Mathematical Modeling of Malaria: Theories of Malaria Elimination

Geoffrey Louis Chi-Johnston

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2012
ABSTRACT

Mathematical Modeling of Malaria: Theories of Malaria Elimination

Geoffrey Louis Chi-Johnston

This dissertation describes the development and application of a new mathematical model for simulating the progression of *Plasmodium falciparum* infections in individuals with no malarial acquired immunity. The model allows for stochastic simulation of asexual and sexual parasitemias as well as the onset of fever and human to mosquito infectivity on a daily time scale. The model components for the asexual and sexual stages were developed elsewhere but are here extended to allow for simulation of the full range of dynamics observed in a subset of malaria therapy patients. As a first application of the model, I calculate the human component of malarial $R_0$, the basic reproductive number. I then compare this value to those from three other models and describe how this quantity can be used to model malaria transmission. The second application of the model incorporates the effects of drug treatment on progression of infection by utilizing modeled pharmacokinetic and pharmacodynamic properties of a variety of antimalarials. I utilize a stage specific proportional killing model for sexual stages, informed from recent *in vitro* data. The relationship of effect sizes to treatment coverage and type of treatment in both early and late treatment seeking settings is calculated. In the third chapter, I consider the economic and epidemiological ramifications of antimalarial and rapid diagnostic subsidization for malaria control. For the epidemiological modeling I utilize a semi-mechanistic model of the spread of drug resistance parameterized from historical malaria mortality data; for the economic model I consider the effect of rapid diagnostics on the intensive and extensive margins of antibiotics and antimalarials, as well as the benefits to improved targeting of both. I find that rapid diagnostic testing is justified given our baseline assumptions for areas with low proportions of malarious individuals among all treatment-seekers, but that caution is necessary before deployment worldwide. For antimalarial subsidization, we find that this is a
cost-effective method for reducing mortality in developing countries, though efforts to delay the onset and slow the spread of resistance are urgently needed.
### Table of Contents

**List of Figures** ................................................................. iii  
**Acknowledgements** ............................................................ vi  
**Dedication** ................................................................. vii  
**Preface** ................................................................. viii  
**Chapter 1: Malaria's Missing Number: Calculating the Human Component of $R_0$ with a Mechanistic Model**  
**Title Page** ................................................................. 1  
**Abstract** ................................................................. 3  
**Introduction** ................................................................. 5  
**Methods** ................................................................. 8  
**Results** ................................................................. 14  
**Discussion** ................................................................. 19  
**References** ................................................................. 23  
**Acknowledgements** ............................................................. 26  
**Figure Legends** ................................................................. 28  
**Figures** ................................................................. 31  
**Tables** ................................................................. 36  
**Text S1** ................................................................. 39  
**Chapter 2: Modeling Prospects for Malaria Elimination in Low-Endemicity Areas Through Early Treatment with Transmission-Blocking Drugs**  
**Title Page** ................................................................. 62  
**Abstract** ................................................................. 63  
**Introduction** ................................................................. 64  
**Methods** ................................................................. 66  
**Results** ................................................................. 68  
**Discussion** ................................................................. 73  
**References** ................................................................. 77  
**Acknowledgements** ............................................................. 80  
**Figure Legends** ................................................................. 83  
**Figures** ................................................................. 87  
**Tables** ................................................................. 93  
**Text S1** ................................................................. 97
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>75</td>
</tr>
<tr>
<td>Discussion</td>
<td>81</td>
</tr>
<tr>
<td>References</td>
<td>85</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>90</td>
</tr>
<tr>
<td>Supporting Information</td>
<td>91</td>
</tr>
<tr>
<td>Figure Legends</td>
<td>95</td>
</tr>
<tr>
<td>Figures</td>
<td>98</td>
</tr>
<tr>
<td>Tables</td>
<td>103</td>
</tr>
<tr>
<td>Text S1</td>
<td>104</td>
</tr>
<tr>
<td>Supplemental Figures</td>
<td>125</td>
</tr>
<tr>
<td>Supplemental Tables</td>
<td>129</td>
</tr>
<tr>
<td>Text S2</td>
<td>131</td>
</tr>
</tbody>
</table>

**Chapter 3: Epidemiological and Economic Considerations for Worldwide Deployment of Rapid Diagnostic Testing and Antimalarial Subsidies for Malaria Control**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>142</td>
</tr>
<tr>
<td>Abstract</td>
<td>143</td>
</tr>
<tr>
<td>Introduction</td>
<td>144</td>
</tr>
<tr>
<td>Methods</td>
<td>145</td>
</tr>
<tr>
<td>Model Specification</td>
<td>146</td>
</tr>
<tr>
<td>Consistency of Model Output</td>
<td>151</td>
</tr>
<tr>
<td>Description of the Effects of RDTs</td>
<td>152</td>
</tr>
<tr>
<td>Results</td>
<td>158</td>
</tr>
<tr>
<td>Discussion</td>
<td>161</td>
</tr>
<tr>
<td>Figure Legends</td>
<td>166</td>
</tr>
<tr>
<td>References</td>
<td>169</td>
</tr>
</tbody>
</table>
Table ................................................................................................. 172
Figures ................................................................................................. 173
Text S1 ................................................................................................. 179
Supplemental Figures ........................................................................ 185
List of Figures

Chapter 1: Malaria’s Missing Number: Calculating the Human Component of $R_0$ with a Mechanistic Model

1. Outputs from the within-host mechanistic model for three simulated individuals
2. Mean infectivity over time and distribution of net infectivity
3. Comparison of cumulative distribution functions for the durations of infection
4. Comparison of mean infectivity over time for three models
5. Comparison of distribution of net infectivity for three models

Chapter 2: Modeling Prospects for Malaria Elimination in Low-Endemicity Areas Through Early Treatment with Transmission-Blocking Drugs

1. Outputs from the within-host mechanistic model for three treated and untreated individuals
2. Pharmacokinetic and pharmacodynamic outputs
3. Distribution of net infectivity for treated individuals
4. Distribution of net infectivity with treatment prophylactic effect
5. Effect sizes of treatments as a function of treatment coverage in early and late treatment seeking settings

S. Gametocyte clearance after antimalarial treatment in four settings
2.S Percentage of individuals gametocytic from model simulations and field data in Thailand and Myanmar
3. Gametocyte densities after treatment from model simulations and in four treatment settings
4. Graphical user interface for mechanistic malaria model

Chapter 3: Epidemiological and Economic Considerations for Worldwide Deployment of
Rapid Diagnostic Testing and Antimalarial Subsidies for Malaria Control

Figure 3.1 Malaria-attributable mortality estimates, 1980-2010
Relative shares of failing first-line therapies, bed nets, and ACTs on modeled mortality

Figure 3.2 Effects of RDT targeting of antibiotics and antimalarials on mortality

Figure 3.3 Four scenarios for private sector ACT subsidization

Figure 3.4 Malaria mortality averted by ACTs per year, including resistance

Figure 3.5 Cost-effectiveness of antimalarials and effectiveness of RDTs

Figure 3.6 Etiology of fever and malaria mortality attribution

Figure 3.S1 Effects of rapid diagnostic testing on overtreatment with ACTs
Acknowledgements

There are many individuals who helped bring this work to its denouement. First I would like to thank the Fidock Lab for all of their support, both intellectual as well as emotional. Eric Ekland taught me about the variety of human immune responses to malaria infections; Andrew Lee explained the intricacies of DNA repair (as well as listened to my complaining). Sophie Adjalley provided references on gametocyte development, and Ines Peteresen described how parasites are competed against each other in vitro. Phillip Heinrich and I experimented with modeling the structures of drug transporters. David Fidock described the various life stages of the parasite and their idiosyncrasies, as well as explained the processes by which drug resistant mutants arise. He encouraged me to approach my academic tasks with laser-like focus and an extreme attention to detail, advice for which I am very grateful. I also appreciate his generosity in listening to my eccentric theories about vaccine development or new experiments. More than anything else though, the members of the Fidock Lab acted as a family, always willing to listen and share a story or heartfelt advice. I do and will appreciate their support.

I would like to thank David Smith, without whom this thesis could not have been written. When we first met, I was convinced that I could improve upon malaria modeling without fully understanding any of the models themselves. I had a dream to build a spatio-temporal model of malaria transmission, but only the barest outlines of how such a task might be accomplished. Dave was extremely patient with me, even though he had no reason to be. He first showed me how little I knew about the field, and then encouraged me to go to the literature to learn. I read many of his papers, marveling at how he was able to weave uncomplicated but profound analytic models to describe aspects of malaria transmission and apply them to field data. It was he who encouraged me to review the models of Molineaux and Dietz, and he who first conceived of the within-host simulator. He was always there with an encouraging word, and I appreciate his input.
I would also like to thank the Sustainable Development doctoral program, especially Solomon Hsiang. Sol is a true mensch; there is no other way to put it. Sol helped to massively improve my MATLAB modeling skills, and his tutorials on the Science of Sustainable Development informed my understanding of the many types of science utilized by the field. Sol also explained some of the intricacies of econometrics to me in a very patient and understanding way. He encouraged me to enroll in this program, and I fondly remember sleeping on an air mattress in his apartment when I visited. I admire his professionalism and his commitment to integrity, and I know that the future holds much promise for him. I would also like to thank Ram Fishman, who introduced the SIR modeling framework to me, as well as Wolfram Schlenker and Eric Verhoogen, who taught me the basics of environmental and development economics. I would also thank John Mutter, the program’s Director of Graduate Studies, for his advice and assistance, Doug Almond, who was patient with a student who thought he knew more than he did about economics, Mona Khalidi, the program’s administrator, and Scott Barrett and Jeffrey Sachs for pushing me to achieve beyond my own expectations. Thanks as well to Xiaojia Bao and Kyle Meng, who spent many late nights with me in the bowels of Lehman Library, working economics problem sets.

Finally, I would like to thank my family, my grandparents, and my wife Charlene for their support and love. “No man is an island, entire of itself; every man is a piece of the Continent, a part of the main,” as John Donne so eloquently put it. I appreciate all of the good and wonderful things that you brought into my life. Thanks as well to Br. Joseph Chvala, who taught me English in high school and provided an example of a man who was good at what he did, and did what he thought good.
~ For my family ~
Preface

Malaria is a disease that is as old as the human species. It has taken countless lives over thousands of years and plagues developing counties to the present day, exacting a severe human and economic toll. The disease is caused by infection with one of five species in the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. The first of these species is the most virulent, responsible for the vast majority of deaths per year, mostly among African children.

Malaria was thought for ages to have been caused by ‘bad air’ emanating from marshlands.\(^1\) It was not until malarial parasites were first observed by Alphonse Laveran in 1880 that the etiologic agent of malaria was identified.\(^2\) Further, the mechanism of transmission of malaria was unproven until 1897, when Ronald Ross discovered malarial parasites in mosquito midguts. After this discovery, serious control efforts began to be formulated, applying ancient techniques such as swamp draining, bed nets, and improved sanitation. Ronald Ross himself further contributed to malaria control efforts by formulating mathematical models describing the equilibrium dynamics of malaria in human and mosquito populations.

As the World Wars raged, understanding of malaria steadily improved, and new drugs for treatment of malaria were developed, such as chloroquine. The insecticidal properties of DDT were discovered in 1939, allowing for mass spraying of wide areas to eliminate or severely reduce mosquito populations. Further, from 1950-1952 the malariologist George MacDonald was modifying and building upon the mathematical work that Ross had pioneered.\(^3\) The model that he developed, called the Ross-MacDonald model, as well as the insights he gleaned from

---

\(^1\) [http://www.cdc.gov/malaria/about/history/laveran.html](http://www.cdc.gov/malaria/about/history/laveran.html)

\(^2\) ibid.

working with Ross, were then pressed into service during the first Global Malaria Eradication Program (GMEP).

The GMEP, begun in 1955, was an effort to eliminate malaria from many parts of the world outside of Africa, and was successful in many places. However, the project lost steam and by the early 1970s was abandoned, never having made a serious attempt to eliminate malaria in Africa (though significant pilot projects were run in Pare-Taveta from 1957-1959 and in Garki, Nigeria from 1969-1976). After this program was abandoned, efforts toward controlling malaria languished. Resistance to the first-line antimalarial, chloroquine, and the second-line antimalarials, such as sulphadoxine-pyrimethamine, spread widely; further, resistance to DDT began to spread as well. By the 1990s malaria was resurging throughout Africa and Southeast Asia and the number of deaths was approaching two million per year.

Not all is gloom and doom, however. In the early 2000s researchers perfected the use of artemisinin derivatives, a newly-discovered class of highly potent antimalarials, and artemisinin-based combination therapies were deployed worldwide. Relying on these new antimalarial combinations, along with pyrethroid treated bed nets and insecticide spraying, massive improvements in controlling malaria mortality and morbidity were achieved, and by the end of the century significant progress had been made.

However, this progress, although impressive, is tenuous. Antimalarial and insecticide resistance are once again on the horizon, and funding sources are drying up as recessions slow economic growth worldwide. Progress toward an antimalarial vaccine has proved difficult, and money for vaccine development is scarce. In order to make the best use of limited resources, mathematical models may once again be relied upon to help direct control efforts. Further,
models are needed to help predict how resistance might arise and how its spread might be limited.

In this dissertation, we describe two papers that apply recently developed modeling techniques to help improve the modeling and mapping of malaria transmission. In Chapter 1, we describe the parameterization of a new within-host model of the development of malaria infection. We utilize this model to predict the infectivity of human populations in the absence of preimmunity. The model was parameterized using malaria therapy data, in which individuals with tertiary syphilis were infected with \textit{P. falciparum} in order to induce a fever. The net human infectivity that we calculate is a critical component of the $R_0$, or basic reproductive number, for malaria. We then compare our predicted net infectivity to the net infectivity predicted by other malaria models. Our improved estimate was used to inform recent malaria mapping work of the worldwide $R_0$ for malaria.\footnote{Gething, P. et al., “A new world malaria map: \textit{Plasmodium falciparum} endemicity in 2010,”\textit{ Malaria J.}, 10:378.}

For Chapter 2, we analyze the effectiveness of artemisinin-based combination therapies as well as new antimalarial combinations at reducing malaria transmission. Specifically, we utilize our within-host model as well as the pharmacokinetics and pharmacodynamics of a variety of antimalarials to predict how drug treatment would reduce net human infectivity. We simulate treatment in two different treatment-seeking contexts (early and late treatment), corresponding to observed patterns of treatment in Thailand and Myanmar, respectively. We find that malaria control efforts benefit from gametocytocidal treatments (treatments that kill the sexual forms of the parasite). However, there is only a 1.5-fold increase in reduction in transmission that is achieved from adding gametocytocidal drugs to artemisinin-based combination therapies in the early treatment setting (less in the late treatment setting).
reduction needs to be weighed against the costs of such drugs, as well as possible side effects.

We also develop a framework that allows for comparing the transmission reductions achievable from different drug combinations to those from improving treatment coverage and/or treatment seeking behavior. This framework will allow for a cost-benefit analysis of whether to focus on introducing a new drug or improving existing delivery mechanisms in a given area.

