articles must contain sufficient detail, and material/information must be made available, to permit the work to be repeated by others. Supply of materials should be in accordance with laws and regulations governing the shipment, transfer, possession, and use of biological materials and must be for legitimate, bona fide research needs. Links to, and information regarding, these laws and regulations can be found at http://www.asm.org/ under the Public Policy tab. We ask that authors pay particular attention to the NSAR Select Agent/Toxin list on the CDC website http://www.selectagents.gov/index.html and the NSABB criteria for identifying dual use research of concern in the report “Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information” on the Office of Biotechnology Activities website http://oba.od.nih.gov/biosecurity/pdf/Framework%20for%20transmittal%200807_Sep07.pdf (p. 17–22).

Ethical Guidelines

ASM requirements for submitted manuscripts are consistent with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, as last updated by the International Committee of Medical Journal Editors in April 2010 (http://www.icmje.org).

Authors are expected to adhere to the highest ethical standards. The following sections of these Instructions include detailed information about ASM’s ethical standards. Failure to comply with the policies described in these Instructions may result in a letter of reprimand, a material constitutes the substance of a paper and therefore renders the manuscript unacceptable for publication. In brief, a paper is not acceptable for submission to an ASM journal if it, or its substance, has been published/posted in:

Plagiarism. Misappropriating another person’s intellectual property constitutes plagiarism. This includes copying sentences or paragraphs verbatim (or almost verbatim) from someone else’s work, even if the original work is cited in the references. The NIH ORI publication “Avoiding Plagiarism, Self-Plagiarism, and Other Questionable Writing Practices: a Guide to Ethical Writing” (http://ori.dhhs.gov/education/products/plagiarism/) can help authors identify questionable writing practices.

Plagiarism is not limited to the text; it can involve any part of the manuscript, including figures and tables, in which material is copied from another publication without permission and attribution. An author may not reuse his or her own previously published work without attribution; this is considered self-plagiarism.

Fabrication, manipulation, and falsification of data. As a member of the Committee on Publication Ethics (COPE), ASM encourages authors to consult COPE’s “Guidelines on Good Publication Practice” (http://publicationethics.org/static/1999/1999pdf13.pdf). Fabrication, manipulation, and falsification of data constitute misconduct. As defined by the U.S. Department of Health and Human Services, fabrication is “making up data or results and recording or reporting them,” and falsification is “manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record” (42 Code of Federal Regulations, §93.103). All sources and methods used to obtain and analyze data, including any electronic preprocessing, should be fully disclosed; detailed explanations should be provided for any exclusions.

Primary publication. Manuscripts submitted to the journal must represent reports of original research, and the original data must be available for review by the editor if necessary.

By submission of a manuscript to the journal, the authors guarantee that they have the authority to publish the work and that the manuscript, or one with substantially the same content, was not published previously, is not being considered or published elsewhere, and was not rejected on scientific grounds by another ASM journal. It is incumbent upon the author to acknowledge any prior publication, including his/her own articles, of the data contained in a manuscript submitted to an ASM journal. A copy of the relevant work should be submitted with the paper as supplemental material. Whether the material constitutes the substance of a paper and therefore renders the manuscript unacceptable for publication is an editorial decision.

In brief, a paper is not acceptable for submission to an ASM journal if it, or its substance, has been published/posted in:
• A serial, periodical, or book
• A conference report or symposium proceedings
• A technical bulletin or company white paper
• A nonpersonal website
• Any other retrievable source

The following do not preclude submission to, or publication by, an ASM journal, as long as the posted data do not constitute the substance of a submission:

• Posting of a method/protocol on a nonpersonal website
• Posting of a limited amount of original data on a personal/university/corporate website or websites of small collaborative groups working on a problem
• Posting of unpublished sequence data on the Internet (the URL where the sequence is posted should be included in the text)
• Preliminary disclosures of research findings as meeting posters, webcast as meeting presentations, or published in abstract form as adjuncts to a meeting, e.g., part of a program
• Posting of theses and dissertations on a personal/university-hosted website

Availability of materials. By publishing in the journal, the authors agree that, subject to requirements or limitations imposed by laws or governmental regulations of the United States, any DNAs, viruses, microbial strains, mutant animal strains, cell lines, antibodies, and similar materials described in the article are available from a national collection or will be made available in a timely fashion, at reasonable cost, and in limited quantities to members of the scientific community for noncommercial purposes. The authors guarantee that they have the authority to comply with this policy either directly or by means of material transfer agreements through the owner.

Similarly, the authors agree to make available computer programs, originating in the authors’ laboratory, that are the only means of confirming the conclusions reported in the article but that are not available commercially. The program(s) and suitable documentation regarding its (their) use may be provided by any of the following means: (i) as a program transmitted via the Internet, (ii) as an Internet server-based tool, or (iii) as a compiled or assembled form on a suitable medium (e.g., magnetic or optical). It is expected that the material will be provided in a timely fashion and at reasonable cost to members of the scientific community for noncommercial purposes. The authors guarantee that they have the authority to comply with this policy either directly or by means of material transfer agreements through the owner.

Permissions. The corresponding author is responsible for obtaining permission from both the original author and the original publisher (i.e., the copyright owner) to reproduce or modify figures and tables and to reproduce text (in whole or in part) from previous publications. Permission(s) must be obtained no later than the modification stage. The original signed permission(s) must be identified as to the relevant item in the ASM manuscript (e.g., “permissions for Fig. 1 in AAC00123-11”) and submitted to the ASM production editor on request. In addition, a statement indicating that the material is being reprinted with permission must be included in the relevant figure legend or table footnote of the manuscript. Reprinted text must be enclosed in quotation marks, and the permission statement must be included as running text or indicated parenthetically.

It is expected that the authors will provide written assurance that permission to cite unpublished data or personal communications has been granted.

For supplemental material intended for posting by ASM (see “Supplemental Material”), if the authors of the AAC manuscript are not also the owners of the supplemental material, the corresponding author must send to ASM signed permission from the copyright owner that allows posting of the material, as a supplement to the article, by ASM. The corresponding author is also responsible for incorporating in the supplemental material any copyright notices required by the owner.

Authorship. All authors of a manuscript must have agreed to its submission and are responsible for its content (initial submission and any subsequent versions), including appropriate citations and acknowledgments, and must also have agreed that the corresponding author has the authority to act on their behalf in all matters pertaining to publication of the manuscript. The corresponding author is responsible for obtaining such agreements and for informing the coauthors of the manuscript’s status throughout the submission, review, and publication process. Submitting a paper before all authors have read and approved it is considered an ethical violation, as is failure to credit someone who qualifies as a coauthor; however, ASM does not itself investigate or attempt to resolve authorship disputes.

An author is one who made a substantial contribution to the overall design and execution of the experiments; therefore, ASM considers all authors responsible for the entire paper. Individuals who provided assistance, e.g., supplied strains or reagents or critiqued the paper, need not be listed as authors but may be recognized in the Acknowledgments section.

A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations if desired) of the contributing members only may be given in a footnote linked to the study group name in the byline or as a separate paragraph in the Acknowledgments section.