In Chapter 3, we predict how subsidies for artemisinin-based combination therapies and rapid diagnostic testing will affect malaria mortality using epidemiological and economic modeling. For the epidemiological modeling we used historical trends in malaria mortality to understand the impact of first-line drug resistance on mortality during the period of 1980-2005 and projected the possible impacts of artemisinin resistance using these trends. For the economic modeling we considered how rapid diagnostic testing might affect the intensive and extensive margins of antibiotic and antimalarial usage and how rapid diagnostic testing results might be used to improve antibiotic and antimalarial targeting. We found that rapid diagnostic testing is justified in areas where there are relatively low proportions of malarious individuals among all treatment-seeking individuals. If we incorporate the benefits of reduced overtreatment using rapid diagnostics for delaying the onset of artemisinin resistance, the net effects of rapid diagnostics might recommend their usage worldwide. However, given the uncertainties involved, we state that a risk-averse social planner would deploy rapid diagnostic testing in a limited number of regions give our baseline parameter assumptions and the lack of confidence in the benefits of overtreatment for delaying resistance. Regarding antimalarial subsidization, we find that this is a cost-effective method for reducing mortality in developing countries; however, serious effort needs to be employed to delay the onset and slow the spread of resistance.
The results described in this thesis form the basis for a more thorough understanding of theories of elimination and eradication. In order to proceed with such a campaign, mathematical models are useful tools to help guide control efforts, and the results herein have helped and will continue to help improve model predictions and estimates. Further, the within-host modeling framework described here is an ideal tool to help predict how drug resistant malaria might spread, and efforts are ongoing to utilize results from such modeling efforts. Our results concerning antimalarial and rapid diagnostic subsidization show the need for caution before deploying these interventions worldwide, given the large number of feedbacks and uncertainties that are associated with these policies. We hope that we might continue to build upon the results described herein to facilitate elimination/eradication programs going forward.
Chapter 1: Malaria’s Missing Number: Calculating the Human Component of $R_0$ with a Mechanistic Model
Malaria’s Missing Number: Calculating the Human Component of $R_0$ with a Mechanistic Model

Geoffrey L. Johnston$^{1,2}$, David L. Smith$^{3,5\dagger}$, and David A. Fidock$^{1,4\dagger}$

$^1$Department of Microbiology and Immunology, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
$^2$School of International and Public Affairs, Columbia University, New York, NY 10027, USA
$^3$Bloomberg School of Public Health, John Hopkins University, Baltimore, MD 21205, USA
$^4$Division of Infectious Diseases, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
Abstract

**Background:** Human malaria is caused by infection with one of five species of parasites of the genus *Plasmodium*. *Anopheles* mosquitoes are the primary host of *Plasmodium* and transmit parasites by biting their intermediate hosts, humans. Modeling transmission thus requires an analysis of parasite development in both mosquitoes and humans. The mosquito-borne components of transmission are described by their vectorial capacity. The components of transmission describing human infectivity to mosquitoes over time are less well understood.

**Methods and Findings:** We have developed and analyzed a comprehensive simulation model of *P. falciparum* within-host malarial infection and transmission in immunologically-naïve humans. Our model incorporates the entire lifecycle of *P. falciparum*, starting with the asexual blood stage forms responsible for disease, the onset of symptoms, the development and maturation of sexual stage forms (gametocytes) that are transmissible to *Anopheles* mosquitoes, and human to mosquito infectivity. These model components were parameterized from malaria therapy data and from a range of other studies to simulate individual infections such that the ensemble is statistically consistent with the full range of patient responses to infection. Human infectivity was modeled over the course of untreated infections and the effects were examined in relation to transmission intensity expressed in terms of the basic reproductive number $R_0$. We then compared our model results to implicit calculations of human infectiousness from three other studies.

**Conclusions:** We find that mean human infectivity varies from 7.2 fully infectious days in the model of Okell et al., ~32 days using the current model, 45 days in the Garki model, and 94 days in the model of Lawpoolsri et al. This ten-fold difference reflects a diversity of modeling assumptions as well as the possible effects of immunity on transmission. Our current model
provides a credible estimate of the human component of R₀ and a sound basis for quantifying the
effect sizes of antimalarial drugs. Further, the novel mechanistic modeling framework described
here will enable careful studies into theories of eradication allowing for variation in infection
responses within a human population.
Introduction

Approximately 2.5 billion people live in areas whose native ecology permits transmission of *Plasmodium falciparum* [1]. This range encompasses an incredible diversity of settings, from the arid Sahel to the jungles of the Congo; from rice paddies of India to the refugee camps of Western Thailand; indeed, malaria was once transmitted Central Park, London, and to the edge of the Arctic Circle. Efforts have been ongoing to map the historical and current limits of this transmission [1-3]; however, applications of these maps are complicated by the diversity of vectors in such areas, the state of the health systems, and the levels of human immunity and the extent of control efforts that are applied.

Epidemiologists have long recognized the importance of developing mathematical techniques to quantify the extent of disease transmission in an area. The most important mathematical parameter for theories of eradication is $R_0$, the basic reproductive number [4]. This value represents the number of secondary cases that an index case would generate in a population without previous exposure to the disease. For malaria, $R_0$ has been expressed as the product of the vectorial capacity (i.e. the number of infectious bites that would eventually arise from all the mosquitoes that bite a fully infectious human on a single day), the duration of infectious period, and the efficiency of transmission. Vectorial capacity can be estimated directly over time by mosquito capture [5,6]. Changes in vectorial capacity can also be inferred from rapid changes in malaria endemicity corresponding to weather shocks [7] and less reliably from long-term endemicity responses to changing climate [3,8,9]. Statistical models have been developed that allow for prediction of vectorial capacity and malaria endemicity given other covariates such as the periodicity and intensity of rainfall, temperature, vegetation, etc. [10].
However, the human component of malaria transmission is more difficult to quantify, in part, because net infectiousness varies over the course of an infection. There are at least three aspects of human biology that affect human to mosquito infectiousness: immunity that is generated over the course of a single infection, the multiplicity of infection of a given host, and malaria-specific immunity acquired by repeated infections over time. The extent and mechanisms by which humans acquire immunity over time to malaria infections is a matter of current investigation and debate, with no consensus on the functional relationship between acquired immunity and progression of subsequent infection [11-13]. It is also not clear how infectious multiply-infected individuals are over time, though some estimates have been made that found no interaction among multiply-infected individuals in Africa [14].

Malaria elimination, however, is based on conditions of low endemicity, when the latter two types of human response to infection are less relevant than the first [15,16]. In areas of naturally low endemicity, or in areas of previously high endemicity where control measures have been effective and preimmunity has waned over time [6,11], population immune responses will be similar to those observed in malaria-naïve individuals. Indeed, $R_0$ itself is defined as the number of secondary infections among a non-infected population and serves as a threshold criterion for transmission: if the $R_0$ of an area is below 1, the disease will eventually become extinct; if above 1, the disease will spread.

Given the recent gains that have been made against malaria [17,18] public health and malaria experts have been increasingly promoting the agenda of malaria elimination and drawing up campaigns to reduce transmission [19,20]. To assess the likelihood of eliminating malaria from an area, quantitative approaches involve determining the intensity of interventions needed to eventually bring this value to less than 1, either through simulation or through estimation of $R_0$. 
and control effect sizes. For such calculations, it is important that human component of transmission be carefully quantified.

In order to simulate the range of human responses to malaria and calculate their infectiousness on to mosquitoes, we developed a stochastic, mechanistic model that incorporates within-host infections and human to mosquito infectivity. This framework simulates the progression of blood-stage parasitemia after emerging from the liver, as well as conversion of asexual forms to sexual forms (gametocytes), onset of symptoms, and human to mosquito infectiousness. Molineaux and Dietz first developed the asexual and gametocyte components of our model from malaria therapy data, in which individuals with tertiary syphilis were infected with *P. falciparum* to induce a fever and clear the syphilis [21-23]. This framework has been used to simulate the effects of vaccines on transmission [21-25]. However, their original model required that the parameters be fitted to an individual patent’s case history before simulation. We have extended their work by choosing stochastic distributions for parameters that allow for within-host simulations that generate an ensemble consistent with the observed population of infections.

Once we developed this model, we quantified the levels of human to mosquito infectivity over time and isolated the host-related determinants of the basic reproductive number, *R₀*. This novel analysis of *R₀* allowed us to analyze overlooked aspects of transmission relevant for elimination campaigns. Predictions were compared to outputs derived from other models [15,26,27]. We find that mean human infectivity varies from 7.2 fully infectious days in the model of Okell et al., ~32 days using the current model, 45 days in the Garki model, and 94 days in the model of Lawpoolsri et al. Our modeling work provides the most careful estimate yet of the distribution of human responses to malaria infection and the mean human contribution to *R₀*. 
and also provides a framework to examine how altering human infection dynamics affects malaria transmission.

**Methods**

*Plasmodium falciparum*, the parasite that causes the deadliest form of human malaria, has a complex life-cycle that presents both challenges and opportunities for controlling malaria transmission [28]. Parasites enter the blood through the bite of an *Anopheles* mosquito and travel to the liver where they develop and replicate. Upon emerging from the liver, parasites then enter the blood as merozoites, which infect red blood cells, develop, replicate, burst from the infected cell, and then repeat the cycle of blood-stage infection. Some of these infected red blood cells sequester in the microvasculature to develop further and differentiate into sexual stages ('gametocytes'). Once mature, these gametocytes enter the bloodstream [29]. Mature male and female gametocytes are then primed to form gametes and mate in the *Anopheles* mosquito following blood meal ingestion.

**Model Description**

We chose to utilize and extend a previously developed mechanistic modeling framework to reproduce the entire range of dynamics exhibited in the malaria therapy data, an extensive dataset describing deliberate malaria infections induced under clinical conditions. Our model simulates an infection by extending, combining, or modifying previously published models of: 1) bloodstream infections with the replicating asexual parasite forms; 2) onset of symptoms; 3) production and bloodstream dynamics of gametocytes; 4) the net infectiousness to mosquitoes as a function of gametocyte densities.

1. **Bloodstream Infections with Asexual Parasites**
Asexual parasitemia was modeled as a system of discrete (two-day time interval) difference equations previously elaborated by Molineaux et al. [21], which was also extended by Smith et al. [25]. The model simulates parasite densities in 50 different subpopulations aggregated by var gene expression type; switching occurs among the types in response to immune pressure. Asexual parasitemias are regulated by three immune responses: an innate immune response that establishes an upper limit for parasite density, a PfEMP1 variant-specific response that regulates short-term periodic oscillations in density and a variant-transcending immune response that causes a steady log-linear decrease in density over time, clearing the infection. We do not simulate deaths from malaria as these are so few as to not significantly impact overall transmission.

The data used to parameterize this model comes from malaria therapy, in which individuals with tertiary syphilis and with no malaria preimmunity were inoculated with single strains of *P. falciparum* in order to induce a fever and clear the infection [30,31]. In their original study [21], the model parameters were fitted to individual patient parasitemias to produce a best fit valid for that patient. Our implementation of this model utilizes random distributions for the case-fitted parameters affecting infection duration and the maximum level of parasitemia. Table 1 illustrates the changes in our model from the published parameters.

Our asexual model parameter distributions were chosen so that the model duration of infection matches the duration observed in malaria therapy [32] and so that the maximum parasitemias would follow a truncated lognormal distribution with a maximum parasitemia of approximately 10% (the maximum percent parasitemia observed was 11.7 over 1000 runs) [14]. The model as implemented here is thus stochastic, producing a unique sequence of asexual
parasitemias that mimics the dynamics of parasitemias observed in malaria therapy. The quality of model fit is described below.

2. Onset of Symptoms

Because we are also utilizing this model to simulate drug treatment in low-transmission areas (described in a companion study), treatment-seeking behavior is an important consideration. In the absence of diagnostic testing, fever may serve as an indicator of infection for both patient and clinician [33,34]. We allowed for the first onset of fever to serve as a trigger for treatment of infections. In order to predict when this fever occurs, we utilized the work done by Dietz et al. [35] who fitted probability distributions to the onset of fever as observed in malaria therapy data. In our model, all patients were assumed to be symptomatic and to experience a fever that began a variable number of days before reaching maximum parasitemia. To determine the day of first fever following emergence of parasites from the liver into the blood stream (taken as time zero), we used the uniform distribution based on an individual’s maximum asexual parasitemia as identified by Dietz et al. [25,35].

3. Gametocyte production and blood stream dynamics

We derived our gametocyte model from previous work by Eichner et al. and Diebner et al. [22,23]. This model of gametocyte development calculates daily percentages of gametocyte-infected red blood cells (i.e. gametocytemia) at each stage of development [29]. In human hosts gametocytes are produced by asexual parasites that commit to sexual development while in the host red blood cells. The sexual forms develop through five stages (I-V) over the course of 12-14 days. The first stages sequester in the bone marrow or microvasculature with the final stage(s) being infectious. Our model assumes that gametocytes were produced from asexual parasites at a
rate that varies with each wave of asexual parasitemia. Gametocytes then sequester for a variable length of time before becoming microscopically observable and infectious.

As for the asexual model, the original gametocyte modeling work [22,23] fitted model parameters to each patient’s malaria therapy data. We have modified their model such that model parameters are now either constant or chosen from probability distributions such that the resulting outputs match the observed variability in the malaria therapy data (see goodness-of-fit below). Table 1 illustrates the changes in our model from the published parameters.

4. Infectivity

For our model we estimated the probability of human-to-mosquito transmission as a function of gametocyte levels at a given time. For our simulations, we utilized the nonlinear relationship between gametocytemia and infectivity described by Stepniewska et al., based on mosquito feeding studies on malaria therapy patients [36-38]. According to this function, infectivity rises sigmoidally on a long-linear scale. Thus infectivity rises rapidly at low levels of gametocytemia (1-100 gametocytes per µL) and then more gradually approaches a maximum of 1.

Model fitting

Our modeling framework is designed to replicate both the means and the extremes of the dynamics of *P. falciparum* infections in adult humans with no preimmunity. For our asexual component, we utilized the medians, minima, and maxima of nine malariometric indices derived from the malaria therapy data as our simulation targets [21,39]. We used a bootstrap process to compare model outputs to the data. We first calculated the indices from the best-fit run from 50 samples of 1000 runs (with replacement) as well as the minima and the maxima of the indices of these 50 samples. We then repeated this sampling and the means of 50 such experiments were
recorded. Goodness-of-fit is according to the $\chi^2$ measure. **Table 2** provides a comparison of model outputs to the indices from malaria therapy.

We see that the model as parameterized does a good job of matching the median malaria therapy indices, though the model periods between appearances of PfEMP1 variants are shorter than observed in the data (**Table 2**, index 2-5). Further, the model slightly overpredicts the mean proportion of positive observations in both halves of patency, indicating that the model predicts that infections are more often observable during their duration of patency than observed (**Table 2**, indexes 2-7, 2-8). Given the high number of degrees of freedom in the model and in the data to be fitted, we could not use traditional fitting methods for these parameters, relying instead on a process of trial and error. A further complication encountered was that changing any one component may affect many indices simultaneously, and thus we were satisfied with the levels of fit here achieved.

In addition to these nine indices, we also utilized a study which modeled the durations of infection observed in malaria therapy [32]. This study observed that the distribution of survival times of infections was Gompertz distributed, which informed our choice of distribution for the parameter $\frac{P_m^*}{k_{mg}}$ (**Table 1**).

For our gametocyte target data we relied upon the geometric means, minima, and maxima recorded from fits to malaria therapy as recorded by Eichner et al. [23]. This study recorded these data for three parameters describing gametocyte dynamics; see **Table 3**. We used bootstrapping to compare model outputs, as above, except that 113 samples were chosen from 1000 runs with replacement and the means taken from 50 such experiments. In order to parameterize our model we utilized the reported quantiles for the gametocyte model parameters to help constrain our selection of distributions [22]. We also attempted to reduce the number of
degrees of freedom in the model by setting $\beta$ and $\mu_0$ to their mean values (after correcting for model differences). We note that our model fit the observed indices quite well, although the model predicted a shorter mean maximum duration of circulation for gametocytes ($L$) than was observed in the data. See Text S1 for a full mathematical description of all model components.

As a further check of model outputs, our model predicts that the arithmetic mean duration of time between first fever and first gametocytemia detectable by smear is 12.0 days (with microscopic detection threshold of 10 gametocytes/mL), whereas the measured value from malaria therapy patients was 10 to 11 [40,41].

**Model Illustration**

We illustrate how these dynamics play out in different simulated individuals in **Figure 1. Figure 1A** shows the simulated asexual parasite densities over time for three individuals; densities are in log_{10} parasitized red blood cells (PRBC) per $\mu$L of blood. These individuals display the characteristic peaks and dips associated with PfEMP1 variation. The black line illustrated the lower limit of detectability by microscopy (10 PRBC/$\mu$L). The inset in **Figure 1A** shows the first 50 days of infection along with the first fever day for each individual.

In **Figure 1B**, the daily gametocytemias of the individuals from **Figure 1A** are shown. The gametocytemias are seen to stochastically track the asexual parasitemias after a slight lag. **Figure 1C** illustrates the daily probability that a mosquito bite will produce oocysts in the same individuals as in **Figures 1A and 1B**. Human to mosquito infectivity is seen to fluctuate rapidly in response to changing gametocyte density and infectivity. The large diversity in responses to infections within a population is illustrated in **Figure 2A**, which shows the asexual parasitemias from 1000 runs of the model.
Results

We have developed a mechanistic model of the progression of malaria within a human host, parameterized such that the model reproduces the mean and extremes of the dynamics of infection observed in malaria therapy. Here we describe the mathematical formalisms that relate model outputs to $R_0$ and compare the model outputs to those of three other models.

Classical description of host contributions to $R_0$

The basic reproductive number $R_0$ is one of the most important parameters in infectious disease modeling. This value describes the average number of hosts that would be infected following the full course of infection of a single host. If $R_0$ is greater than one, malaria will tend to be endemic, and if less than one then disease will progress toward elimination.

The classical expression for the $R_0$ of malaria was derived by Macdonald and can be formulated with four terms [16,42]. Potential transmission by a mosquito population is described by its vectorial capacity, $V$, which describes the number of infectious bites that would arise from all the mosquitoes that bite one fully infectious individual on a single day. Two parameters, $b$ and $c$ describe the proportion of blood meals that successfully cause an infection: $b$ is the probability that an infected mosquito will infect an uninfected human upon biting; while $c$ is the probability than an infected human will infect an uninfected mosquito upon biting. In the Ross-Macdonald model, the infectious period of humans is exponentially distributed with a daily clearance rate of $r$ and a mean duration of infection of $r^{-1}$ days. The basic reproductive number of malaria is then described by the classic formula:

$$R_0 = \frac{bcV}{r}$$
The Ross-Macdonald model [16,42] assumes that $c$ is a constant over this period, so the ratio $c/r$ describes the net infectiousness of a simple human infection. This net infectiousness fraction can be interpreted as the equivalent number of days that a person is fully infectious.

**Mean Human Infectivity Over Time**

In reality, neither $V$, $b$, $c$, nor $r$ are constant among individuals over time and $R_0$ is only the first moment of a complicated multivariate distribution. Consider a population of $N$ individuals, none of whom have been previously exposed to malaria. These individuals will differ in their responses to malarial infection, notably in terms of time of fever relative to the initiation of blood stage infection, parasitemias, and time to clearance of infection. We cannot use the ratio $c/r$ in our calculations of $R_0$ to describe the infectiousness of these individuals because we do not assume constant rates of infectiousness and clearance. We let $D_i(t)$ denote the probability that individual $i$ will infect a mosquito upon being bitten at time $t$; this function takes values between 0 and 1. Our mechanistic model allows us to simulate the full variability of $D_i(t)$ for non-immune populations.

If we first consider the mean of the population using the formula

$$D(t) = \frac{1}{N} \sum_{i=1}^{N} D_i(t)$$

then $D(t)$ is a function of time only. We call this function as the mean human infectivity over time. The function $D(t)$ for our mechanistic model is shown in Figure 2A along with the 25th and 75th percentiles of daily infectiousness. The mean $D(t)$ is skewed due to the presence of some individuals exhibiting long-lived infectious periods. Mean human infectivity is an important function for elimination in many contexts. Calculation of $D(t)$ allows for a determination of how likely malaria will be able to persist through droughts or intensive antimalarial campaigns. Our
mechanistic model predicts that individuals are, on average, infectious for a long period of time; see Discussion below.

While $D(t)$ and other functions of infectivity over time are important functions for elimination, there are still other ways of analyzing $D_i(t)$. Indeed, if we integrate over time, rather than over individuals, we are left with the expression

$$D_i = \int_0^\infty D_i(t) \, dt,$$

which describes the net infectivity of each individual. In a natural population there will be a distribution of $D_i$ over individuals. This distribution is shown in Figure 2B.

If we integrate either the mean human infectivity over time with respect to $t$, or the distribution of net infectivity $D_i$ over a population, we arrive at the mean net human infectivity, $D$. Thus, $D$ can be calculated in one of two ways:

$$D = \int_0^\infty D(t) \, dt = \frac{1}{N} \sum_{i=1}^{N} D_i.$$

For our mechanistic model $D$ ranges between approximately 31-34 for a population of 1000 individuals (the mean of 5000 runs was 32.3). The units of $D$ can be considered as fully infectious days, i.e., the number of days in which an individual has a probability of 1 of infecting a mosquito. This value represents the human contribution to $R_0$, and we note here that $D$ is invariant across time and space and ecological setting. Now that we have determined this parameter from our model, we compare this estimate to other models in the literature.

**Comparison of $D(t)$ and $D$ Among Malaria Models**

We will compare the calculation of $D$ reported here to this value as imputed from three other models: the model of Lawpoolsri et al. [26], the model of Okell et al. [27], and the model of Dietz et al. (known as the ‘Garki model’) [15]. The former two models were designed to simulate
the effectiveness of antimalarials at reducing malaria transmission. The model of Lawpoolsri et al. was fit to data from a low-transmission region of Thailand (PfPR ~ 0.0-1.5) [26] while the model of Okell et al. was fit to three regions of medium intensity transmission in Tanzania. Both are compartmental models (Lawpoolsri et al. has one infectious compartment and Okell et al. has four infectious compartments varying in infectivity and clearance rate), and both papers employ their models to predict the constant equilibrium prevalence in untreated and treated cases. We begin first by calculating the function $D(t)$ for these two models.

Lawpoolsri et al. assume that the mean duration of clearance in infectious individuals is $1/188$ day$^{-1}$ with constant daily human to mosquito infectiousness ($c$) of .5. In the model of Okell et al. [27], each of the four infectious compartments in this model had different clearance rates ($1/10.5$, $1/10.5$, $1/31.5$, $1/157.5$ day$^{-1}$) and each compartment had a different proportional infectivity ($1.90$, $3.08$, $1.53$, $0.28$) of the average daily infectivity $c = 0.05$. (We do not weight these durations of infectivity for age or body surface area, i.e., we calculate the unweighted $D(t)$ here.) Figure 3 illustrates the cumulative distributions of the durations of infection/infectiousness for these two models as well that of the mechanistic model. We see that the mechanistic model matches the malaria therapy curve closely (not surprisingly), and that the other two models have significantly heavier tails, indicating that individuals are infected for a longer period of time in these models.

Because we are here examining $R_0$ from a novel perspective, neither of these studies reported $D$ or $D(t)$; however, we can derive $D(t)$ for compartmental models using the curves from Figure 3 and the $c$ values for each compartment. Figure 4A shows $D(t)$ for both of these models as well as our mechanistic model; Figure 4B illustrates the first 200 days of this function for closer inspection. We see that the model of Lawpoolsri et al. predicts that mean infectivity is
above 5% for 433 days, the output from Okell et al. is above this threshold for only 45 days, and
our mechanistic model output is above this value for 151 days. The $D$ values for these three
models are 7.2 fully infectious days for the model of Okell et al., ~32 days using the current
model, and 94 days in the model of Lawpoolsri et al. For the Garki model, we can calculate $D$
from the formula $D = \frac{\epsilon}{\alpha + \delta}$ [15] such that $D = 45.5$ fully infectious days.

Given our calculation of $D$ for Lawpoolsri et al., Okell et al., and our mechanistic model,
we can rescale the plots of $D(t)$ by multiplying each curve by a scaling factor so that the three
models share the same mean net infectivity as the mechanistic model; these results are shown in
Figure 4C. Once the models are rescaled, we can see more clearly that the models of Okell et al.
and the mechanistic model predict that infectiousness is cleared at very similar rates throughout
the population, whereas Lawpoolsri et al. predict a much more gradual loss of infectiousness.
However, the closeness of $D(t)$ for the scaled stochastic representation of Okell et al. and the
mechanistic model is surprising, although Okell et al. do parameterize some of their model
parameters from malaria therapy data.

**Comparison of $D_i$ Among Malaria Models**

In the previous section we calculated the mean responses of individuals over time for the models
of Lawpoolsri et al. and Okell et al. However, since these models are both compartmental, they
can readily be formulated as stochastic, individual-based models by assuming that individuals
are in each infectious compartment for exponentially distributed times. We can thus compute the
distribution of net infectiousness within a population, $D_i$, for both models. **Figure 5A** compares
the distributions $D_i$ for these two models to the distribution generated by our mechanistic model
(red cross indicates mean, green square the median). As implied by the $D(t)$ curve, we see that
the model of Lawpoolsri et al. has some individuals with very high $D$ values, whereas the
distribution generated by the model of Okell et al. is much more centered about its mean. If we scale the distributions $D_i$ to all have the same mean as the mechanistic model, we see that $D_i$ for Lawpoolsri et al. is still much more dispersed than the mechanistic model; however, $D_i$ for Okell et al. matches quite well to that of the mechanistic model (*Figure 5B*).

**Discussion**

We described the development of a novel, stochastic, within-host model of the progression of malaria in patients with no previous infections. This model utilized the difference equations developed by Molineaux and Dietz to simulate the progression of asexual and sexual parasitemias. By fixing some parameters and choosing others from stochastic distributions, we were able to fit the median and extremes of the dynamics of infections observed in malaria therapy, without the need to tune model parameters to individual case histories. We then validated this approach against three data sets from malaria therapy.

There are three caveats to the modeling results described here. First, we are calculating $D$ only among adults; there is no malaria therapy data for children. It is not known how children differ in their overall levels of infectivity from adults, and we do not speculate here. Further research will attempt to address this question. A second caveat is that our assumption that $var$ genes switch in response to immune pressure is most likely incorrect. However, we have chosen to use previously published model structures that were validated against malaria therapy data, and these are the only models reproduce the range of observed responses in the data; we thus inherit their $var$ assumptions.

One existing mechanistic modeling framework, developed and described over three reports [39,43,44], does not allow for simulation of random individuals and thus does not allow
for reproduction of the full range of host-parasite interaction. Given that var switching is extremely complex [45], we are content with a model that may not be correct mechanistically but may reproduce more complicated dynamics by a simpler mechanism. Finally, we note that in order to reproduce the dynamics observed in the data we reduced the degrees of freedom artificially in some cases by setting parameter values to their means. Given the difficulty involved in calculating parameter covariances from the malaria therapy data (they are not reported in Diebner et al. [22]), we chose to fix some parameters to facilitate analysis.

Once our model was formulated, we revisited the Ross-McDonald framework to examine how human infectiousness enters into the formula for the basic reproductive number $R_0$. We then analyzed human infectiousness in three novel ways, calculating $D(t)$, the mean human infectivity over time, the distribution of net infectivity $D_n$, and mean net human infectivity, $D$. We found that $D$ in our mechanistic model is approximately 32 fully infectious days. This quantity is invariant in a population over time and plays a crucial role in determining $R_0$. We have utilized this value in our recent malaria mapping work [1] although a full mathematical treatment of this quantity was left until the present.

Given our interest in these functions, distributions, and integrals, we went back to the literature to determine if we could impute these quantities from other modeling work to examine the reasonableness of our conclusions. We examined the models of Lawpoolsri et al., Okell et al., and the Garki model, and found a wide variance among them. The reasons for the differences in compartmental models have something to do with model structure. Lawpoolsri et al. is constrained functionally by the assumption of only one infectious compartment. Okell et al. uses four infectious compartments and thus encompasses for a much larger class of distributions (the hypoexponential distributions) for the lifetimes of infection. Further, by weighting the infectivity
of each of the duration of infectiousness compartments differently, Okell et al. increase the
degrees of freedom of $D(t)$, allowing them to better fit their target data.

Finally, the differences in $D(t)$ among the models may also have to do with the data being
fitted: the endemicity of the regions being modeled at equilibrium Lawpoolsri et al. are much
lower than those of Okell et al. It is possible that individuals in low endemicity areas are
infectious at higher levels for longer periods than individuals in high endemicity areas, because
preimmunity may limit the severity and density of repeated $P. falciparum$ infections. This effect
may provide a means of identifying the effects of immunity on transmission. However, we would
need to fit a variety of endemic equilibria with hypoexponential models such as that of Okell et
al. to test such a hypothesis; we cannot generate quantitative conclusions from comparing the
models of Lawpoolsri et al and Okell et al directly, given their different model structures. The
fact that the Garki estimate of $D$ is so close to our own may be related to the fact that the
estimate of $D$ for the Garki model is derived from three parameters, two of which were assumed
by the model structure and only one fitted to data from an endemic area [15].

While these models may differ in their conclusions, our new estimate of $D$ is the correct
one for $R_0$, because $R_0$ is a quantity appropriate for a theory of elimination and assumes no
preimmunity, and our model is parameterized solely from malaria therapy. The other models
cannot easily disentangle the effects of preimmunity, multiplicity of infection, and control efforts
from the effects of immunity acting on a single infection, though we have described how future
efforts might begin to disentangle these quantities. In addition to our calculation of the invariant
$D$ and its importance for $R_0$, we also found through calculation of $D(t)$ that human infectiousness
persists for a long period of time at levels sufficient to promote transmission in areas of high
vectorial capacity. While these calculations are for naïve populations, they are relevant for
malaria elimination efforts because antimalarial immunity wanes over time [6,11]. A recent study in Senegal found that persistent infectiousness prevented interruption of transmission even when incidence had been reduced to very low levels through insecticide treated bed nets and usage of ACTs [6]. Our model confirms the relevance of persistent low-level infectiousness for elimination efforts and we will analyze this function further in future work. We hope that the modeling platform and analytic framework we have described here will clarify the different assumptions among malaria models and improve elimination modeling going forward.
References


Acknowledgements
Computing resources were provided in part by the ‘Hotfoot’ High Performance Computing Cluster at Columbia University. DLS was supported by grant 49446 from the Bill and Melinda Gates Foundation.

Financial Disclosure
GLJ received funding from the National Science Foundation Graduate Research Fellowship Program. DLS is supported by a grant from the Bill and Melinda Gates Foundation (#49446) (http://www.gatesfoundation.org). DLS also acknowledges support from the RAPIDD program of the Science & Technology Directorate, Department of Homeland Security, and the Fogarty International Center, National Institutes of Health (http://www.fic.nih.gov). DAF is supported in part by the US National Institutes of Health grant AI079709. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions
GLJ, DLS, and DAF conceived of the research; GLJ developed the mathematical model; GLJ wrote the paper, with input from DLS and DAF.

Competing Interests
All authors have declared that no competing interests exist.

Abbreviations
Parasitized red blood cells, PRBC.
Supporting Information

Text S1. Mathematical description of the model, along with parameter fitting.
Figure Legends

Figure 1. Illustration of asexual, sexual, and infectivity outputs. Our mechanistic *P. falciparum* infection model was run six times to simulate three untreated individuals. (A) Individual log\(_{10}\) asexual parasitemias as a function of the number of days post emergence of parasites from the liver into the bloodstream. The inset depicts the first 50 days of infection; triangles above indicate the first day of fever. The black line is the level of detectability by microscopy (10 PRBC/µL). (B) Daily gametocytemias of the same three individuals. The gametocytemias are usually ~2 orders of magnitude less than the asexual parasitemias a few days prior. (C) Estimated probability of human to mosquito transmission. The x-axis maximum is changed from 700 to 200 as none of the 3 individuals were predicted to be infectious after day 152. The areas under the infectivity curves are 19.3, 25.7, and 23.0 days. Areas under the infectivity curves are equivalent to the number of fully infectious days, \(D\). Although the model predicts the persistence of long-lived low-level and sub-detectable infections (as observed in malaria therapy), (C) illustrates that these infections are usually not transmissible after the initial period of infection.

Figure 2. Modeled Distribution of Human Infectiousness. Using our mechanistic model, we calculated the mean daily human infectiousness to mosquitoes as a function of time post emergence. To calculate this function we simulated the daily infectiousness of 1000 untreated individuals and calculated the arithmetic mean per day. (A) Mean daily infectiousness for the first 300 days in red; the area between the 25\(^{th}\) and 75\(^{th}\) daily infectivity percentiles is shown in blue. (B) We integrated out the temporal component of human infectiousness and calculated the net infectivity for each of 1000 individuals. The distribution of net human infectivity is
represented as a violin plot. The plot extends to the maximum infectivity; the red cross illustrates the arithmetic mean infectiousness; the green box shows median infectiousness.