If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the
group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

All authors must agree to the order in which their names are listed in the byline. Statements regarding equal contributions by two or more authors (e.g., X.J. and Y.S. contributed equally to . . . ) are permitted as footnotes to bylines and must be agreed to by all of the authors. Other statements of attribution may be included in the Acknowledgments section.

A change in authorship (order of listing, addition or deletion of a name, or corresponding author designation) after submission of the manuscript will be implemented only after receipt of signed statements of agreement from all parties involved.

Disputes about authorship may delay or prevent review and/or publication of the manuscript. Should the individuals involved be unable to reach an accord, review and/or publication of the manuscript can proceed only after the matter is investigated and resolved by the authors’ institution(s) and an official report of such and signed statements of agreement are provided to ASM.

Conflict of interest. All authors are expected to disclose, in the manuscript submittal letter, any commercial affiliations as well as consultancies, stock or equity interests, and patent-licensing arrangements that could be considered to pose a conflict of interest regarding the submitted manuscript. (Inclusion of a company name in the author address lines of the manuscript does not constitute disclosure.) Details of the disclosure to the editor will remain confidential. However, it is the responsibility of authors to provide, in the Acknowledgments section, a general statement disclosing financial or other relationships that are relevant to the study. Examples of potentially conflicting interests that should be disclosed include relationships that might detract from an author’s objectivity in presentation of study results and interests whose value would be enhanced by the results presented. All funding sources for the project, institutional and corporate, should be credited in the Acknowledgments section, as described below. In addition, if a manuscript concerns a commercial product, the manufacturer’s name must be indicated in the Materials and Methods section or elsewhere in the text, as appropriate, in an obvious manner.

Copyright

To maintain and protect the Society’s ownership and rights and to continue to afford scientists the opportunity to publish in high-quality journals, ASM requires the corresponding author to sign a copyright transfer agreement on behalf of all the authors on acceptance. Unless this agreement is executed (without changes and/or addenda), ASM will not publish the article.

In the copyright transfer agreement signed by an author, ASM grants to that author (and coauthors) the right to republish discrete portions of his/her (their) article in any other publication (print, CD-ROM, and other electronic forms) of which he/she is (they are) the author(s) or editor(s), on the condition that appropriate credit is given to the original ASM publication. This republication right also extends to posting on a host computer to which there is access via the Internet. Except as indicated below, significant portions of the article may not be reprinted/posted without ASM’s prior written permission, however, as this would constitute duplicate publication.

Authors may post their own published articles on their personal or university-hosted (but not corporate, government, or similar) websites without ASM’s prior written permission provided that appropriate credit is given (i.e., the copyright lines shown at the top of the first page of the PDF version).

The copyright transfer agreement asks that authors who were U.S. government employees and who wrote the article as part of their employment duties be identified. This is because works authored solely by such U.S. government employees are not subject to copyright protection, so there is no copyright to be transferred. The other provisions of the copyright transfer agreement, such as author representations of originality and authority to enter into the agreement, apply to U.S. government employee-authors as well as to other authors.

Copyright for supplemental material (see “Supplemental Material”) remains with the author, but a license permitting the posting by ASM will be sent, along with the article copyright transfer agreement, to the corresponding author for signing at the acceptance stage. If the author of the article is not also the copyright owner of the supplemental material, the corresponding author must send to ASM signed permission from the owner that allows posting of the material, as a supplement to the article, by ASM. The corresponding author is also responsible for incorporating into the supplemental material any copyright notices required by the owner.

Funding Agency Repositories

The National Institutes of Health (NIH) requests that its grantee and intramural authors provide copies of their accepted manuscripts to PubMed Central (PMC) for posting in the PMC Public Access Repository. However, AAC authors are automatically in compliance with this policy and need take no action themselves. For the past several years, ASM has deposited in PubMed Central all publications from all ASM journals. Further, ASM policy is that all primary research articles are made available to everyone, free, 6 months after publication through PubMed Central, HighWire, and international PubMed Central-like repositories. By having initiated these policies, ASM is in full compliance with NIH policy. For more information, see http://publicaccess.nih.gov/. ASM also allows AAC authors whose work was supported by similar funding agencies that have public access requirements like those of the NIH (e.g., the Wellcome Trust) to post their accepted manuscripts in...
publicly accessible electronic repositories maintained by those funding agencies. If a funding agency does not itself maintain such a site, then ASM allows the author to fulfill that requirement by depositing the manuscript (not the typeset article) in an appropriate institutional or subject-based open repository established by a government or noncommercial entity.

Since ASM makes the final, typeset articles from its primary-research journals available free of charge on the ASM Journals and PMC websites 6 months after final publication, ASM recommends that when submitting the accepted manuscript to PMC or a similar public access site, the author specify that the posting release date for the manuscript be no earlier than 6 months after publication of the typeset article by ASM.

Use of Human Subjects or Animals in Research

The use of human subjects or other animals for research purposes is regulated by the federal government and individual institutions. Manuscripts containing information related to human or animal use should clearly state that the research has complied with all relevant federal guidelines and institutional policies. Copies of these guidelines and policy statements must be available for review by the editor if necessary.

Patient Identification

When isolates are derived from patients in clinical studies, do not identify them by using the patients’ initials, even as part of a strain designation. Change the initials to numerals or use randomly chosen letters. Do not give hospital unit numbers; if a designation is needed, use only the last two digits of the unit. (Note: established designations of some viruses and cell lines, although they consist of initials, are acceptable [e.g., JC virus, BK virus, and HeLa cells].)

Nucleotide and Amino Acid Sequences

Newly determined nucleotide and/or amino acid sequence data must be deposited and GenBank/EMBL/DDBJ accession numbers must be included in the manuscript no later than the modification stage of the review process. It is expected that the sequence data will be released to the public no later than the publication (online posting) date of the accepted manuscript. The accession numbers should be included in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for short-form papers. If conclusions in a manuscript are based on the analysis of sequences and a GenBank/EMBL/DDBJ accession number is not provided at the time of the review, authors should provide the sequence data as supplemental material.

It is expected that, when previously published sequence accession numbers are cited in a manuscript, the original citations (e.g., journal articles) will be included in the References section when possible or reasonable.

Authors are also expected to do elementary searches and comparisons of nucleotide and amino acid sequences against the sequences in standard databases (e.g., GenBank) immediately before manuscripts are submitted and again at the proof stage.

Analyses should specify the database, and the date of each analysis should be indicated as, e.g., January 2011. If relevant, the version of the software used should be specified.

See “Presentation of Nucleic Acid Sequences” for nucleic acid sequence formatting instructions.


Proper Use of Locus Tags as Systematic Identifiers for Genes

To comply with recommendations from the International Nucleotide Sequence Database (INSD) Collaborators and to avoid conflicts in gene identification, researchers should implement the following two fundamental guidelines as standards for utilization of locus tags in genome analysis, annotation, submission, reporting, and publication. (i) Locus tag prefixes are systematic gene identifiers for all of the replicas of a genome and as such should be associated with a single genome project submission. (ii) New genome projects must be registered with INSD, and new locus tag prefixes must be assigned in cooperation with INSD to ensure that they conform to the agreed-upon criteria. Locus tag prefixes that are currently in use may be searched at the NCBI locus tag database (http://www.ncbi.nlm.nih.gov/genomes/ltpt.cgi).

Structural Determinations

Coordinates for new structures of macromolecules determined by X-ray crystallography or cryo-electron microscopy must be deposited in the Protein Data Bank and assigned identification codes must be included in the manuscript no later than the modification stage of the review process. It is expected that the coordinates will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send coordinates with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for short-form papers.

The URLs for coordinate deposition are http://rsb-deposit.rutgers.edu/ and http://pdbdep.protein.osaka-u.ac.jp/en/.
Microarray Data

The entire set of supporting microarray data must be deposited in the appropriate public database (e.g., GEO, ArrayExpress, or CIBEX) and the assigned accession number(s) must be included in the manuscript no later than the modification stage of the review process. It is expected that the data will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send the relevant data with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for short-form papers.


Culture Deposition

AAC expects authors to deposit strains used in therapeutic activity assessments and studies of mechanisms of action, resistance, and cross-resistance in publicly accessible culture collections and to refer to the collections and strain numbers in the text. Since the authenticity of subcultures of culture collection specimens that are distributed by individuals cannot be ensured, authors should indicate laboratory strain designations and donor sources as well as original culture collection identification numbers.

Mycobank

New scientific names of fungi along with key nomenclatural and descriptive material must be deposited in MycoBank (http://www.mycobank.org) and the assigned accession number(s) must be included in the manuscript no later than the modification stage of the review process. It is expected that the data will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send the relevant data with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph at the end of the Materials and Methods section for full-length papers and at the end of the text for short-form papers.

Supplemental Material

Supplemental material intended for posting by ASM should be restricted primarily to large or complex data sets or results that cannot readily be displayed in printed form because of space or technical limitations. Such material may include data from microarray, structural, biochemical, or video imaging analyses. In such cases, the manuscript submitted for review should include a distillation of the results so that the principal conclusions are fully supported without referral to the supplemental material.

Supplemental material intended for posting by ASM must be uploaded as a separate Supplemental Material file(s) in the manuscript submission and peer review system and will be reviewed along with the manuscript. The maximum size permitted for an individual file is 8 MB (20 MB for movie files). If your file exceeds this size, you must use the file compression utility WinZip to reduce the file size. The decision to publish (i.e., post online only) the material with the article if it is accepted will be made by the editor. ASM will post no more than 10 individual supplemental files with an individual article. It is possible that a manuscript will be accepted but that the supplemental material will not be.

To ensure broad access, supplemental files should be submitted in the following standard formats:

(i) Text. Word, RTF, or PDF files.
(ii) Figures. TIFF, EPS, high-resolution PDF, JPEG, or GIF format. Supplemental figures should not be embedded in the manuscript text.
(iii) Tables. Word, RTF, or PDF files.
(iv) Data sets. Excel (.xls), RTF, TXT, or PDF files.
(v) Movies. Audio Video Interleave (.avi), QuickTime (.mov), or MPEG files. All movies should be submitted at the desired reproduction size and length.

Unlike the manuscript, supplemental material will not be edited by the ASM Journals staff and proofs will not be made available. References related to supplemental material only should not be listed in the References section of an article; instead, include them with the supplemental material hosted by ASM or posted on a personal/institutional website.

Supplemental material will always remain associated with its article and is not subject to any modifications after publication.

Material that has been published previously (print or online) is not acceptable for posting as supplemental material. Instead, the appropriate reference(s) to the original material should be made available. References related to supplemental material only should not be listed in the References section of an article; instead, include them with the supplemental material hosted by ASM or posted on a personal/institutional website.

Copyright for the supplemental material remains with the author, but a license permitting the posting by ASM must be signed by the corresponding author. If you are not the copyright owner, you must provide to ASM signed permission from the owner that allows posting of the material, as a supplement to your article, by ASM. You are responsible for including in the supplemental material any copyright notices required by the owner.

See also “Publication Fees.”

Warranties and Exclusions

Articles published in this journal represent the opinions of the authors and do not necessarily represent the opinions of ASM. ASM does not warrant the fitness or...
suitability, for any purpose, of any methodology, kit, product, or device described or identified in an article. The use of trade names is for identification purposes only and does not constitute endorsement by ASM.

**SUBMISSION, REVIEW, AND PUBLICATION PROCESSES**

**Submission Process**

All submissions to AAC must be made electronically. In 2011, the ASM journals are switching from Rapid Review to the eJournalPress (eJP) manuscript submission and peer review system. Journals will be transitioned one by one over the course of several months, and the exact timing for AAC has not been determined. When the transition occurs, only new manuscript submissions will be made through the eJP system. If you are returning a modified manuscript and made the original submission in Rapid Review, please use Rapid Review. For up-to-date information about where to submit your manuscript, please refer to the separate html version of Instructions to Authors, [http://aac.asm.org/misc/ifora.dtl](http://aac.asm.org/misc/ifora.dtl), which is updated throughout the year.

**Review Process**

All manuscripts are considered to be confidential and are reviewed by the editors, members of the editorial board, or qualified ad hoc reviewers. To expedite the review process, authors must recommend at least three reviewers who have expertise in the field, who are not members of their institution(s), who have not recently been associated with their laboratory(ies), and who could not otherwise be considered to pose a conflict of interest regarding the submitted manuscript. At least one recommended reviewer must be a member of the journal’s editorial board. Please provide, where indicated on the submission form, contact information for suggested reviewers who are not editorial board members.

Copies of in-press and submitted manuscripts that are important for judgment of the present manuscript should be included as supplemental material to facilitate the review.

When a manuscript is submitted to the journal, it is given a control number (e.g., AAC00047-11 version 1) and assigned to one of the editors. (Always refer to this control number in communications with the editor and the Journals Department.) It is the responsibility of the corresponding author to inform the coauthors of the manuscript’s status throughout the submission, review, and publication processes. The reviewers operate under strict guidelines set forth in “Guidelines for Reviewers” ([http://www.journals.asm.org/misc/reviewguide.dtl](http://www.journals.asm.org/misc/reviewguide.dtl)) and are expected to complete their reviews expeditiously.

The corresponding author is notified, generally within 4 to 6 weeks after submission, of the editor’s decision to accept, reject, or require modification. When modification is requested, the corresponding author must either submit the modified version within 2 months or withdraw the manuscript. A point-by-point response to the reviews must be provided with the revised manuscript, and a compare copy of the manuscript (without figures) should be included as supplemental material if the editor requested one.

Manuscripts that have been rejected, or withdrawn after being returned for modification, may be resubmitted to the same ASM journal if the major criticisms have been addressed. A manuscript rejected by one ASM journal on scientific grounds or on the basis of its general suitability for publication is considered rejected by all other ASM journals; however, a manuscript rejected solely on the basis of scope may be “resubmitted” to a more appropriate ASM journal. A manuscript is considered a resubmission no matter how much (or little) it differs from the rejected or withdrawn manuscript and regardless of how much time has passed.

For all resubmissions (to the same or a different journal, irrespective of the extent of the revisions, and irrespective of the amount of time between rejection and resubmission), the cover letter must state that the manuscript is a resubmission, and the former manuscript control number must be provided. A point-by-point response to the review(s) and a compare copy of the revised manuscript showing the changes must be included as supplemental material. Manuscripts resubmitted to the same journal are normally handled by the original editor. Rejected manuscripts may be resubmitted only once unless permission has been obtained from the original editor or from the editor in chief.