**Figure 3. Comparison of cumulative distributions of durations of infection.** The cumulative distributions of the durations of infection for the malaria therapy data, as well as those of the mechanistic model and the models of Lawpoolsri et al. and Okell et al. are shown. The distribution from the malaria therapy data comes from fitting a Gompertz probability distribution to the durations of infection from 54 patients as reported by Sama et al. The cumulative distribution function of the best-fit Gompertz distribution is plotted in grey. The mechanistic model cumulative distribution was generated by calculating the duration of infections from 1000 runs and plotting the empirical cumulative distribution function. The distributions from Lawpoolsri et al. and Okell et al. were generated running those models according to the mathematical assumptions of each model. The malaria therapy and mechanistic model distributions show relatively tight fits throughout the course of the distribution. The durations of infections for the malaria therapy data and the mechanistic model are defined as the last observable day by smear minus the first observable day; the durations for the compartmental models are defined as the durations of time in infectious compartments.

**Figure 4. Comparison of Mean Net Infectivity over Time, \( D(t) \).** The mean net human infectiousness to mosquitoes is calculated as a function of time for three models: the mechanistic model as well as the stochastic representations of the models of Lawpoolsri et al. [26] and Okell et al. [27]. For each model, the mean daily infectiousness of 1000 untreated individuals was simulated. (A) Distributions for the first 800 days; (B) shows only the first 200 days for closer inspection. These functions were then scaled and plotted (C) so that all three models had the same mean net infectivity (33.5 days) as 1000 runs of the mechanistic model.
Figure 5. Comparison of Net Human Infectivity Distributions, $D_i$. The net human infectivity distributions were calculated for three models: the mechanistic model as well as the stochastic representations of the models of Lawpoolsri et al. [26] and Okell et al. [27]. (A) The infectivity for each of 1000 individuals was integrated over time for each model. These distributions are represented as violin plots; the plot extends to the maximum infectivity. (B) The distributions in (A) were rescaled by multiplying by a scaling factor such that all three distributions had the same mean as that of the mechanistic model. Red crosses illustrate arithmetic mean infectivity; green boxes, median infectivity.
Figure 1

A

Log$_{10}$ parasitized red blood cells per µL

B

Log$_{10}$ gametocytes per µL

C

Probability of transmission
Figure 2

A

Number of days after emergence from liver

Asexual parasite densities (parasitized red blood cells/μL)

B

Days Post Emergence

Mean Daily Human Infectivity

C

Distribution of Net Infectiousness of Simulated Infections

Net Infectious Days
Figure 3

![Graph showing P(Duration of Infection ≤ x Days) vs Days. The graph compares different datasets: Mechanistic Model, Malaria therapy data, Lawpoolsri et al., and Okell et al.](image)
Figure 4

(A) Mean Daily Human Infectivity

(B) Mean Daily Human Infectivity

(C) Mean Daily Human Infectivity

Mechanistic Model
Lawpoolsri et al.
Okell et al.
Figure 5

A

B

Net Infectious Days

Johnston et al. mechanistic  Lawpoolsri et al. one compartment  Okell et al. four compartment

Net Infectious Days

Johnston et al. mechanistic  Lawpoolsri et al. one compartment  Okell et al. four compartment
Table 1. Best-fit Model Parameter Constants and Distributions

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter name</th>
<th>Reference value</th>
<th>Current value/distribution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asexual</td>
<td>$k_c$</td>
<td>0.2 $^1$</td>
<td>0.164</td>
<td>Affects levels of innate immune response to total parasitemia</td>
</tr>
<tr>
<td></td>
<td>$\sigma$</td>
<td>0.02 $^1$</td>
<td>0.15</td>
<td>Affects decay rate of acquired immune response to PEMP1 variant</td>
</tr>
<tr>
<td>Asexual</td>
<td>$P_c^* / k_c$</td>
<td>Fitted to case history $^1$</td>
<td>truncated ln($\mu, \sigma^2$) $</td>
<td>\mu = \ln(10^{14.8}), \sigma = 1.148$, truncation point = 5.5</td>
</tr>
<tr>
<td>Asexual</td>
<td>$P_m^* / k_m$</td>
<td>Fitted to case history $^1$</td>
<td>Gompertz($\alpha, \theta$) $</td>
<td>\alpha = 0.0311, \theta = 0.0004$</td>
</tr>
<tr>
<td>Asexual</td>
<td>$m_0$</td>
<td>truncated $\mathcal{N}(\mu, \sigma^2)$ $</td>
<td>\mu = 16, \sigma = 10.4$, truncation point = 1 $^1$</td>
<td>truncated $\mathcal{N}(\mu, \sigma^2)$ $</td>
</tr>
<tr>
<td>Gameocyte</td>
<td>$D_s$</td>
<td>Fitted to case history $^2$</td>
<td>round(truncated $\mathcal{N}(\mu, \sigma^2)$) $</td>
<td>\mu = 7, \sigma = 1.5$, truncation points = 4, 12</td>
</tr>
<tr>
<td>Gameocyte</td>
<td>$\gamma$</td>
<td>Fitted to case history $^2$</td>
<td>truncated ln($\mu, \sigma^2$) $</td>
<td>\mu = -6, \sigma = 4$, truncation point = .189</td>
</tr>
<tr>
<td>Gameocyte</td>
<td>$\alpha_0$</td>
<td>Fitted to case history $^2$</td>
<td>$U(0.06,1)$</td>
<td>Rate at which age affects gametocyte mortality</td>
</tr>
<tr>
<td>Gameocyte</td>
<td>$\beta$</td>
<td>Fitted to case history $^2$</td>
<td>0.0013</td>
<td>Effects of previous asexual parasitemias on gametocyte death rates</td>
</tr>
<tr>
<td>Gameocyte</td>
<td>$\mu_0$</td>
<td>Fitted to case history $^2$</td>
<td>0.03</td>
<td>Initial age-related component of total gametocyte mortality rate</td>
</tr>
</tbody>
</table>


Table 1. Best-fit Model Parameter Constants and Distributions. The best-fit parameters for the asexual and gametocyte components of our mechanistic model are shown. These parameters are either constants or chosen from probability distributions. The original values for these parameters are also provided, along with a description of their usage. ‘Fitted to case history’ indicates that the model was run with this parameter as a free parameter and the best-fit value chosen after fitting outputs to the case history of an individual treated with malaria therapy.
<table>
<thead>
<tr>
<th>Table 2. Asexual Model Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Minimum observed from 34 malaria therapy patients</td>
</tr>
<tr>
<td>Median observed from 34 malaria therapy patients</td>
</tr>
<tr>
<td>Maximum observed from 34 malaria therapy patients</td>
</tr>
<tr>
<td>Mean minimum model values</td>
</tr>
<tr>
<td>Mean median model values</td>
</tr>
<tr>
<td>Mean maximum model values</td>
</tr>
<tr>
<td>Model values for mean of best-fits to median malaria therapy values</td>
</tr>
<tr>
<td>2-1 Initial slope</td>
</tr>
<tr>
<td>2-2 Log density at first local maximum</td>
</tr>
<tr>
<td>2-3 Number of local maxima</td>
</tr>
<tr>
<td>2-4 Slope of local maxima</td>
</tr>
<tr>
<td>2-5 Geometric mean (GM) of the intervals between consecutive local maxima</td>
</tr>
<tr>
<td>2-6 SD of the logs of the consecutive local maxima</td>
</tr>
<tr>
<td>2-7 Proportion of positive observations in the first half of the interval between first and last positive day</td>
</tr>
<tr>
<td>2-8 Proportion of positive observations in the second half of the interval between first and last positive day</td>
</tr>
<tr>
<td>2-9 Last positive day</td>
</tr>
</tbody>
</table>


2 Model values are from the mean of 50 trials, each utilizing 50 draws from 1000 runs with replacement. Goodness-of-fit is according to the pseudo-$\chi^2$ statistic.

Table 2. Asexual Model Validation. The nine malarometric indices described by Molineaux et al. from analysis of malariatherapy data are listed at left. The next three columns provide the minimum, median, and maximum values observed for each of these indices from 34 malariatherapy patients. In order to examine the model fit, we bootstrapped our model outputs as follows. The mechanistic malaria model was run with default parameters 1000 times and samples of 50 runs each were selected. The maximum and minimum values for the nine indices, as well as the best-fit run, were generated from these 50 runs. This procedure was then repeated 50 times, and the mean of the minimum, best-fit, and maximum values were calculated. These values are displayed at right. The end time for all runs was 801 days.
### Table 3. Gametocyte Model Validation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D</th>
<th>gbar</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Table 1, N = 113&lt;sup&gt;1&lt;/sup&gt; Simulated&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Table 1, N = 113&lt;sup&gt;1&lt;/sup&gt; Simulated&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Table 1, N = 113&lt;sup&gt;1&lt;/sup&gt; Simulated&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.0</td>
<td>4.04</td>
<td>2.70E-04</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>7.4</td>
<td>6.93</td>
<td>0.0064</td>
</tr>
<tr>
<td>Maximum</td>
<td>12.0</td>
<td>10.78</td>
<td>0.135</td>
</tr>
</tbody>
</table>


<sup>2</sup> Model values are from the mean of 50 trials, each utilizing 113 draws from 1000 runs with replacement.

**Table 3. Gametocyte Model Validation.** A comparison of three gametocytemic parameters is shown. The parameters are D, the gametocyte sequestration time in days; gbar, the gametocyte conversion rate as a proportion of asexuals; and L, the length of time gametocytes persist in circulation, are given. The first row for each parameter lists their values from fitting the model of Eichner et al. to the data from 113 malarial therapy patients; the second row gives the values from the mechanistic malaria model using default assumptions. The end time for all
Supplemental Text S1: Model equations and distributions

I) Alternative Mechanistic Model Frameworks

II) Model of Asexual Parasitemia
   a. Basic asexual parasite dynamics
   b. Switching rates among PfEMP1 variants
   c. Host immune functions
   d. Model fitting
   e. Malaria mortality

III) Model of Gametocytemia
   a. Model fitting

IV) Distribution of First Fever Onset

V) Host Infectivity to Mosquitoes

VI) Fitting Untreated Model to Malaria Therapy Data

VII) Mathematical Description of Mean Transmissive Capacity Function

VIII) Coding Specifications
I) Alternative Mechanistic Model Frameworks

When developing this model, our desiderata included four characteristics. Firstly, we wanted to develop a model that was parameterized from clinical and/or field data. Secondly, we wanted our model to be stochastic to allow for simulation of the full range of host-parasite responses observed in our target dataset. Thirdly (and fourthly) we wanted our model to simulate the daily levels of both asexual and sexual stage parasites. These features would allow us to simulate human infectiousness over time as well as calculating the effect sizes of various antimalarials.

We are aware of three mechanistic modeling frameworks that have been built to simulate the within-host progression of malaria from infection through human-to-mosquito transmission. However, none of these frameworks satisfied all of our desiderata. One existing mechanistic modeling framework, developed and described over three reports [1-3] does not allow for simulation of random individuals and thus does not allow for reproduction of the full range of host-parasite interaction. In a second framework, developed and described in two reports [4,5], infectivity is a function of the asexual parasite density, and gametocyte densities are not modeled. While this approach is sensible for vaccine modeling, we need the capability of simulating gametocyte densities in order to determine the effects of drugs against both asexual and gametocyte densities. The third mechanistic framework [6] does not allow for stochastic simulation of parasite-host dynamics; the model is mean-fitting only and does not simulate of the range of diversity demonstrated in the malaria therapy data.

Since our model includes these four features, our model allows for daily calculations of infectivity for treated and untreated individuals of varying resistance levels and types. We describe this model in detail below.
II) Model of Asexual Parasitemia

a. Basic Asexual Parasite Dynamics

The model used to calculate asexual parasitemia is a within-host model that simulates the course of an infection once merozoites have emerged from the liver (sporozoites are not modeled). This model was developed by Molineaux et al. and is described in more detail here [7]. The model was fit to malaria therapy patients, in which infections are monoclonal and there is no superinfection. Although the authors had data on 334 malaria therapy patients, they chose to fit their model to 35 *P. falciparum* infections, which were those classified as ‘spontaneous cures’ (although some received low-dose suppressive treatments). Thus, this asexuals model best reproduces the time course of asexual parasitemias in naïve adult male patients who exhibited strong native immune responses, and we note that some parameters are possibly biased by these low-dose treatments.

The number of parasitized red blood cells (PRBCs) is calculated every two days. Red blood cells exhibit antigenic variation by displaying different *PfEMP1* profiles. There are three immune functions, each of which responds to a different set of stimuli (described below). Each variant is assumed to grow at a different rate $m_i$. This assumption has elicited some discussion and controversy [1,7,8]; however, there is experimental evidence that some *PfEMP1* isotypes grow faster than others *in vivo* (specifically, those isotypes that are associated with severe disease have been shown to grow faster than those that are not) [9]. These net growth rates might be caused by differential sequestration, faster intrinsic growth, etc.; however, we do not model the specific processes causing variant-specific growth rates here. We also note that the assumption that infections are monoclonal may not be overly restrictive; a modeling study
showed that parasite dynamics in multiply infected individuals may behave similarly to monoclonal infections [10].

i. **Quantities Modeled**

$P_i(t)$ is the number of red blood cells infected by *Plasmodium falciparum* parasites displaying PfEMP1 type $i$ at time $t$.

$P_c(t)$ is the cumulative number of number of red blood cells infected at time $t$.

ii. **Constants and Parameters**

$s$: Probability isotype will switch $var$ expression in the next period

$v$: Number of isotypes ($Pf$EMP1 variants)

$\mu_m, \sigma_m^2$: Parameters for normal distribution describing isotype-specific growth rates

$minparasit$: Minimum parasitemia allowed by model

$m_i$: Growth rates of different $Pf$EMP1 variants; **stochastic** with distribution $N(\mu_m, \sigma_m^2)$ truncated so that $m_i \geq 1$

iii. **Equations Determining Asexual Parasitemia**

$$P_i(t + 2) = \left(1 - s\right)P_i(t) + sp_i(t)\sum_{j=1}^{v} P_j(t)m_jS_c(t)S_i(t)S_m(t)$$

$$P_i(t + 2) = \begin{cases} P_i(t + 2) & \text{if } P_i(t + 2) \geq \text{minparasit} \\ 0 & \text{otherwise} \end{cases}$$

$$P_c(t) = \sum_{i=1}^{v} P_j(t)$$

b. **Switching Rates Among PfEMP1 Variants**

As mentioned above, $Pf$EMP1 isotype levels are explicitly modeled. The model assumes that all isotypes share a fixed probability (the parameter $s$) of switching to a different isotype in the next
period. However, the isotype that they switch to in the next period is assumed to be inversely related to the immune response against that type.

This assumption that PfEMP1 switching is influenced by factors outside of the RBC has generated considerable discussion [1,7,11,12]. Though it is possible that var expression is mediated by external factors, we do not need to assume that immune responses affect var switching in vivo, even though our model implicitly uses immune responses to mediate switching. Complicated models of var switching utilizing branching pathways have recently been constructed that allow for var gene switching without signaling [13]. These models allow for parasites to avoid exhausting their var gene repertoire early in the course of infection. Parasites can thus persist for much longer than if they were switching among all var genes with an equal probability.