**Notification of Acceptance**

When an editor has decided that a manuscript is acceptable for publication on the basis of scientific merit, the author and the Journals Department are notified. A PDF version of the accepted manuscript is posted online as soon as possible (see “AAC Accepts”).

The text files undergo an automated preediting, cleanup, and tagging process specific to the particular article type, and the illustrations are examined. If all files have been prepared according to the criteria set forth in these Instructions and those in the online manuscript submission system, the acceptance procedure will be completed successfully. If there are problems that would cause extensive corrections to be made at the copyediting stage or if the files are not acceptable for production, ASM Journals staff will contact the corresponding author. Once all the material intended for publication has been determined to be adequate, the manuscript is scheduled for the next available issue. The editorial staff of the ASM Journals Department completes the editing of the manuscript to bring it into conformity with prescribed standards.

**AAC Accepts**

For its primary-research journals, ASM posts online PDF versions of manuscripts that have been peer re-
Page Proofs

Page proofs, together with a query sheet and instructions for handling proofs, will be made available to the corresponding author electronically via a PDF file that can be accessed through a unique password. Since corresponding authors will be notified of the availability of their PDF proofs, instructed how to access information about page charges, reprints, and color figure charges (if applicable), and assigned their unique password via e-mail, an e-mail address must be supplied in the corresponding author electronically via a PDF file that can be accessed through a unique password. Since corresponding authors will be notified of the availability of their PDF proofs, instructed how to access information about page charges, reprints, and color figure charges (if applicable), and assigned their unique password via e-mail, an e-mail address must be supplied in the corresponding author footnote. Failure to do so may result in a delay in publication. The PDF page proofs must be printed out, and corrections must be written on the hard copy. Queries must be answered on the query page or on a separate sheet of paper, and any changes related to the queries must be indicated on the proofs. Note that the copy editor does not query at every instance where a change has been made. Queries are written only to request necessary information or clarification of an unclear passage or to draw attention to edits that may have altered the sense. It is the author’s responsibility to read the entire text, tables, and figure legends, not just items queried. As soon as the page proofs are corrected and signed by the person who proofread them (within 48 h), they should be mailed or sent by a courier service such as FedEx, not faxed or sent as an e-mail attachment, to the ASM Journals Department, 1752 N St., N.W., Washington, DC 20036-2904. The proof stage is not the time to make extensive corrections, additions, or deletions. Figures as they appear in the proofs are for validation of content and placement, not quality of reproduction or color accuracy. Print output of figures in the PDF page proofs will be of lower quality than the same figures viewed on a monitor. Please avoid making changes to figures based on quality of color or reproduction in proof.

Important new information that has become available between acceptance of the manuscript and receipt of the proofs may be inserted as an addendum in proof with the permission of the editor. If references to unpublished data or personal communications are added, it is expected that written assurance granting permission for the citation will be included. Limit changes to corrections of spelling errors, incorrect data, and grammatical errors and updated information for references to articles that have been submitted or are in press. If URLs have been provided in the article, recheck the sites to ensure that the addresses are still accurate and the material that you expect the reader to find is indeed there.

Questions about late proofs and problems in the proofs should be directed to the ASM Journals Department (e-mail, nlin@asmusa.org; telephone, 202-942-9231). Questions about accessing or viewing your PDF proofs should be directed to Katie Gay of Cadmus Communications at 804-261-3155 or gayk@cadmus.com.

PDF Files

A corresponding author who has included an e-mail address in his/her “corresponding author” footnote will have limited access (10 downloads, total) to the PDF file of his/her published article. An e-mail alert will automatically be sent to him/her on the day the issue is posted. It will provide a URL, which will be required to obtain access, and instructions. An article may be viewed, printed, or stored, provided that it is for the author’s own use.

Should coauthors or colleagues be interested in viewing the paper for their own use, the corresponding author may provide them with the URL; a copy of the article may not be forwarded electronically. However, they must be made aware of the terms and conditions of the ASM copyright. (For details, go to http://www.journals.asm.org/misc/terms.dtl.) Note that each such download will count toward the corresponding author’s total of 10. After 10 downloads, access will be denied and can be obtained only through a subscription to the journal (either individual or institutional) or after the standard access control has been lifted (i.e., 6 months after publication).

Publication Fees

Page charges. Authors whose research was supported by grants, special funds (including departmental and institutional), or contracts (including governmental) or whose research was done as part of their official duties (government or corporate, etc.) are required to pay page charges (based on the number of typeset pages, including illustrations, in the article).

For a corresponding author who is an ASM member, page charges are currently $67 per page for the first eight pages and $125 per page for each page in excess of eight (subject to change without notice). To obtain the
member rate, the corresponding author must be an ASM member.

For a nonmember corresponding author, page charges are currently $80 per page for the first eight pages and $250 for each page in excess of eight (subject to change without notice). A corresponding author who is not an ASM member may join ASM to obtain the member rate.

If the research was not supported by any of the means described above, a request to waive the charges may be sent to the Journals Department, ASM, 1752 N St., N.W., Washington, DC 20036-2904, USA (fax, 202-942-9355; e-mail, aluckey@asmusa.org). The request must include the manuscript control number assigned by ASM and indicate how the work was supported. Waivers apply only to page charges; responsibility for color charges and other publication fees remains with the author.

Minireviews, Commentaries, and Comment Letters to the Editor are not subject to page charges. New-Data Letters to the Editor are subject to page charges.

Color charges. The cost of publishing in color must be borne by the author.

For a corresponding author who is an ASM member, color charges are currently $170 per color figure (subject to change without notice).

For a nonmember corresponding author, color charges are currently $375 per color figure (subject to change without notice). A corresponding author who is not an ASM member may join ASM to obtain the member rate.

Minireviews, Commentaries, and Comment Letters to the Editor are not subject to color charges. New-Data Letters to the Editor are subject to color figure charges.

Reprints. Reprints (in multiples of 100) may be purchased by all coauthors. In the proof notification e-mail, the corresponding author will be instructed how to access information about reprints.

The corresponding authors of Minireviews and Commentaries may receive 100 free reprints of their contribution; additional reprints (in multiples of 100) may be purchased if desired. As for regular articles, the corresponding author will be instructed, in the proof notification e-mail, how to access information about reprints.

Supplemental material fee. Authors are charged a flat fee for posting supplemental material as an adjunct to their published article. For 2011, the fee is $190. (Exceptions: No fee is charged for supplemental material associated with Minireviews or Commentaries.)

Optional open access fee. Author-paid optional open access (OOA) is now available for all article types. The 2011 fee is $2,000. This fee is in addition to any page charges, color charges, or supplemental material charges and permits immediate public access to both the preliminary “Accepts” version and the copyedited, typeset version published in the online journal. This option is in addition to the open access already provided through NIH’s PubMed Central repository; all primary research published in ASM journals is freely available through PubMed Central 6 months after publication.

ORGANIZATION AND FORMAT

Editorial Style

The editorial style of ASM journals conforms to the ASM Style Manual for Journals (American Society for Microbiology, 2011, in-house document) and How To Write and Publish a Scientific Paper, 6th ed. (Greenwood Press, Westport, CT, 2006), as interpreted and modified by the editors and the ASM Journals Department.

The editors and the Journals Department reserve the privilege of editing manuscripts to conform with the stylistic conventions set forth in the aforesaid publications and in these Instructions.

On receipt at ASM, an accepted manuscript undergoes an automated preediting, cleanup, and tagging process specific to the particular article type. To optimize this process, manuscripts must be supplied in the correct format and with the appropriate sections and headings.

Type every portion of the manuscript double-spaced (a minimum of 6 mm between lines), including figure legends, table footnotes, and references, and number all pages in sequence, including the abstract, figure legends, and tables. Place the last two items after the References section. Manuscript pages must have line numbers; manuscripts without line numbers may be editorially rejected by the editor, with a suggestion of resubmission after line numbers are added. The font size should be no smaller than 12 points. It is recommended that the following sets of characters be easily distinguishable in the manuscript: the numeral zero (0) and the letter “oh” (O); the numeral one (1), the letter “el” (l), and the letter “eye” (I); and a multiplication sign (×) and the letter “ex” (×). Do not create symbols as graphics or use special fonts that are external to your word processing program; use the “insert symbol” function. Set the page size to 8½ by 11 inches (ca. 21.6 by 28 cm). Italicize any words that should appear in italics, and indicate paragraph lead-ins in boldface type.

Authors who are unsure of proper English usage should have their manuscripts checked by someone proficient in the English language.

Manuscripts may be editorially rejected, without review, on the basis of poor English or lack of conformity to the standards set forth in these Instructions.

Full-Length Papers

Full-length papers should include the elements described in this section.

Title, running title, and byline. Each manuscript should present the results of an independent, cohesive study; thus, numbered series titles are not permitted.
Exercise care in composing a title. Avoid the main title/subtitle arrangement, complete sentences, and unnecessary articles. On the title page, include the title, the running title (not to exceed 54 characters and spaces), the name of each author, the address(es) of the institution(s) at which the work was performed, each author’s affiliation, and a footnote indicating the present address of any author no longer at the institution where the work was performed. Place an asterisk after the name of the author to whom inquiries regarding the paper should be directed (see “Correspondent footnote,” below).

**Study group in byline.** A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations if desired) of the contributing members may be given in a footnote keyed to the study group name in the byline or as a separate paragraph in Acknowledgments.

If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

**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 (× 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 hard-copy 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.


2. Cox, C. S., B. R. Brown, and J. C. Smith. J. Gen. Genet., in press. {Article title is optional; journal title is mandatory.}


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.)


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.}


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.


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. Weatherers, 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.}
... 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.

(iii) References related to supplemental material. References that are related only to supplemental material hosted by ASM or posted on a personal/institutional website should not be listed in the References section of an article; include them with the supplemental material itself.

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


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:


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.

**Commentaries**

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.

**Errata**

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.

**Authors’ Corrections**

The Author’s Correction section provides a means of correcting errors of omission (e.g., author names or citations) and errors of a scientific nature that do not alter the overall basic results or conclusions of a published article (e.g., an incorrect unit of measurement or order of magnitude used throughout, contamination of one of numerous cultures, or misidentification of a mutant strain, causing erroneous data for only a [noncritical] portion of the study). Note that the addition of new data is not permitted.

For corrections of a scientific nature or issues involving authorship, including contributions and use or ownership of data and/or materials, all disputing parties must agree, in writing, to publication of the Correction. For omission of an author’s name, letters must be signed by the authors of the article and the author whose name...
was omitted. The editor who handled the article will be consulted if necessary.

Submit an Author’s Correction via the online manuscript submission and peer review system (see “Submission, Review, and Publication Processes”). Select Erratum as the manuscript type; there is no separate selection for an Author’s Correction, but your Correction will be published as such if appropriate. In the Abstract section of the submission form (a required field), put “Not Applicable.” Upload the text of your Author’s Correction as a Microsoft Word file. Please see a recent issue for correct formatting. Signed letters of agreement must be supplied as supplemental material (scanned PDF files).

Retractions

Retractions are reserved for major errors or breaches of ethics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Submit Retractions 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 Retraction as a Microsoft Word file. Letters of agreement signed by all of the authors must be supplied as supplemental material (scanned PDF files). The Retraction will be assigned to the editor in chief of the journal, and the editor who handled the paper and the chairperson of the ASM Publications Board will be consulted. If all parties agree to the publication and content of the Retraction, it will be sent to the Journals Department for publication.

ILLUSTRATIONS AND TABLES

Illustrations

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.

We strongly recommend that before returning their modified manuscripts, authors check the acceptability of their digital images for production by running their files through Rapid Inspector, a tool provided at the following URL: http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp. Rapid Inspector is an easy-to-use, Web-based application that identifies file characteristics that may render the image unusable for production.

If you require additional information, please send an e-mail inquiry to digitalart@cadmus.com.

Minimum resolution. It is extremely important that a high enough file resolution is used. All separate images that you import into a figure file must be at the correct resolution before they are placed. (For instance, placing a 72-dpi image in a 300-dpi EPS file will not result in the placed image meeting the minimum requirements for file resolution.) Note, however, that the higher the resolution, the larger the file and the longer the upload time. Publication quality will not be improved by using a resolution higher than the minimum. Minimum resolutions are as follows:

- 300 dpi for grayscale and color
- 600 dpi for combination art (lettering and images)
- 1,200 dpi for line art

Size. All graphics should be submitted at their intended publication size; that is, the image uploaded should be 100% of its print dimensions so that no reduction or enlargement is necessary. Resolution must be at the required level at the submitted size. Include only the significant portion of an illustration. White space must be cropped from the image, and excess space between panel labels and the image must be eliminated.

Maximum width for a 1-column figure: 3⅛ inches (ca. 8.4 cm)
Maximum width for a 2-column figure: 6⅝ inches (ca. 17.4 cm)
Minimum width for a 2-column figure: 4¼ inches (10.8 cm)
Maximum height: 9½ inches (23.0 cm)

Contrast. Illustrations must contain sufficient contrast to be viewed easily on a monitor or on the printed page.

Labeling and assembly. All final lettering and labeling must be incorporated into the figures. On initial submission, illustrations should be provided as PDF files, with the legend beneath each image, to assist review. At the modification stage, production quality digital figure files must be provided, along with text files for the legends. Put the figure number well outside the boundaries of the image itself. (Numbering may need to be changed at the copyediting stage.) Each figure must be uploaded as a separate file; i.e., rather than uploading a separate file for each panel in a figure, assemble all panels into one file; i.e., rather than uploading a separate file, and any multipanel figures changed at the copyediting stage.) Each figure must be bound at the print journal may not match that in the online journal 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 10³, and M for 10⁶, 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 “10⁴ cells per ml” (not “cells per ml × 10⁻⁴”). Likewise, an enzyme activity of 0.06 U/ml might be shown as 6 accompanies 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

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Fraction</th>
<th>ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/mg of protein</td>
<td>Total U</td>
</tr>
<tr>
<td>Control</td>
<td>Depleted membrane</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>Concentrated supernatant</td>
<td>0.134</td>
</tr>
<tr>
<td>E1 treated</td>
<td>Depleted membrane</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Concentrated supernatant</td>
<td>0.11</td>
</tr>
</tbody>
</table>

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/biblig/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.