This branching-process mediated var gene switching may result in var genes being expressed as if expression were directly mediated by antigenic response. The asexuals model employed here thus makes a simplification that may in practice mimic the much more complicated picture of var gene expression within the host.

i. **Quantities Modeled**

$p_i(t)$: Probability that an infected red blood cell will display PfEMP1 on its surface at time $t + 2$.

ii. **Constants and Parameters**

$q$: Parameter for geometric distribution affecting isotype-dependent switching probability

$minresponse$: Minimum immune response provoking var switching

iii. **Equations Determining Switching Probability**
\[ p_t(t) = \begin{cases} 
0 & \text{if } S_t(t) < \text{minresponse} \\
\frac{q^t S_t(t)}{\sum_{j=1}^{q^t} S_j(t)} & \text{otherwise}
\end{cases} \]

\[ S_c(t) \] represents the innate immune response to the total parasite load, irrespective of PfEMP1 type. The two other immune responses modeled here are acquired and are dependent on antibody production. \( S_i(t) \) represents the PfEMP1 variant-specific immune response. This response is mediated by IgM and IgG and develops in response to one specific PfEMP1 isotype. \( S_m(t) \) represents the acquired PfEMP1 variant-transcending immune response. This immune response is provoked by the conserved regions of PfEMP1 (since PfEMP1 variants have been shown to induce cross-reactivity) as well as conserved surface proteins (such as MSP-1) and other antigenic variants.

i. **Quantities Modeled**

\( S_c(t) \) is the PfEMP1 variant-transcending innate immune response. \( S_i(t) \) is the PfEMP1 variant-specific acquired function response. \( S_m(t) \) is the PfEMP1 variant-transcending acquired immune function response.

ii. **Constants and Parameters**

\( k_c \): Affects levels of innate immune response to total parasitemia

\( P_c^* \): Affects levels of innate immune response to total parasitemia

\( k_y \): Affects levels of acquired immune response to PfEMP1 variant

\( P_y^* \): Affects levels of acquired immune response to PfEMP1 variant

\( \sigma \): Affects decay rate of acquired immune response to PfEMP1 variant

\( k_m \): Parameter determining levels of acquired immune response to cross-reactive epitopes
δ_v: Delay in onset of acquired immune response to PfEMP1 variant

β: Affects levels of acquired variant-transcending immune response

P_m*: Affects levels of acquired variant-transcending immune response

δ_m: Delay in onset of variant-transcending acquired immune response

ρ: Affects decay rate of variant-transcending acquired immune response

C: Level of parasitemia above which variant-transcending immunity does not increase

\( \frac{P_c}{k_c} \): Asexual parasite density at the first peak of parasitemia (almost always also the maximum asexual parasitemia); **stochastic** following a truncated log-normal distribution with a mean of \( 10^{4.79} \) and a standard deviation of 1.148 [10]

\( \frac{P_m}{k_m} \): First asexual parasitemia observation day minus the last asexual parasitemia observation day; **stochastic** following a Gompertz distribution with shape parameters (.0311, .0004) chosen to best match the data from Sama et al. [14]

### iii. Equations Determining Host Immune Functions

\[
S_c(t) = \left( 1 + \left( \frac{1}{P_c^*} \sum_{\tau=0}^{t-\delta_v} P_l(\tau) e^{-\sigma(t-\tau-\delta_v)} \right) k_v^{\delta_v} \right)^{-1}
\]

\[
S_l(t) = \left( 1 + \left( \frac{1}{P_v^*} \sum_{\tau=0}^{t-\delta_v} P_l(\tau) e^{-\sigma(t-\tau-\delta_v)} \right) k_v^{\delta_v} \right)^{-1}
\]

\[
S_m(t) = (1 - \beta) \left( 1 + \left( \frac{1}{P_m^*} \sum_{\tau=0}^{t-\delta_m} \hat{P}_c(\tau) e^{-\rho(t-\tau-\delta_m)} \right) k_m^{\delta_m} \right)^{-1} + \beta
\]

\[
\hat{P}_c(t) = \begin{cases} 
P_c(t) & \text{if } P_c(t) < C \\
C & \text{otherwise}
\end{cases}
\]

Case-specific parameters:
\[ P_c^* = k_c \cdot (\text{first maximum local density}) \]
\[ P_m^* = k_m \cdot [(\text{last pos. day}) - (\text{first pos. day})] \]

d. Model Fitting

In order to ensure that the model accurately simulates the time-course of parasitemia in an immunologically naïve individual, we consulted the paper of Molineaux et al. to examine how their model was fit to data. In this paper, the authors define 9 quantities from a parasitemia time-course as indices (such as initial slope of the parasitemia curve); we use these indices here as well [7]. Note that we define a local ‘peak’ in asexual parasitemia for the purpose of developing an index value as a parasitemia a) greater than the 6 values preceding it and b) greater or equal to the 6 values following. This is the definition of a peak found in Molineaux et al. [7]; however, Eichner et al. add a third criterion, that the parasitemia needs to be c) greater than or equal to 100 PRBC/µL [15]. This latter criterion is added when using the asexual parasitemia to calculate gametocytemias.

In the original paper, the authors attempted to fit their model to 35 actual case histories, according to how well their model fit these 9 indices. Because we are hoping to reproduce the range of phenomena seen in the malaria therapy trials, our simulation targets consist of the medians of the indices generated from the entire set of 35 malaria therapy trials.

As a measure of fit, the authors construct a pseudo-\( \chi^2 \) statistic, such that the distance between a model run and the experimental data is given by the formula

\[ D(x_1, ..., x_9) = \sum_{i=1}^{k=9} \frac{(x_i - E_i)^2}{E_i} \]

where \((x_1, ..., x_9)\) are the 9 index values for the model run and \((E_1, ..., E_9)\) are indices from the experiment (here, the median malaria therapy values) [7]. We call this statistic a ‘pseudo-\( \chi^2 \).
statistic’ for the following reason. If we used the variance of each index in the denominator, and
assumed that $x_i$ were distributed normally, then the value ($D$) will have a $\chi^2$ distribution. This
sum $D$ would then be known as a $\chi^2$ statistic. Also, if we knew that each of these indices is
Poisson distributed, and that the means ($E_1, ..., E_9$) were large (>5, so that the Poisson was an
acceptable approximate to the normal) then the above formula for $D$ would also be distributed as
$\chi^2$ (approximately). However, since we have no reason to assume that all of these 9 indices are
Poisson distributed, and even if they were their means are small in some cases, we cannot
assume that the variance of each index is equal to its mean. Thus, the above sum may not
converge to a $\chi^2$ distribution. We therefore call the above statistic a ‘pseudo-$\chi^2$ statistic,’ since it
takes the general form of a $\chi^2$ statistic, but is not necessarily $\chi^2$ distributed, and we employ it as
the measure of goodness of fit.

A smaller distance between generated and observed values yields a smaller pseudo-$\chi^2$
value and indicates a better fit. If the statistic were a true $\chi^2$ statistic, i.e., $D(x_1, ..., x_k) \sim \chi^2(k)$,
then with $k = 9$, the p-value for $\alpha = .05$ would be 16.92. This p-value gives a rough estimate of
when model and empirical values differ significantly.

To generate Table 2, we used this measure of goodness of fit to evaluate which runs best
matched the medians of the malaria therapy data. In the original paper by Molineaux et al., after
parameters were fit to each patient’s malaria therapy data, the authors ran their stochastic model
and chose the best fit from 50 runs for each patient. These ‘best simulations’ were then compared
to the malaria therapy data for statistical analysis. We utilize a similar methodology here but
bootstrap our simulations in order to reduce random variation. The mechanistic malaria model
was run with default parameters 1000 times and a sample of 50 runs was selected from this pool.
The maximum and minimum values for the nine indices, as well as the best-fit run, were
selected. This procedure was then repeated 50 times, and the mean of the minimum, best-fit, and maximum values were calculated. These values are displayed in Table 2. The end time for all runs was 801 days.

A further validation of the asexual component of the model is found in Figure 3. In this figure the cumulative distribution of the durations of infection for malaria therapy patients is generated in red. This distribution (Gompertz) resulted from a series of fitting exercises to the malaria therapy data; details are found in Sama et al. [14]. The mechanistic model cumulative distribution is shown in blue, generated using default assumptions; the model generates infections that last approximately 10 days less than observed in malaria therapy; however, the fit throughout most of the distribution is relatively tight.

e. Malaria Mortality

Mortality from malaria is highest among children in highly endemic areas, whereas in low endemicity regions the burden of mortality is spread more uniformly throughout the age distribution [16]. However, in some low endemicity regions, few individuals actually die from any particular malaria infection. An epidemiological study in a region of western Thailand with low and seasonal transmission (estimated incidence rate of 1.0 infections per person per year) yielded a case fatality rate of 1.9 deaths per 1000 infections, with the chance of death declining exponentially with age [17]. Further, mortality was low even though many adults were hyperparasitemic (defined as >4% parasitized red blood cells by smear). Indeed, of the 133 adults hospitalized during the study period, 82 were hyperparasitemic [17]. This mortality level is probably an underestimate, as this area benefitted from effective health care; however, the magnitude of this value demonstrates that mortality is likely not a significant factor affecting malaria transmission by adults in low transmission settings with adequate health care. Because
our model reproduces the dynamics of malaria in low transmission areas such as western Thailand, we do not include mortality in our model.

III) Model of Gametocytemia

Gametocyte levels are modeled as a function of past asexual parasitemias. The gametocytemia model used here is that described by Diebner et al. and Eichner et al. (although the two models differ slightly; see below) [15,18].

Each wave of asexual parasitemia produces gametocytes with a characteristic frequency. The fraction of gametocytes produced from each wave is determined by a function $\gamma()$. Gametocytes are assumed to sequester for a variable number of days as they develop. Only fully mature gametocytes are counted toward the total number of gametocytes. Once the mature gametocytes emerge, they are cleared by the immune system or die on their own. The lifetimes of the gametocytes, in absence of immune response related to asexual parasitemia, follow a Gompertz distribution [18]. The level of immune response is assumed to be related to the cumulative levels of asexual parasitemia.

i. Quantities Modeled

$G(t)$ is the number of mature gametocytes circulating in the bloodstream. The gender of gametocytes is not specifically modeled (although mature gametocytes are often found in male:female ratio of 1:4, and so gender could be predicted if needed) [19].

ii. Constants and Parameters

$D_s$: Sequestration time for gametocyte maturation; **stochastic** with truncated normal distribution (lower limit, 1; upper limit, 33, mean, 7; standard deviation, 1.5) then truncated again to lie in interval (4, 12)
\( \gamma \): Asexual to sexual conversion probability, peak specific; **stochastic** following log-normal distribution with location parameter of -6 and a scale parameter of 4 in natural log space

\( \alpha_G \): Rate at which age affects gametocyte mortality; **stochastic** with uniform distribution between .06 and 1

\( \beta \): Effects of previous levels of asexual parasitemias on gametocyte death rates

\( \mu_0 \): Initial age-related component of total gametocyte mortality rate

**iii. Equations Determining Gametocytemia**

\[
G(t) = \sum_{\tau = D_s+1}^{t} \gamma (\tau - D_s) P_c(\tau - D_s) \cdot e^{-\left(\frac{\mu_0}{\alpha_G}(e^{\alpha_G(1-\tau)}-1) - \beta \sum_s^{\tau} \log(P_c(s)+1)\right)}
\]

Diebner et al. and Eichner et al. disagree on whether \( \log(A(s) + 1) \) or \( \log(A(s) + 1) \) should be used in the above equation [15,18]. The latter formulation is correct, as we now show. (We use the notation \( A(t) \) for the asexual parasitemia at time \( t \) for this proof, in line with the above references.)

To prove this proposition, we shall assume that \( \log(A(s) + 1) \) is correct and show that this assumption gives a result in line with their shared description of gametocyte-related death rates.

To derive the asexual density-dependent component of the equation determining gametocytemia, we assume that the hazard (i.e. death) rate is \( \lambda(t) = \beta \log(A(t) + 1) \) and so the cumulative hazard function is \( A(t) = \int_{\tau}^{t} \beta \log(A(t) + 1) \), where \( \tau \) is the starting time.

However, we have by definition that \( A(t) = -\log(S(t)) \), so \( \int_{\tau}^{t} \beta \log(A(t) + 1) = -\log(S(t)) \), and thus \( S(t) = e^{-\beta \sum_s^{\tau} \log(A(s)+1)} \), where \( S(t) \) is the survival function (i.e. the probability that a gametocyte will survive to time \( t \)). Note that in this model we use the daily sum as an approximation for the integral.
If we consider only one day’s contribution to the survival probability, i.e., the probability that a gametocyte will survive one more day given that it has already survived to that point, we have\[P(S(t)|P(S(t - 1) = 1)) = e^{-\beta \log(A(t) + 1)} = (A(t) + 1)^{-\beta}.\]

Thus, the probability that a gametocyte will die in a one day interval is \(1 - S(t) = 1 - (A(t) + 1)^{-\beta}\), in agreement with Eichner et al. In order to account for this discrepancy, we adjusted the value of \(\beta\) given in Diebner et al. by multiplying a conversion factor \(\left(\frac{1}{\ln 10}\right)\).

a. Model Fitting

Although the models described in Eichner et al. and Diebner et al. are similar, their parameter estimates differ. The gametocyte model utilizes five different parameters (described in Diebner et al.): \(\gamma, D_s, \alpha_G, \beta, \mu_0\). Both of the papers use malaria therapy data to parameterize their models, but the two differ in their choice of which patients to include in their analysis.

In the paper by Diebner et al., the gametocytogenesis model was fit to a subset of the malaria therapy data, choosing patients who had \(P.falciparum\) inoculations and at least four positive gametocyte observations. Out of 334 malaria therapy patients with \(P.falciparum\) inoculations, 262 had at least four gametocyte positive observations. Diebner et al. reported the quantiles of the five parameters from fits to their selected subset of patients; however, the model was fit to the first 100 days of infection only [18].

In the paper by Eichner et al., the model was fit to a smaller subset of the malaria therapy data [15]. The result of this selection was that out of the same 334 malaria therapy patients, only 113 were chosen for the parameter fitting. Due to their exclusion criterion (iv), it is probable that the infections included in the Eichner analysis were less severe than those included in the Diebner study. (The study by Eichner et al. omitted all patients with gametocytemia densities >
100/µl). The end result of the different exclusion criteria is that a model using the parameters from Diebner et al. may need to be adjusted to fit the data as reported in Eichner et al.

In order to assess how well the model outputs match the malaria therapy data, two indices were developed. These are the average asexual-sexual conversion probabilities, \( \bar{g} \), and average mature gametocyte circulation times, \( L \) [15]. Eichner et al. reported minimum, mean, and maximum values for \( D_s \), \( \bar{g} \), and \( L \) as estimated from their subset of patients, along with information about the observed distributions of these parameters. We use \( \bar{g} \), and \( L \) as our simulation targets.

We decided to allow three of the parameters \( (D_s, \gamma, \alpha_G) \) to be stochastic. Our choice of these three parameters was driven partly by the data: Eichner et al. reported that the sequestration delay parameter \( D_s \) roughly followed a normal distribution, and Diebner et al. reported that their model did not fit well with a constant value of \( \gamma \) for each infection. For \( D_s \) we chose a truncated normal distribution for our model (with endpoints from the min and max observed by Diebner et al.). To fit the standard deviation, we created a loss function from the absolute value of the difference between the observed quantiles in Diebner et al. and generated quantiles from various normal distributions. When we included the last quantiles in Diebner et al. (33 days for sequestration), the normal fits were not satisfactory, so we omitted that point and used the remaining quantiles for our loss function (data not shown). It was found that a standard deviation of 1.5 best fit the quantiles from Diebner et al. However, because we regarded the tails of this distribution to be biologically implausible, after the above fitting we then truncated this distribution again to fit between the minimum (4 days) and maximum (12 days) as reported by Eichner et al. For the distributions for \( \gamma \) and \( \alpha_G \), we noted that Eichner et al. reported that \( \bar{g} \) roughly followed a lognormal distribution, and we chose parameters and distributions that gave
best fits to the gametocytemic targets $g$ and $L$. Because our system was overdetermined (more parameters than usable data), we set $\beta$ and $\mu_0$ to be fixed at their median values as reported by Diebner et al. (subject to the correction in section c above). Table 2 compares the results of running the model using these distributions against the target data.

IV) Distribution of First Fever Onset

Our model of the effects of antimalarials on transmission assumes that the day of first fever occurrence can be predicted. To determine when the fever occurs, we utilized a study by Dietz et al. [8]. In this analysis, the authors developed a model to describe the first wave of parasitemia from malaria therapy patient data.

Dietz et al. built a mathematical model of the first wave of parasitemia and parameterized it from the first wave in the malaria therapy patients. The model-predicted asexual parasitemias were then correlated with the occurrence of fever. To predict when a fever would occur, the authors utilized a fever threshold, i.e., a level above which an individual would have a fever. The authors ran their model 2000 times to generate a set of asexual parasitemias, and fever thresholds were then randomly chosen for each individual according to a uniform probability distribution. The resulting distribution of fever times from the 2000 simulated cases was found to visually match the distribution of the fever times from the original 100 patients quite well [8].