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.bsgc.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., hisF._coli or hisK._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., desClp).

(vi) Deletions are indicated by the symbol Δ placed before the deleted gene or region, e.g., ΔtrpA432, Δ(aroP-aceE)419, or Δ(hisQ-hisI)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-lac95). Likewise, Φ(arab-’lacZ\(^+\))6 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). An insertion of an E. coli his gene into plasmid pSC101 at zero kilobases (0 kb) is shown as pSC101 Ω(0kb::K-12hisB)4. An alternative designation of an insertion can be used in simple cases, e.g., gatT236::Tn5. 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\(^-\)), Δmut cts, or mal::Δmut cts::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, pa-
rentheses (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\(^{8}(gal^+)\).


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 analogy to the style used for recording transposon insertions (zeb) 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, subtitles 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., ScURA3 versus CaURA3); 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 Aam11 int2 red114 cI857; this strain carries mutations in genes cI, int, and red and an amber-suppressible (am) mutation in gene A. A strain designated λ att\(^{343}\) imm\(^{-}\) 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 Szymbalski and Szybalski (Gene 7:217–270, 1979) and Echols and Muri-aldo (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::Tn5, 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-specifed 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) demonstrated that XYZ cells grow at pH 6.8,” “Figure 2 shows that ABC cells failed 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 inhibited . . . .” 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 (dicyethylaminoethyl); EDTA (ethylene diaminetetraacetic acid); EGTA (ethylene glycol-
bis(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)
- approx (approximately)
- avg (average)
- concn (concentration)
- diam (diameter)
- exp (experiment)
- exp(t) (experimental)
- ht (height)
- mo (month)
- mol wt (molecular weight)
- no. (number)
- 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; cephalor, CEC; cefadroxil, CFR; cefamandole, FAM; cefazolin, CZF; cefdinir, CDR; cefditoren, CDN; cefepime, MEP; cefetamet, FET; cefixime, CFM; cefmetazole, CMZ; cefonicid, CID; cefoperazone, CFP; cefotaxime, CTX; cefotetan, CTI; cefoxitin, FOX; cefpodoxime, CPD; cefprozil, CPR; cefazidime, CAZ; ceftriaxone, CBT; cefixime, CFX; ceftizoxime, ZOX; ceftriaxone, CRO; cefuroxime (axetil or sodium), CFX; cephalexin, LEX; cephalothin, CEF; cephradin, HAP; cephradine, RAD; chloramphenicol, CHL; cinoxacin, CIN; ciprofloxacin, CIP; clarithromycin, CLR; clindamycin, CLX; clindamycin, CLI; colistin, CST; daptomycin, DAP; dicloxacinil, DCX; dirithromycin, DTM; doxycycline, DOX; enoxacin, ENX; erythromycin, ERY; fleroxacin, FLE; fosfomycin, FOE; gatifloxacin, GAT; gentamicin, GEN; grepafloxacin, GRX; imipenem, IPM; kanamycin, KAN; levofloxacin, LVX; linezolid, LZD; lomefloxacin, LOM; loracarbef, LOR; meropenem, MEM; metillicillin, 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; pipracillin-tazobactam, TZP; polymixin B, PMB; quinupristin-dalfopristin (Synercid), Q-D; rifabutin, RBF; rifampin, RIF; rifampenitne, RFP; sparfloxacin, SFX; spectinomycin, SPT; streptomycin, STR; teicoplanin, TEC; telithromycin, TEL; tetracycline, TET; ticarcillin, TIC; ticaricillin-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; clofazimine, CLT; fluconazole, 5FC; fluconazole, FLC; itraconazole, ITC; ketoconazole, KTC; nystatin, NFT; 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 cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions), Cmax/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 > 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., fAUC) 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 × 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 ≥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 eva-

(4) 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 modifications 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 if they occur during the trial; the results for regimens with and without superinfections 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.

(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 − β) 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.


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 mmol or μmol. 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, “μmol/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., 14CO2, H2O, and H235SO4). Brackets are not used when the isotopic symbol is introduced is placed in square brackets directly preceding the name of a compound that in its natural state does not contain the element (e.g., 32S-ATP) or to a word that is not a specific chemical name (e.g., 131I-labeled protein, 14C-amino acids, and 3H-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:

[14C]urea
| [γ-32P]ATP
1-[methyl-14C]methionine
UDP-[U-14C]glucose
[2,3-3H]serine
E. coli [15P]DNA
[ε-14C]lysine
fructose 1,6-[1-32P]phosphate

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 not 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.

Reviewing 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.


• 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]"

Manuscript Submission
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.

Permissions
Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

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

Article by DOI

Book

Book chapter

Online document

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.isssn.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.

**Permissions**
If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.
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

**Electronic Supplementary Material**
Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author’s article, as certain information cannot be printed or is more convenient in electronic form.
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.
To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

**Audio, Video, and Animations**
Always use MPEG-1 (.mpg) format.

**Text and Presentations**
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).

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

Collecting Multiple Files
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
- Authors' agreements

Article written in good English
Manuscript has been 'spellchecked'
References are in the correct format for this journal
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)
Colour figures are clearly marked as being intended for colour reproduction or to be reproduced in black-and-white

For further information please contact the Author Support Department at [authorsupport@elsevier.com](mailto:authorsupport@elsevier.com)
Editorial process

On receipt in the Editorial Office your manuscript will be subject to detailed scrutiny with respect to both format and content. The Editors will assign the article to subject experts for peer review. The purpose of this review is to guide the editors in their decisions. If it is considered appropriate the comments will be made available to the authors and will guide in any revision.

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.

Authors in Japan kindly note that, upon request, Elsevier Japan will provide authors with a list of people who can check and improve the English of their paper (before submission). Please contact our Tokyo office: Elsevier K.K., 4F Higashi-Azabu, 1-Chome Bldg, 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106-0044, Japan, tel.: (+81) (3) 5561 5037; fax: (+81) (3) 5561 5047, e-mail: jp.info@elsevier.com

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.

Results. The results should be precisely presented once in the text, tables or figures without discussion of their significance. When results are presented in tables or figures the text should comment only on the important points. Tables and figures with legends should be able to stand alone.

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
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.

### 2.5. References

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.

**Text.** Indicate the references by superscript numbers in the text. The actual authors can be referred to, but the reference number(s) must always be given.

**List.** Number the references in the list in the order in which they appear in the text.

**Examples:**

Reference to a journal publication:

Reference to a book:

Reference to a chapter in an edited book:

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](http://www.nlm.nih.gov/tsd/serials/terms_cond.html)

**Online references**

Such article citations should include DOI (digital object identifier). The DOI is a persistent identifier, which remains with the article even after it is published in print. See [http://www.dx.doi.org](http://www.dx.doi.org)


### 2.6. Tables

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].

2.7. Figure legends

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).

2.8. Figures

See detailed note below.

3. Specific journal style

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.

Mathematical formulae. Present simple formulae in the line of normal text where possible. In principle, variables are to be presented in italics. Use the solidus (/) instead of a horizontal line, e.g. \( X_p/Y_m \).

Powers of \( e \) are often more conveniently denoted by exp.

Number consecutively any equations that have to be displayed separate from the text (if referred to explicitly in the text).

Nomenclature and units. Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUB: Biochemical Nomenclature and Related Documents http://www.chem.qmul.ac.uk/iupac/biblog/white.html for further information.

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.

When reporting percentages in the text both the numerator and denominator should be included. When the sample size is greater than 100 percentages should be reported to no
more than one decimal place. When the sample size is 100 or less, percentages should be reported in whole numbers.

Statistical methods with which readers may not be familiar should be fully referenced and details of any statistical software packages used should be given, e.g. Epi Info (CDC, Atlanta, GA, USA). Precise values of P should be given where possible, to no more than two significant figures, but P less than 0.001 should be used instead of smaller values.

*Drug names.* Generic names of drugs should be used. The proprietary name may be used together with the generic name where it is first mentioned in the text and details of the manufacturer should be given (name, city, state, country).

*DNA sequences and GenBank Accession numbers.* Gene accession numbers refer to genes or DNA sequences about which further information can be found in the databases at the National Center for Biotechnical Information (NCBI) at the National Library of Medicine. Authors wishing to enable other scientists to use the accession numbers cited in their papers via links to these sources, should type this information in the manner set out below.

For each and every accession number cited in an article, authors should type the accession number in bold, underlined text. Letters in the accession number should always be capitalised. (See Example below). This combination of letters and format will enable Elsevier's typesetters to recognize the relevant texts as accession numbers and add the required link to GenBank's sequences.

Example: ‘(GenBank accession nos. AI631510, AI631511, AI632198 and BF223228), a B-cell tumor from a chronic lymphatic leukemia (GenBank accession no. BE675048), and a T-cell lymphoma (GenBank accession no. AA361117).’

Authors are encouraged to check accession numbers very carefully. An error in a letter or number can result in a dead link. In the final version of the printed article, the accession number text will not appear bold or underlined. In the final version of the electronic copy, the accession number text will be linked to the appropriate source in the NCBI databases enabling readers to go directly to that source from the article.

*Preparation of supplementary data.* We welcome submission of electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier web products, including ScienceDirect at [http://www.sciencedirect.com](http://www.sciencedirect.com). In order to ensure that your submitted material is directly usable, please ensure that data are provided in our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit [http://www.elsevier.com/authors](http://www.elsevier.com/authors).

4. *Preparation of electronic illustrations*

Submitting your artwork in an electronic format helps us to produce your work to the best possible standards, ensuring accuracy, clarity and a high level of detail.

*General points*
- Make sure you use uniform lettering, symbols and sizing in your original artwork
- Arial font should be used in illustrations if possible
- Where possible figures should be designed to fit a single column (80 mm width) with the degree of reduction to be determined by the Publisher
- The axes of graphs should be carefully chosen so as to occupy the space available to the best advantage
- Line drawings should be as simple as possible: many computer-generated figures, e.g. three-dimensional graphs, fine lines, gradations of stippling and unusual symbols, cannot be reproduced satisfactorily when reduced
- The lettering and symbols, as well as other details, should have proportionate dimensions, so as not to become illegible or unclear after reduction
• Number the illustrations according to their sequence in the text
• Use a logical naming convention for your artwork files
• Provide all illustrations as separate files
• Provide legends to illustrations separately

A detailed guide on electronic artwork is available on our website: http://www.elsevier.com/artworkinstructions

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats
Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

• EPS: Vector drawings. Embed the font or save the text as 'graphics'
• TIFF: Colour or greyscale photographs (halftones): always use a minimum of 300 dpi
• TIFF: Bitmapped line drawings: use a minimum of 1000 dpi
• TIFF: Combinations bitmapped line/half-tone (colour or greyscale): a minimum of 500 dpi is required
• DOC, XLS or PPT: If your electronic artwork is created in any of these Microsoft Office applications please supply 'as is'

Please do not: • Supply embedded graphics in your document
• Supply files that are optimised for screen use (like GIF, BMP, PICT, WPG); the resolution is too low
• Supply files that are too low in resolution
• Submit graphics that are disproportionately large for the content

Colour illustrations
If, together with your accepted article, you submit usable colour figures then Elsevier will ensure, at no additional charge, that these figures will appear in colour on the web (e.g. ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in colour in the printed version. For colour reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.

Please note: Because of technical complications that can arise by converting colour figures to 'grey scale' (for the printed version should you not opt for colour in print) please submit in addition usable black and white illustrations corresponding to all the colour illustrations.

5. Online submission to the journal

These instructions apply to all articles submitted to the journal. Variations applicable to some types of article are noted in the appropriate sections.

Authors should upload their article via the journal's homepage (http://ees.elsevier.com/trstmh/), where you will be guided stepwise through the creation and uploading of the various files. Once the uploading is done, the system generates an electronic (PDF) version of the article which is used for the reviewing process. Authors, Reviewers and Editors send and receive all correspondence by e-mail and no paper correspondence is necessary.

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.

Please submit, with the manuscript, the names and e-mail addresses of two potential referees. You may also mention persons who you would prefer not to review your paper.

6. Formal requirements

These matters relate to the integrity of the publication process. You need to be aware of these
matters.

6.1. Authorship

For articles published in this journal, a person listed as an author must have made a substantial contribution to:
- the conception and design of the study, or the analysis and interpretation of data
- drafting the article or revising it critically for intellectual content
- giving final approval of the version to be published

ALL these conditions must be met.

Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.

Designation as an author infers a responsibility for the integrity and accuracy of all the data published. One or more of the authors should be listed as guarantors of the paper. The guarantor accepts full responsibility for the conduct of the study, had access to the data and controlled the decision to publish. Normally the corresponding author will be a guarantor but this must be explicitly stated.

At the end of each paper the contributions of each author to the study and its publication must be listed. We encourage intending authors to discuss this amongst themselves and agree the precise nature of each person's contribution. Authors must ensure that all authors listed meet the above criteria for authorship and that there is no one else who fulfils the criteria but has not been included as an author.

When a large, multi-centre group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship defined above.

It is the duty of the corresponding author/guarantor to ensure that each author has signed a declaration concerning his or her individual contribution. Any consequences that result from failure to do so will be the sole responsibility of the author/guarantor who is advised to keep copies of these declarations on file in case of dispute. The following format is suggested: "I declare that in this article entitled {title of article} I participated in {here list contributions made to the study} and that I have seen and approved the final version. I have the following competing interests {here list competing interests}." You will be required to send us signed copies of these statements. These signatures need not be dated and the documents must be submitted via the online submission system.

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.

Acknowledgements. You should acknowledge here anyone who has contributed towards the study by providing study materials or helped in data acquisition or analysis, or helped care for patients, but who does not meet the criteria for authorship. Persons providing purely technical help or writing assistance should be listed in this section.

Authors should obtain permission to acknowledge from all those named in the Acknowledgements.

 Alterations to authorship or acknowledged contributors. All authors must approve any change in authors and acknowledged contributors after initial submission. This applies to additions, deletions, change in order of authors, or contributions being attributed differently. The editor may contact any of the authors or contributors to ascertain whether they have agreed to any alteration.
6.2. Funding

Please list the source(s) of funding for the study and for each author, and for the manuscript preparation. If the funding body contributed in any way to study design, or the collection, analysis, and interpretation of data, or the writing of the manuscript, and/or the decision to submit the manuscript for publication, this should be explicitly stated. Full details of the funding bodies must be given, i.e. name, city, state, country and any grant/reference numbers or other identifiers included.