We note that some of the malaria therapy patients for which data was available were excluded from their modeling: of the 334 malaria therapy patients, 100 were included in their analysis. It is unclear whether or how their selection criteria introduced selection bias into the fever model. However, all of the individuals included in their analysis had a fever in the first wave of parasitemia, and this was not necessarily the case for all of the patients in general. Thus,
it is possible that fevers might occur later in the course of parasitemia than the model would predict. The effect of this selection bias then may be to predict a fever day that is too early, which would have the effect of increasing the effectiveness of ACTs at interrupting transmission (because there would be a lower parasitemia at the predicted fever day than in actuality).

i. Quantities Modeled

feverday: The predicted first fever day of an individual

\( P_{star} \): Fever threshold; this value is stochastic and follows a uniform distribution

fevconst: Lower limit of uniform distribution determining fever threshold

\( max(P_{tot}) \): Maximum level of asexual parasitemia

ii. Constants and Parameters

iii. Equations Determining First Fever Onset

\[ P_{star} = max(P_{tot}) \cdot 10^{(\log_{10}(fevconst))}, \]  where \( U(a, b) \) is a draw from a uniform random variable with lower bound \( a \) and upper bound \( b \)

V) Host Infectivity to Mosquitoes

In the Ross-MacDonald model, infectivity of humans to mosquitoes is parameterized by a constant, \( c \) [20]. Note that a successful human to mosquito infection can be defined by a variety of endpoints, whether the production of oocysts in the midgut or the development of (functional) sporozoites. For this paper we define a successful infection of a mosquito as the production of an oocyst, following Jeffery [21].

The relationship between gametocyte levels and host infectivity to mosquitoes has been quantified by a variety of mosquito feeding studies, in which mosquitoes are fed on infected individuals whose gametocyte levels have been assessed. Using these relationships, simulated
individuals’ infectiousness to mosquitoes can be calculated, given their predicted gametocyte levels. Stepniewska et al. report their results of curve-fitting the percentage of mosquitoes infected when fed on individuals with various gametocyte levels [22]. Stepniewska et al. report two infectivity curves: the relationship between gametocytemia and infectivity in malaria therapy patients and that found among Gambian children [21,23]. Because we have assumed immunologically naïve hosts for this modeling process, we utilize the relationship derived from the malaria therapy patients. These empirical gametocyte-infectivity relationships use the total number of peripheral gametocytes to predict infectivity; however, the relationships do not necessarily assume that all peripheral gametocytes are infectious. Rather, these relationships are highly non-linear, and that the causes of this non-linearity are still under analysis. We account for the fact that 2 gametocytes need to be present in a bite in order to allow for transmission by setting the minimum gametocytemia that permits transmission to be 2 gametocytes per 3 µl (where 3 µl is the approximate average volume of blood in a mosquito bite [24]). The malaria therapy infectivity curve predicts only a 1.7 percent chance of human to mosquito transmission at this level of gametocytemia.

Many other factors besides gametocyte density have been postulated to affect infectivity to mosquitoes. For this paper, we assume that there are no other factors that affect transmission besides gametocyte levels. Three possible factors not modeled here that might also affect transmission are the presence of fever, the effects of drugs on gametocytes beyond simple killing, and host immune factors. Regarding the effects of fever, there is some evidence that fever reduces the effectiveness of gametocytes at successfully transmitting in *P. vivax* [25]. However, according to an analysis of the malaria therapy data using *P. falciparum*, there is no direct relationship between fever and host infectivity to mosquitoes [26]. Thus, we have not
included an effect of fever on transmission in our simulations. Further, we are not aware of any human feeding studies that have examined whether gametocytes surviving exposure to artemether-lumefantrine are able to transmit as efficiently as untreated gametocytes. However, field evidence seems to support the proposition that gametocytes that survive drug exposure are still capable of transmission, and we assume that these gametocytes capable of forming viable sporozoites later in their lifecycle [27]. Finally, it is very likely that host antibodies may interfere with transmission and thus affect the relationship between gametocytemia and infectivity [28]. Because we are simulating transmission among immunologically naïve patients, however, antibodies to gametocytes will not be present before infection. And for those antibodies that arise during the course of an infection, the malaria therapy feeding study data implicitly include their effects on transmission.

A final note regarding infectivity is that of Jeffery and Eyles in their original 1955 study of mosquito feedings on malaria therapy patients [21]. First, the authors report that gametocytes generally become observable 10–15 days after parasite patency (recall that observability implies densities $\geq 10/\mu l$ for the purposes of this paper). When we ran the model as described above 100 times, we found similar values (although the model also generated larger values).

However, the authors also observe that, in the first two to four days after gametocytes are observable in the bloodstream of infected patients, individuals are not infectious to mosquitoes (what we call the “Jeffery-Eyles effect”). The authors attribute this phenomenon to the fact that, when gametocytes are first becoming patent, they are still immature and are thus unable to infect mosquitoes. To account for the observed non-infectivity of gametocytes appearing very early in the course of infection, we decided to adjust infectivity profiles slightly so that for individuals in which the difference between the first observable asexual and sexual parasitemias was 15 days or
less, these individuals would become infectious two days after gametocyte observability. For individuals with larger differences between asexual and gametocyte patency, or that never have an observable gametocytemia, we assume that individuals are not infectious until more than 17 days after initial asexual patency have passed. This adjustment roughly corresponds to the feeding study data reported by Jeffery and Eyles. The effect of this adjustment on total transmission is minimal; however, we have included this effect in our model.

i. Quantities Modeled

$c(x)$ is the infectivity of humans to mosquitoes (i.e. the percent chance that a mosquito bite will produce oocysts in the mosquito midgut), where $x$ is the level of gametocytes

ii. Constants and Parameters

$\text{mintrans}$: minimum gametocyte level that allows for transmission

iii. Equations Determining Host Infectivity

$$c(x) = \begin{cases} 
1.08 \cdot e^{-0.86 \cdot \log_{10}(x) - 1.48} & \text{if } x \geq \text{mintrans} \\
0 & \text{if } x > \text{mintrans}
\end{cases}$$

VI) Fitting Untreated Model to Malaria Therapy Data

With the model as specified above, we tested the outputs against a variety of malarialometric databases. The first data we used as comparison was developed by Molineaux et al. from malaria therapy patient data [7]. Molineaux et al. describe the development of nine malarialometric indices used to categorize and quantify the malaria therapy data. The authors then use this data to calibrate their asexuals model; these indices were also used by Gatton et al. [2] for model validation. In the model of Molineaux et al., the model parameters had to be set for to match the data for each patient; however, our model is stochastic and does not need to be calibrated on an individual basis. We designed the model so that our model would reproduce the median values of
the indices as seen in the malaria therapy data as well as exhibiting a similar range of variation. (Table S1) compares our model outputs using the default settings to the parameters from the model of Molineaux et al. as fit to the malaria therapy data; the model shows excellent agreement with both the median as well and the minimum and maximum values. This fit was achieved by careful choice of distributions for the stochastic parameters in the asexuals component of the model.

In order to examine the degree of fit of model outputs to gametocytemic data, we utilized data from Eichner et al. [7]. In this study, the authors utilized their gametocytemic model to generate gametocyte profiles from the asexual parasitemias of malaria therapy patients. They fit their model to match each patient’s relationship between asexual and sexual parasitemias, and reported quantiles of the three variable parameters: \( D \), the length of delay before gametocyte emergence; \( g \), the asexual to sexual parasite production ratio; and \( L \), the length of time gametocytes are observed in the circulation. (Table S2) shows the fits of the model to the maximum, minimum, and geometric means of these parameters; the model was run using default parameters. These results indicate that the model fits mean, minimum, and maximum asexual and sexual parasitemia levels for non-immune individuals quite well, using default assumptions. The length of time gametocytes are circulating matches not only the malaria therapy data but also model recent \textit{in vivo} data from the field as well [29].

VII) Mathematical Description of Mean Transmissive Capacity Function
Assume that there is a population of \( N \) individuals living in an area with \( j \) species of mosquito vector, each with vectorial capacity \( V_j(t) \). Let us further assume that individuals are infectious to
each species at a proportion $c_j$ of net their infectiousness; for simplicity, we assume $c_j = 1$, for all $j$. We define $T(i, t)$ of an individual $i$ at time $t$ as

$$T(i, t) = \sum_j b c_j V_j(t) D(i, t) = \sum_j b V_j(t) D(i, t)$$

We call $T(i, t)$ the transmission capacity of an individual $i$ at time $t$. The mean transmissive capacity of a population over time is

$$T(t) = \frac{\sum_i \left( \sum_j b V_j(t) D(i, t) \right)}{N}$$

For an individual $i$, their basic reproductive number $R_0(i)$ has a natural expression using this terminology

$$R_0(i) = \int_0^\infty T(i, t) dt$$

and the basic reproductive number for the population is

$$R_0 = \frac{\sum_i R_0(i)}{N} = \int_0^\infty T(t) dt = D \int_0^\infty \sum_j b V_j(t)$$

Note that the commutativity of sums and integrals in this expression is a result of the well-mixing assumption (i.e., humans and mosquitoes are well-mixed).

VIII) Coding Specifications

The model was built in MATLAB (Mathworks, Version R2011a). The model outputs include the random number generator seed values so that runs may be replicated exactly.
References


Chapter 2: Modeling Prospects for Malaria Elimination in Low-Endemicity Areas Through Early Treatment with Transmission-Blocking Drugs
Modeling Prospects for Malaria Elimination in Low-Endemicity Areas Through Early Treatment with Transmission-Blocking Drugs

Geoffrey L. Johnston\textsuperscript{1,2}, David L. Smith\textsuperscript{3\#}, and David A. Fidock\textsuperscript{1,4\#}

\textsuperscript{1}Department of Microbiology and Immunology, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
\textsuperscript{2}School of International and Public Affairs, Columbia University, New York, NY 10027, USA
\textsuperscript{3}Bloomberg School of Public Health, John Hopkins University, Baltimore, MD 21205, USA
\textsuperscript{4}Division of Infectious Diseases, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
Abstract

**Background:** Malaria elimination is a priority of increasing importance to the global public health community. However, a roadmap to achieve elimination is still unclear, especially in areas where the local ecology is favorable for high-level transmission of the most lethal human malarial parasite *Plasmodium falciparum*. Furthermore, evidence from Southeast Asia suggests that *P. falciparum* has begun to acquire resistance to artemisinin-based combination therapies (ACTs), a backbone of malaria control efforts. Mathematical modeling provides a tool to determine which interventions would have the greatest impact in driving towards malaria elimination.

**Methods and Findings:** We have developed a mechanistic, within-host model that makes use of recent pharmacokinetic (PK) and pharmacodynamic (PD) data to simulate the effects of ACTs and other drug regimens on parasite transmission. Human infectivity was modeled over the course of infections with and without drug treatment initiated at variable times, and the effects were examined in relation to transmission and the basic reproductive number $R_0$. These calculations were performed for both early and late treatment-seeking contexts in low transmission settings. Our model predicts that if all symptomatic individuals are treated early, then transmission is reduced 3.4-fold with drugs that target only the pathogenic asexual blood stage parasites, 5.5-fold with artesunate-mefloquine that targets asexuals and early stage sexual forms (immature gametocytes), and 7.0-fold with artesunate-mefloquine plus the transmission-blocking drug primaquine that acts on mature gametocytes. In late treatment settings, the fold reductions are 1.5, 2.1, and 2.7, respectively.

**Conclusions:** Malaria control efforts benefit substantially from the use of gametocytocidal drugs, including ACTs. Adding primaquine and other transmission-blocking agents to ACTs would
marginally enhance their transmission reduction effectiveness. In areas of low transmission ($R_0$ close to 1), antimalarials combining asexual and gametocytocidal action can be a highly effective tool for promoting elimination, if the majority of symptomatic individuals are treated promptly. However, the reductions in transmission are diminished if treatment is delayed or treatment coverage with effective antimalarials is poor (<50% infected individuals treated).
Introduction

Plasmodium falciparum, the most virulent of the Plasmodium species that cause malaria in humans, is responsible for hundreds of millions of cases and hundreds of thousands of deaths per year. The exact number of malarial deaths is a matter of considerable debate, with estimates for 2010 ranging from 655,000 to 1,238,000 [1,2]. Both of these studies agree that overall levels of morbidity and mortality have declined over the past decade, due at least in part to the worldwide scaling up of insecticide-treated bed nets and the use of artemisinin-based combination therapies (ACTs). ACTs are now the first-line antimalarial drugs in almost all of the malaria-endemic world and pair fast-acting yet short-lived artemisinin derivatives with a longer-lasting partner drug [3,4].

Given the substantial reductions in malaria morbidity and mortality achieved with these tools, public health and malarial infection experts are increasingly promoting elimination in areas of low transmission [5] while planning significant reductions in higher-transmission areas [6,7]. Major obstacles, however, stand in the way. These include insecticide and drug resistance [8,9], underdeveloped health systems, shifting public funding priorities, donor fatigue [5], malaria importation [10] and economic constraints. The complex life-cycle of P. falciparum also presents both challenges and opportunities for controlling malaria transmission [11]. The various life stages of P. falciparum differ in their levels of metabolic activity, their within-host locations, and their susceptibilities to antimalarials [12]. Mathematical modeling can help guide elimination efforts by providing quantitative predictions to assess the feasibility of achieving malaria elimination [13-16].

Here we attempt to clarify the currently complicated literature regarding the effects of ACTs on malaria transmission. We also attempt to quantify the extent to which transmission can
be reduced by adding late-stage gametocytocides such as primaquine and methylene blue to current ACTs. These drugs and their analogues are currently receiving considerable interest within the malaria community as to how they might be deployed to help interrupt transmission in certain areas [17-19].

To examine the role that antimalarials can play in reducing transmission, we report the development and utilization of a stochastic, mechanistic model that simulates the within-host progression of a malarial infection and its treatment. This model builds upon earlier work by Molineaux and Dietz, who developed much of the within-host malaria simulation and first evoked its possible extension to modeling antimalarials [20-24]. Our model also shares the same general design as other simulation models [25,26] though our model differs substantially in its specific within-host and PD assumptions. Our simulations begin approximately one cycle of replication after parasites have emerged from the liver and entered the blood as merozoites. These merozoites infect red blood cells, replicate, and develop before bursting from the infected cells to repeat the cycle of blood-stage infection. Some of these infected red blood cells sequester in the microvasculature to develop further and differentiate into sexual stages (‘gametocytes’); once mature, these gametocytes enter the bloodstream [27]. Mature male and female gametocytes are then primed to form gametes and mate in the *Anopheles* mosquito following blood meal ingestion. Our model simulates the total numbers of both asexual and gametocyte forms and allows us to estimate human infectivity over time [24,28-32].

To this model we have also added the PK-PD properties of existing and hypothetical artemisinin-based combination therapies (ACTs) against both asexual and sexual forms. Treatment is individual-specific and is based upon treatment-seeking behavior. We utilized field data [33] in the model calibration and validation as well as a new set of *in vitro* stage-specific
antimalarial gametocytemic data [17]. Predictions were compared to outputs derived from other models [34,35].

Using this combined model we have estimated the effectiveness of ACTs at reducing onward malaria transmission. Simulated combinations include artesunate-mefloquine (AM), AM plus primaquine, and AM plus methylene blue [3,36]. We find that highly gametocytocidal combination therapies are twice as effective at reducing human infectiousness than ‘schizonticidal’ drugs that act only on the pathogenic asexual stages under conditions where all symptomatic individuals are treated early. Thus, we confirm that gametocytocidal drugs can play an important role in reducing onward transmission of malaria [34] but that these reductions depend heavily on the timing of treatment: late treatment diminishes most of the transmission reductions achieved through ACT therapy.

**Methods**

**Model Description**

To simulate the effects of drug treatment on transmission, we chose to utilize and extend a previously developed, mechanistic within-host model developed by Molineaux, Dietz, and others [20-24] to reproduce the time courses of malaria therapy infections. For the malaria therapy data utilized in their model, immunologically naïve individuals with tertiary syphilis were infected with various strains of *P. falciparum* to induce a fever, and resulting asexual parasitemias and gametocytemias were tracked over time. Some individuals received non-curative drug treatments in instances where infections were judged to be dangerous [20-24]. The model incorporated three types of immune factors: innate immunity, and immunity that was PfEMP1 variant-specific or
variant-transcending. In their original publications, the authors modified parameters in order to fit each individual patient’s case history.

We extended this model by drawing parameters from probability distributions such that the resulting model outputs matched the whole range of observed dynamics without the need to tailor the model to any particular patient. We also extended the Molineaux and Dietz model to allow predictions of first fever [37], and used data from mosquito feeding experiments to translate gametocyte densities into a probability of human-to-mosquito infection [38,39]. The process used to select and fit these distributions to malaria therapy data will be presented elsewhere (Johnston et al., manuscript in preparation). Here we describe the addition of drug PK and PD data to this model framework. Figure 1 illustrates the progressions of asexual parasitemias, gametocytemias, and human-to-mosquito infectivity over time for three uninfected individuals (labeled). Figure 1A shows the log_{10} number of parasitized red blood cells (PRBC) per μL of blood, while Figures 1B and 1C depict the daily gametocytemias and human-to-mosquito infectivity.

In some areas asymptomatic infections are relatively common and these infections might serve as an important reservoir of parasitemia [40,41]. However, in a study conducted in a region of western Thailand with low and seasonal transmission, most infections (87%) were found to be symptomatic [42]. For our low-transmission analysis, we used this 87% value as an estimate of the proportion of individuals exhibiting symptoms (and potentially seeking treatment). Our model does not include the simulation of deaths from malaria and its complications because fatalities do not significantly affect malaria transmission at the population level [35].

1. Antimalarial Drug Pharmacokinetics
We chose to simulate the PK properties of chloroquine (CQ) as well as two types of ACTs, artesunate-mefloquine (AM) and artemether-lumefantrine (AL). AM has been a frequently used first-line therapy in parts of Southeast Asia [43], while AL has recently become the most widely-used ACT worldwide [3]. To model the PK properties of these drugs, we relied upon field studies that measured drug concentrations in plasma following a course of antimalarial treatment. These data were then fitted to one- or two-compartment models when the data were sufficient. For CQ we modeled the concentrations of both CQ and its active metabolite, monodeethyl-chloroquine (mdCQ), using data from [44] in which Papua New Guinean children with uncomplicated malaria were treated with CQ and a single dose of sulphadoxine-pyrimethamine (we assumed no PK interactions between the two).

The PK properties of both AM and AL have been well characterized in adults. For AL we utilized data from the treatment of uncomplicated individuals in western Thailand (Mae La) [45]. For AM we utilized two studies, one in Thailand [46] and one in Peru [47]. For AM we modeled both fixed (coformulated) and loose (non-coformulated) formulations. For the current study, we assumed treatment with only the loose formulation. Figure 2 illustrates the baseline PK-PD profiles of chloroquine, mefloquine (MFQ) and lumefantrine (LMF) as assumed in the model. In Figure 2A, the daily plasma concentrations of chloroquine are shown in blue, while the PD effects against asexuials are shown in green. The horizontal black line illustrates the gametocytocidal threshold. In Figures 2B and 2C show these data for mefloquine and lumefantrine respectively. Because of its longer half-life (12 vs. 5 days for mefloquine vs. lumefantrine respectively) these profiles predict that mefloquine is active against asexuials and gametocytes for a much longer duration than is lumefantrine, even though the maximum plasma concentrations of mefloquine are considerably lower. Our model also allows for the simulation
of PK variation among individuals. A complete description of the baseline PK modeling assumptions is included in Text S1.

2. Pharmacodynamics

In this paper, the effects of antimalarial drug concentrations on each life stage were modeled separately. For the PD properties of the artemisinins and their partners against asexual parasites, we assumed that the antimalarial dose-response curves against asexual parasites (excepting the artemisinins) follow the form: 

\[ PRR \cdot K(x) = PRR \cdot \left( \frac{(a-b)}{1+(x/c)^d} + b \right), \]

where \( a \) is fixed to be 0, \( b \) is fixed to be 1, \( c \) is what we term the ‘EC\(_{50}\),' \( d \) is the Hill slope, \( x \) is the plasma concentration of the drug, and \( PRR \) is the square root of the log\(_{10}\) of the 48 hour maximum parasite reduction ratio (PRR) for the drug. To determine asexual parasite densities during treatment, we subtract the quantity \( PRR \cdot K(x) \) from the log\(_{10}\) asexual parasite densities during drug treatment. If multiple drugs are present at a given time point we assume that the effects are strictly additive (i.e. no synergism).

We set the PRRs of dihydroartemisinin, artesunate, and artemether to be \( 10^4 \), those of mefloquine and lumefantrine were set to \( 10^2 \), and for chloroquine we used a value of \( 10^3 \) [48]. Given the short half-lives of the artemisinins, we assumed that they were either fully or not active. We used EC\(_{50}\) values of 90, 235, and 600 ng/ml for CQ, LMF, and MFQ, respectively; these values were derived from the ratios of in vitro 50% inhibitory concentrations (IC\(_{50}\)) and the assumption that the EC\(_{50}\) MFQ was 600 ng/ml [49,50] (see Text S1). Note that a 600 ng/ml EC\(_{50}\) for MFQ assumes a slightly MFQ-resistant background; this is the default value for simulations by Simpson et al. [45] and thus we used the same default value here. As a simplification, we also assumed all Hill slopes were the same as that of MFQ (2.5; value taken from [42]).
For drug activity against gametocytes, we assumed a binary activity model: antimalarials act against gametocytes only if plasma concentrations are above a given threshold. We chose $5 \times$ the *in vitro* IC$_{50}$ against asexual parasites as our threshold; for CQ, LMF, and MFQ, these values are 40, 174, and 322 ng/ml, respectively (Text S1). This choice of threshold gives sufficiently long active periods against gametocytes (Figure 2); further, the $5 \times$ threshold was utilized in a recent *in vitro* gametocyte inhibition study [17].

Gametocytes mature over the course of approximately 15 days through stages I-V, with each stage differing in metabolic activity and drug susceptibility [17]. In our model, we allow for variable stage-specific effects of drugs against all stages (I-V) of gametocytes; this feature is novel to the literature so far as we know, although a recent article did allow for a differential effect on uncirculating vs. circulating gametocytes [51]. We are able to implement variable stage-specific killing in our model because we compute gametocytemias every day, allowing for differential effects of drugs against gametocytes of various ages. For the stage-specific activities of the drugs, we assumed two types of baseline gametocytocidal effects, which we call ‘simplified’ and ‘*in vitro*’. In brief, the simplified assumptions assume a simplified stage-specific killing function for AM and AL: artemisinins are assumed to kill 100% percent of stage I-II gametocytes, 50% of stage III per day, and have no effect on stages IV-V [51,52]; mefloquine and lumefantrine were assumed to have no effect at any stage. For the *in vitro* parameterization, we utilized recent *in vitro* data that characterizes the responses of stages I-V to a variety of antimalarials [17].

**Figure 1** also shows the effect of drug treatment on three individuals infected with *P. falciparum*. As for the untreated cases, **Figure 1A** shows the log$_{10}$ PRBC/µL, while **Figures 1B** and 1C depict the daily gametocytemias and human-to-mosquito infectivities. Treatment with
AL was assumed to start two days after the onset of fever; dosage was assumed to follow the Coartem dosing prescription (twice daily for three days). The effects of treatment are seen immediately on the asexual population, which shows a steep drop after dosing, as well as the gametocytemias that show a slightly more delayed effect. Net infectivity is 3.3, 5.7, and 3.9 days for treated and 19.3, 25.7, and 23.0 days for untreated individuals, respectively, demonstrating how reductions in gametocytemia translate into reductions in infectivity. Figure 1 utilizes the \textit{in vitro} stage-specific gametocytocidal parameterization.

We also modeled AM plus one dose of primaquine (PMQ; combination abbreviated AM+PQ) or one dose of methylene blue (MB). For our three-drug combinations (AM+PQ, AM+MB), we simulated the third drugs’ effects only against gametocytes (as ACTs are already potent schizonticides). We assumed that PQ was active for three days, including the day of dosing [34,53]; for methylene blue, we assumed activity for five days total [54].

3. Description of parameterization of modeled stage-specific gametocytemic effects

Once the model was parameterized as above, we attempted to reproduce the patterns of gametocyte clearance observed after drug treatment in a variety of settings. The data sources used to parameterize the effects of drug treatment on gametocyte clearance are: 1,175 patients treated with an ACT (artesunate-mefloquine and artemisinin-piperaquine) in Thailand [33]; 397 patients treated with ACTs + PQ (artesunate–amodiaquine, dihydroartemisinin–piperaquine, AL, and AM (fixed and loose formulations)) and 411 patients treated with ACTs alone in Myanmar [55]; 279 children treated with non-ACTs and 249 children treated with either sulphadoxine-pyrimethamine + artesunate (SP+AS) or AL in Kenya [56]; and 53 children treated with SP + AS and 53 children treated with SP+AS+PQ in Tanzania [57]. All of these studies tracked the percentages of individuals gametocytemic by smear over time; the Kenya and Tanzania studies
also tracked gametocyte densities after treatment (using both smear and PCR). Percentages and proportions gametocyte positive over time for all data sets are shown in Figure S1. For our model calibration simulations we assumed treatment with either AM or AM+PQ as it was not possible to replicate such diversity of treatments here; further, the partner drug in an ACT has less of an effect on gametocyte densities than does the artemisinin and/or primaquine component [17].

The first step in utilizing the field data to parameterize our model was to match the starting gametocytemias in the model and the field data. We see from Figure S1 that the gametocytemias of patients upon admission range from .36 gametocytes per µL in Kenya to 7900 gametocytes per µL in Myanmar; thus there are approximately 21,000 times more gametocytes per µL at admission among the treated Myanmar population than the Kenyan population. In order to match these starting gametocytemias with our model, we adjusted the model day of treatment such that gametocytemias of simulated individuals at treatment matched the admission characteristics of the field population. For example, we found that individuals treated 15-16 days after emergence of parasites into the bloodstream yielded a good fit between model and Thai populations at admission. Treatment 15-16 days post emergence corresponded to treatment approximately 5 days after first fever; Table S1 provides the model and Thai field data fits at admission. To match the high gametocytemias observed in Myanmar, however, we had to delay treatment until 23 days after emergence; even then we could not replicate such high gametocytemias and we further assumed that only individuals with the highest gametocytemias were treated (top ~20%). The Myanmar study itself acknowledged that such treatment-seeking behaviors might have caused the high gametocytemias upon admission [55].
Once we had configured the model such that the starting gametocytemias approximated admission gametocytemias from the field, we used an iterative fitting approach: we first fit the model clearance behavior to the Thai field data, examined how the model behaved relative to the other data sets, and then adjusted the model to give a reasonable fit to the Thai data while matching the other data sets as well. We used the Thai data as our primary data set because our model assumes no malarial preimmunity, and the Thai data is from low transmission areas; the Thai data tracks individuals for many days post treatment; and the Thai field study also provided a variety of data on asexual densities as well as treatment-seeking behavior prior to admission. (The Thai data could be used to calibrate only MFQ and DHA, as PMQ was not administered there.) Indeed, fitting underdetermined models is as much art as science (there are approximately 15 stage-specific killing parameters for each drug); however, using the in vitro, Thai, and field data iteratively in this way we could reduce the degrees of freedom so that finding best-fit parameters was possible. We used the model-predicted Thai and Myanmar treatment-seeking behavior patterns for our ‘early’ and ‘late-treatment seeking’ results below as Thai treatment was quite prompt while the Myanmar treatment was significantly delayed.

Results

In this paper we report the development of the first mechanistic malaria model that incorporates the PK and PD properties of antimalarials against both asexual parasites and the five stages of gametocytes. In order to build this model, we expanded upon a model of the within-host progression of *P. falciparum* infections parameterized to malaria therapy. To this model we coupled the effects of antimalarials against asexual parasites as well as stage-specific
gametocytocidal effects, parameterizing the model with recently published *in vitro* data as well as a variety of field data.

1. **Modeled stage-specific gametocytemic effects**

In the Methods we described the data sets and methods used to fit the model to field data. After fitting, we found that neither the simplified nor the *in vitro* gametocytocidal models matched the Thai gametocyte clearance data well. Indeed, to match the field clearance data we needed to assume that the antimalarials were more efficient at removing gametocytes than the *in vitro* data indicated. **Figure S2** illustrates the model-predicted clearance of gametocytes versus the field clearance data using the best-fit clearance parameters. For these best-fit parameters, we found that on average the model-predicted MFQ clearance parameter was .9 times the *in vitro* value [17] (we assumed that MFQ/LMF/CQ all shared the same gametocyte stage-specific clearances); the model-predicted DHA clearance was .67 times the *in vitro* value [17]; and the model-predicted PMQ was .15 times the *in vitro* value [17]; see **Table S2** for a comparison of best-fit and *in vitro* clearance parameters. (For MB, we assumed the same improved efficacy in the field as for DHA.) We note here that a qualitative difference among the drugs comes from their effects against late stages; crudely, MFQ/LMF/CQ are not gametocytocidal against late stages, DHA is gametocytocidal against early stages but only slightly so against later stages, and PMQ is strongly gametocytocidal against all stages **Table S2**.

A variety of factors could have caused the model-predicted clearance values to be lower than the *in vitro* values. Host immune responses to gametocytes could have been greater in the field than *in vitro*, although the model does include gametocyte-specific immune responses. Further, if the antimalarials damaged gametocytes without killing them, these would perhaps be more readily cleared in the body than *in vitro*. However, if immunity alone were the driving
factor, the model-predicted parameters for all drugs should have differed by approximately the same amount from the \textit{in vitro} data; however, we see that PMQ is much more effective in the field than \textit{in vitro}. This is likely due to the action of a metabolite of PMQ, and thus we find here that it is very likely that there is a much more active metabolite of PMQ that is responsible for much of its gametocytemic killing.

Once the stage-specific gametocyte killing parameters were set, we then ran 1000 individual simulations for each combination of drug and treatment timing. Figure 3A shows the time course of asexual parasitemias over time for untreated individuals, and Figure 3B shows the effects of treating 80\% of symptomatic individuals with AM in the early-treatment scenario (15-16 days after emergence, or approximately 4 days after first fever). Visually, there are far fewer parasitemias that progress over time in the treated and untreated cases; however, in order to establish a comparison, these differences needed to be quantified.

In order to quantify the transmission reductions achieved, we used the daily gametocytemia counts, translated them to estimated daily human-to-mosquito infectivity probabilities, and summed these over time. The result is the net infectivity, which is equivalent to the number of days an individual would be fully infectious to mosquitoes (i.e. the number of days that a mosquito bite would be infectious and produce oocytes with a probability of 1). The net infectivity is the human-determined component of $R_0$, the basic reproductive number, or the number of secondary infections that a typical index infection would cause, in the absence of preimmunity [58]. We then took the ratio of untreated to treated net infectivity, $R_0/R_C$, where $R_C$ is the number of secondary infections per index infection under control. This ratio is called the effect size of a treatment [34]; its utility for control is that this term is the fold change in transmission from a given baseline $R_0$. Thus, if the $R_0$ in an area is 10, and the effect size is 5,
then the $R_0$ after control will be 2; a disease eventually become eliminated in an area if its $R_0$ can be reduced below 1 for a sufficient period of time.

**Figure 3C** illustrates the distribution of net infectivity in a population receiving early treatment with AM; simulations include treatment of all infected individuals, symptomatic individuals, and proportions thereof. The distribution of net infectivities of untreated individuals is shown for comparison. We find that there is a 18-fold decrease in mean net infectivity ($R_0/R_c = 18$) between untreated and treated populations if all simulated individuals are treated with AM. This effect size is larger than some other studies: Okell et al. [35] calculated individuals were 15.5% percent as infectious when treated with ACTs versus baseline treatment with failing antimalarials, whereas the ratio here is 5.5%. However, these results are not directly comparable, because our current modeling framework allows us to vary the treatment seeking behavior of the population and does not rely upon a baseline of semi-treatment, whereas earlier work cannot identify the effects of drugs alone.