6.3. Conflicts of interest

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](http://www.icmje.org/sponsor.htm).

6.4. Ethical issues

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 institutional guidelines.

Studies on patients or volunteers require ethics committee approval and informed consent which should be documented in your paper. Patients have a right to privacy. Therefore identifying information, including patients¿ images, names, initials, or hospital numbers, should not be included in videos, recordings, written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and you have obtained written informed consent for publication in print and electronic form from the patient (or parent, guardian or next of kin where applicable). If such consent is made subject to any conditions, Elsevier must be made aware of all such conditions. Written consents must be provided to Elsevier on request. Even where consent has been given, identifying details should be omitted if they are not essential. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note. If such consent has not been obtained, personal details of patients included in any part of the paper and in any supplementary materials (including all illustrations and videos) must be removed before submission.

6.5. Clinical trials registration

All randomised controlled trials submitted for publication in Transactions should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart. Please refer to the CONSORT statement website at [http://www.consort-statement.org](http://www.consort-statement.org) for more information. Transactions has adopted the proposal from the International Committee of Medical Journal Editors (ICMJE) which require, as a condition of consideration for publication of clinical trials, registration in a public trials registry. Trials must register at or before the
onset of patient enrolment. The clinical trial registration number should be included at the end of the summary and in the Materials and Methods section of the text. For this purpose, a clinical trial is defined as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g. phase I trials) would be exempt. Further information can be found at [http://www.icmje.org](http://www.icmje.org).

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.

2. [http://www.clinicaltrials.gov](http://www.clinicaltrials.gov)
3. [http://www.ISRCTN.org](http://www.ISRCTN.org)
4. [http://www.umin.ac.jp/ctr/index/htm](http://www.umin.ac.jp/ctr/index/htm)
5. [http://www.trialregister.nl](http://www.trialregister.nl)


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.

A trial will be considered as ongoing if investigators were still collecting, cleaning or analysing data as of 1 July 2005. All ongoing trials require registration before being submitted to the Transactions.

6.6. Copyright

Upon acceptance of an article, authors will be asked to sign a "Journal Publishing Agreement" (for more information on this and copyright see [http://www.elsevier.com/authorsrights](http://www.elsevier.com/authorsrights)). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail (or letter) will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form.

If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: contact Elsevier's Rights Department, Philadelphia, PA, USA: Tel. (+1) 215 238 7869; Fax (+1) 215 238 2239; e-mail healthpermissions@elsevier.com. Requests may also be completed online via the Elsevier homepage ([http://www.elsevier.com/locate/permissions](http://www.elsevier.com/locate/permissions)).
**Funding body agreements and policies**
Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit [http://www.elsevier.com/fundingbodies](http://www.elsevier.com/fundingbodies)

**Sponsored Articles:**
Journal Name offers authors the option to sponsor non-subscriber access to their articles on Elsevier's electronic publishing platforms. For more information please view our [Sponsored Articles information page](#) Appendix 2

**Proofs**
One set of page proofs in PDF format will be sent by e-mail to the corresponding author, to be checked for typesetting/editing. No changes in, or additions to, the accepted (and subsequently edited) manuscript will be allowed at this stage. Proofreading is solely your responsibility. A form with queries from the copyeditor may accompany your proofs. Please answer all queries and make any corrections or additions required. Return corrections within 5 days of receipt of the proofs. Should there be no corrections, please confirm this. The Publisher reserves the right to proceed with publication if corrections are not communicated. When the edited manuscript is received by the Publisher it is considered to be in its final form. Proofs are not to be regarded as 'drafts'. Elsevier will do everything possible to get your article corrected and published as quickly and accurately as possible. In order to do this we need your help. When you receive the (PDF) proof of your article for correction, it is important to ensure that all of your corrections are returned to Elsevier in one communication. Subsequent corrections will not be possible, so please ensure your first sending is complete. Note that this does not mean you have any less time to make your corrections just that only one set of corrections will be accepted.
American Journal of Tropical Medicine and Hygiene

Instructions for Authors

Manuscripts and correspondence can be submitted at http://mc.manuscriptcentral.com/ajtmh. Questions about the submission process can be directed to cbs15@cwru.edu or Support@ScholarOne.com.

Authors who are unable to submit via the Manuscript Central website may send an electronic copy of their manuscript by e-mail or on a disk. However, we prefer that authors submit their own manuscripts electronically. Self-submission by the corresponding author allows authors greater control over the submission process. In addition, authors can provide us with helpful information, such as suggested and excluded reviewers, contact information for all authors, and comments to the Editor upon submission.

Cover Letter and Signatures
All manuscripts should be accompanied by a cover letter with the following information:

- The title of the paper
- Significance of the paper to the readers of the Journal
- A statement that the material has not and will not be submitted for publication elsewhere so long as it is under consideration by the American Journal of Tropical Medicine and Hygiene
- Written disclosure of any relationships or support which might be perceived as constituting a conflict of interest
- 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:

- By mailing it to The American Journal of Tropical Medicine & Hygiene, Wolstein Research Building, Room 4120, 10900 Euclid Avenue, Cleveland, Ohio 44106-4983 USA.
- By faxing it to 216-368-6987.
- By uploading the signed letter as a jpeg or tif file upon submission of the first draft of a new manuscript. PDF files cannot be uploaded to the site but can be sent by email to the editorial office.

In addition, the corresponding author must sign and return the copyright form upon submission. This form can be accessed on the journal's submission site.

Authorship
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.

Please do NOT upload .pdf files, as we may need to make some changes to the paper during copyediting. The journal does not accept Microsoft Word 2007 documents at this time. Please use Word's "Save As" option to save your document as an older (.doc) file type.

The converted .pdf and .jpg files will be the files evaluated during the review process. All original files that you upload, as well as their associated converted files marked as Not for Review at the end of the manuscript submission process, will be available for access by the journal office if necessary.

You may upload other file types such as LaTeX files, QuickTime movies, or other image types, but Manuscript Central will not convert these. The peer review participants will only be able to view these unconverted files if they have the appropriate software on their computers.

Short Reports
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
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.

AJTMH requests that authors of manuscripts published BEFORE 2010 do the following to comply with this policy:

1. Deposit the FINAL published paper in PubMed Central, not your author manuscript or galley proofs.

2. Request that PubMed Central honor the journal’s 12-month embargo on free access to current content, unless you have paid the author open access fee, in which case your manuscript may be deposited upon publication.

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.

If an author does not pay the open access fee but wants the journal office to deposit their article, the author should inform the journal office of this request at the time of publication. The journal office can deposit the article but will put a twelve-month hold on the release to coincide with the journal's embargo on free access to current content.
Please note that this only applies to NIH-funded, Wellcome Trust-funded, or other research funded by an agency that has repository guidelines for its authors. If you are not sure, please check with the agency that funded your research to see if they require a manuscript deposit in their repository.

SPECIFIC MANUSCRIPT GUIDELINES
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:**

**Examples of books:**

**Chapter in a book:**

**Web reference:**

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 = intraperitoneal, 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. 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 triatomines 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.
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