**Figure 3D** compares treatment of 80% of the symptomatic early-treatment population treated with either AM; AM+MB; AM+PQ; CQ; a non-gametocytocidal (NG) antimalarial; or a fully transmission-blocking drug. For the pure schizonticide NG, we removed the gametocytocidal effects of AM and simulated its action on asexuals; for TB we assume that all gametocytes are killed upon treatment, regardless of age. We find essentially no difference in effect size among the ACT treatments and TB; any transmission reductions are lost in model stochasticity due to including untreated individuals in the effect size calculations. However, the strongly gametocytocidal treatments are somewhat better at reducing transmission than CQ or NG. This is due to the fact that, in the early-treatment setting, there is little time for gametocytes to appear, and so there is little advantage to gametocytocidal drugs.
ACTs and other antimalarial drugs work on *P. falciparum* infections and reduce transmission in three ways: by killing the asexual stages of the infection and thus preventing continued production of gametocytes, by killing existing gametocytes and preventing infection of the mosquito, and by post-treatment drug prophylaxis wherein residual drug levels can prevent some new infections [11]. In Figure 4A we calculate the prophylactic effects of AM treatment. We assume that individuals were infected and successfully treated such that all parasites from the primary infection have cleared and only the residual partner drug remains. Then, during the period when only the partner drug is present, parasites emerge into the blood stream from a bite separate from the one that caused the primary infection; for simplicity we assume here that there is no cross-immunity between the primary and secondary bites. Emergence happens a variable number of days after treatment of the primary infection. We can see that by day 10 most of the prophylactic effects of AM have disappeared due to clearance of MFQ from the plasma; the prophylactic effect protective period is less than that observed in the literature [59], possibly because we assume a somewhat mefloquine-resistant baseline EC$_{50}$ for MFQ and/or because we ignore cross-immunity between primary and secondary infections.

Figure 4D demonstrates the prophylactic effect of various treatments with secondary infections emerging 10 days after treatment of primary infection. The prophylactic effects of all of the ACTs are similar, indicating that the prophylactic effect is due mainly to the action of the drug on asexuals. Indeed, the prophylactic effect of CQ is much greater, even though it is only weakly schizonticidal, since the remaining concentrations are sufficient to terminate some of the secondary infections, given its greater PRR ($10^3$ vs. $10^2$). The gametocytocidal properties of the combinations only affect the gametocytes remaining during the unprotected half-lives, but so few
gametocytes are produced before the partners are cleared that the effects on transmission are small in comparison to the effect of interrupting the subsequent infection.

The final figure (Figure 5) shows the treatment effect sizes of all combinations on primary infections in both early- and late-treatment seeking contexts. Given early-treatment seeking behavior, we find a very large effect size for gametocytocidal drugs; if all infected individuals were treated with AM+PQ, we find an 85-fold reduction in transmission (Figure 5A). Indeed, AM+PQ and AM+MB approach the maximum reductions in transmission theoretically possible at reasonable treatment coverage levels in early-treatment settings. However, given the reciprocal relationship between $R_c$ and the effect size, very large reductions in transmission are achievable only at high levels of coverage. The shaded red region of Figure 5 illustrates the effect sizes that are achievable only through treatment of non-symptomatic individuals. The blue region highlights the effect sizes from treatment of 80% of the symptomatic cases, which range from 2.29-fold reductions for NG versus 3.18-fold reductions for AM+PQ (Figure 5A).

The late-treatment seeking effect sizes tell a slightly different story than those from the early-treatment context. Here, the maximum effect sizes achievable by drugs are much smaller, since treatment is delayed approximately 10 days versus the early-treatment context and so some of the transmission happens before the treatment is received. However, because the gametocyte counts at admission are much higher, the differential effect of the gametocytocidal drugs becomes evident: the late-stage gametocytocidal combinations are more effective at reducing transmission.

These results are consistent with those of Lawpoolsri et al. [34], who find that $R_0/R_c \sim 50$ if 99% of individuals are treated with AM+PQ (whereas we find $R_0/R_c = 42.3$ in the early-treatment setting). However, we find that the proportionate increase in effect size at 99%
coverage between no treatments versus AM (14.4-fold) is greater than the increase from AM+PQ versus AM (2.94-fold). Thus we find that the addition of a late-stage gametocytocidal drug is not as significant a benefit in the early-treatment setting as Lawpoolsri et al. Further, Lawpoolsri et al. assumes that individuals are treated with AM immediately after asexual parasite density reached $10^4$ PRBC/mL (and treating with PMQ 8 days later) \[33\], whereas we extend these results to two treatment contexts with a variety of different treatments (some of which have not been used in the field). The results here concerning the late-treatment seeking setting have no parallel in the existing literature. Finally, the study by Gething et al. \[60\] reports an average effect size of 1.1-1.8 of ACTs versus failing pre-ACT treatment across contexts; here we disaggregate by treatment-seeking behavior and coverage level, and so our results are an extension of those estimates.

**Discussion**

We described the development of a within-host model of malaria infection in naïve patients coupled to an antimalarial drug model. Our model includes the PK-PD profiles of a variety of antimalarial treatments against both asexual and sexual stages, including CQ, AM, AM+PQ, AM+MB, a hypothetical non-gametocytocidal schizonticide, and a hypothetical fully transmission blocking drug. The effects of antimalarials against all of the five stages of gametocytes were derived from fitting model output to field data from four settings, using recent *in vitro* data as a starting point. The model described here differs from other recent work in that we are modeling the effects of drugs against all of the life-stages, rather than assuming that drug treatment kills 100% of parasites immediately \[61\].
One caveat to our model is that we assume that individuals have no preimmunity to malaria infections. This assumption confines the applicability of our results to low transmission settings, where preimmunity does not significantly affect the course of infection. However, as control efforts in high transmission areas reduce incidence, the levels of preimmunity will decrease because antimalarial immunity wanes over time [8,62]. Thus, as control efforts are more efficacious, malarial infections will behave more like those infections simulated by our mechanistic model and our model results will become applicable. Therefore we might describe the results here as describing a theory of elimination, rather than a theory of control, because these results are most relevant after endemicity has declined [63,64].

A further caveat to these ACT effect size calculations is that the model developed in this paper does not consider the potential oocidal and sporonticidal actions of antimalarials in the mosquito. Tafenoquine [65], dihydroartemisinin, lumefantrine, and methylene blue [17] have been shown to be oocidal to varying degrees. Thus the effect sizes reported here could be underestimating the effectiveness of ACTs at controlling transmission.

Our first set of results concerned the determination of stage-specific gametocytocidal effects for MFQ, DHA, and PMQ; we found that the drugs acted more strongly in vivo than in vitro, even accounting for some level of gametocyte-directed immune response. In the case of PMQ, the discrepancy was so large that this result confirms our suspicion that much of the action of PMQ against gametocytes in vivo is caused by a metabolite of PMQ [19]. We also calculated the prophylactic efficacy of the modeled antimalarials assuming emergence of secondary infections 10 days after treatment for a primary infection and found that the more schizonticidal treatment was much more effective than the more gametocytocidal treatments.
For our third set of outputs we calculated the effect sizes of the various modeled treatments in both early and late treatment seeking settings. The modeling results described here provide a context for the various ACT effect size calculations reported previously [34,35,51,60] and paint a complex picture for the utility of antimalarials for malaria elimination. Our model predicts that, if all symptomatic individuals are treated early (approximately 5 days after first fever), non-gametocytocidal schizonticides reduce transmission 3.4-fold, artesunate-mefloquine reduces transmission 5.5-fold, while artesunate-mefloquine plus primaquine reduces transmission 7.0-fold. These results are in agreement with the 4-fold decrease in incidence observed with ACT usage in Senegal before the introduction of bed nets [8]. For comparison, Gething et al. estimate that the effect sizes of insecticide treated bed nets are approximately 5-15 at coverage levels of 40-60% [60]. However, in late treatment settings, where treatment is delayed approximately 10 days past that in the early treatment setting, the reductions are 1.5, 2.1, and 2.7, respectively.

Thus we find that in areas where infections are treated early and treatment coverage is high, ACTs may have a significant impact at reducing transmission, especially in areas of low $R_0$, even approaching the lower range of the effect sizes of bed nets. These effect sizes are much higher than earlier estimates indicate [60]. However, much of these transmission reductions are lost if treatment is delayed approximately 10 days on average (Figure 5). We also find that late-stage stage gametocytocides improve the effectiveness of ACTs, reducing transmission an additional 1.5-fold, and that these gains are intermediate relative to other types of interventions. For example, a 1.5-fold transmission reduction can also be achieved by increasing treatment coverage from 40% to 73% of infected individuals in the early treatment setting, or from 40% of individuals treated late to 69% of individuals treated early. Figure 5 visually illustrates the types
of tradeoffs that need to be made among treatment type, treatment coverage, and treatment seeking behavior in order to achieve a given level of transmission reduction.

Given these conclusions, serious efforts to eradicate malaria will require intensive planning and sustained support [5]. We note the hopeful result that our model confirms previous field observations that moderately gametocytocidal combinations such as ACTs substantially reduce onward transmission [8,66] and given high levels of drug coverage antimalarials may be sufficient to interrupt transmission (especially if complimented by bed net distribution). We also note that the addition of gametocytocidal drugs to ACTs reduces onward transmission even further. Thus, in areas of drug sensitivity, there may be a benefit to introducing strongly gametocytocidal drugs to the ACT regimen, although such a determination would depend on a cost-benefit analysis of such deployment versus other means of achieving needed reductions, such as improved treatment coverage, shorter time to treat, or increased bed net coverage, to name a few. The results here can also be combined with recent mapping work in order to help plan and coordinate control efforts [67]. Efforts are also ongoing to utilize this model to predict the effects of rising artemisinin resistance [9,68] on malaria transmission and control.
References


Acknowledgements

Computing resources were provided in part by the ‘Hotfoot’ High Performance Computing Cluster at Columbia University. DLS was supported by grant 49446 from the Bill and Melinda Gates Foundation.

Financial Disclosure

GLJ received funding from the National Science Foundation Graduate Research Fellowship Program. DLS is supported by a grant from the Bill and Melinda Gates Foundation (#49446) (http://www.gatesfoundation.org). DLS also acknowledges support from the RAPIDD program of the Science & Technology Directorate, Department of Homeland Security, and the Fogarty International Center, National Institutes of Health (http://www.fic.nih.gov). DAF is supported in part by the US National Institutes of Health grant AI079709. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

GLJ, DLS, and DAF conceived of the research; GLJ developed the mathematical model; GLJ wrote the paper, with input from DLS and DAF.

Competing Interests

All authors have declared that no competing interests exist.

Abbreviations
ACT, artemisinin-based combination therapy; AL, artemether-lumefantrine; AM, artesunate-mefloquine; AM+MB, artesunate-mefloquine plus methylene blue; AM+PQ, artesunate-mefloquine plus primaquine; DHA, dihydroartemisinin; MFQ, mefloquine; PMQ, primaquine; CQ, chloroquine; LMF, lumefantrine; EC50, 50% effective concentration; IC50, 50% inhibitory concentration; PRBC, parasitized red blood cells; PRR, parasite reduction ratio; NG, non-gametocytocidal antimalarial; TB, transmission-blocking antimalarial.

Supporting Information

Text S1. Mathematical description of the model, along with parameter fitting.

Text S2. User guide for use of the graphical user interface of the model.

Table S1. Validation of the simulated Thai population outputs. The severity of malaria among a set of Thai individuals treated with ACTs is provided. The model was then run assuming similar treatment-seeking behavior. Model outputs are compared to the Thai data in order to validate the ACT-treated asexual and gametocyte models’ performance against field data.

Figure S1. Gametocyte field data. A variety of data sets tracking the clearance of gametocytes from treated individuals are shown [33,55-57] (see Methods for description). (A) The percent of individuals who tested gametocyte positive by smear. (B) The proportion of individuals who tested gametocyte positive by smear, normalized to day 0 (day of admission). (C) The percentage of individuals testing gametocyte positive, by smear in Thailand and Myanmar, by polymerase
chain reaction (PCR) in Kenya and Tanzania. (D) The proportion of individuals gametocyte positive, normalized to day 0, by smear or PCR. The average numbers of gametocytes per microliter at admission are also shown. (E) The effects of primaquine in the Myanmar setting on gametocyte clearance. The arrow indicates that the clearance curve is shifted due to the gametocytocidal action of primaquine. (F) Clearance of gametocytes. The Myanmar data (E) shows the distinct effect that primaquine treatment has on gametocyte clearance, all other covariates being equal. However, a variety of factors influence measured rates of clearance, including the gametocytemia at admission, the type of drugs used, the method of detection, and the levels of immunity.

**Figure S2. Comparison of percent of individuals gametocyte positive.** (A) The percentages of individuals smear positive for gametocytes in both modeled and field settings. Simulated individuals are treated with no treatment, non-gametocytocidal treatment, or three different parameterizations for artemether-mefloquine (AM). The model assumes treatment began days 15-16 after parasites emerged from the liver; this treatment-seeking behavior best fit the observed field data. The model parameterizations for AM (simplified, *in vitro*, scaled *in vitro*) assume different levels of stage-specific gametocyte killing (see Methods). Simulated treatments are compared to the clearance of gametocytes from Thai field data [33]. (B) The data from panel (A) are plotted after dividing by the percentage of pretreatment individuals who are gametocytemic (model day 0). (C) Model predicted data for the proportion of individuals gametocytemic by smear is compared to field data from Myanmar [55]; the proportions were derived by simulating the percent of individuals gametocytemic pre- and post-treatment and dividing by the day 0 percentages. Model treatment was assumed to begin on day 23; some
individuals with lower gametocytomas were omitted in order to match the admission gametocytomas in Myanmar. The model was run assuming a variety of different treatment types (NG, non-gametocytocidal antimalarial; CQ, chloroquine, MB, methylene blue; PQ, primaquine); the Myanmar individuals were all treated with either AM or AM+PQ. (D) The the percentages of individuals treated with either AM or AM+PQ who were gametocytemic over time was normalized to day 0 for both the model and field data. The difference of these proportions was then calculated and graphed to isolate the effect of PQ treatment on gametocytemia.

Figure S3. Comparison of modeled gametocyte densities in treated individuals to two field data sets. (A) The natural log of the geometric mean gametocytemia among individuals PCR positive for gametocytes is shown for modeled and field treated individuals. Simulated individuals were treated 15-16 days after emergence of parasites from the liver, according to the behavior best matching Thai individuals (classified as ‘early treatment’). The threshold for PCR detection is assumed to be .02 gametocytes per µL, versus 10 per µL for smear. Modeled individuals were treated with either AM, artemether-mefloquine; a non-gametocytocidal antimalarial; CQ, chloroquine, MB, methylene blue; PQ, primaquine, or a combination thereof. The field data come from gametocyte clearance among treated Kenyan or Tanzanian children [56] [57]. (B) Modeled and field data from (A) were subtracted by the log gametocyte densities at admission to yield normalized densities among gametocytemic over time. (C) The daily normalized densities from (B) were normalized again in a data dependent manner: for the model data, the densities in (B) were subtracted by the normalized densities among CQ treated individuals; for both sets of field data, the densities were subtracted by the normalized densities among non-ACT treated children in Kenya. The resulting data illustrates the effects of strongly
gametocytemic treatments on gametocyte densities, controlling for the combined effects of schizonticidal treatment and immunity on gametocytemia. Gametocytemias among modeled untreated gametocyte positive individuals continue to rise versus the normalized data. (D-F) The same analyses as in (A-C) were run assuming highly gametocytemic modeled individuals treated 23 days after emergence of parasites from the liver, according to the behavior best matching Myanmar individuals (classified as ‘late treatment’). These data were then compared to the field data from Kenya and Myanmar; all normalizations in (D-F) are identical to those in (A-C).

**Figure S4. Graphical user interface for model standalone software.** Screen capture of the mechanistic malaria model user interface. The interface allows users to adjust a variety of parameters affecting the relationship between treatment and transmission, including the parasite reduction rate of dihydroartemisinin and its partner drug, the PD properties of dihydroartemisinin and its partner against asexuals and gametocytes, treatment-seeking behavior such as the delay in treatment and the probability of treatment. The software is designed for standalone operation on both Windows and Mac operating systems and allows for saving of outputs including asexual and gametocyte densities as well as daily and net infectiousness.

**Dataset S1.** Standalone model designed for Windows operating systems.

**Dataset S2.** Standalone model designed for Macintosh operating systems.
Figure Legends

Figure 1. Illustration of asexual, gametocyte, and human-to-mosquito infectivity model outputs. The *P. falciparum* infection model was run six times to simulate three untreated individuals and another three treated with artemether-lumefantrine two days after the onset of fever. Treated individual data are colored red/magenta/orange; untreated are blue/violet/green. (A) Individual log_{10} asexual parasitemias as a function of the number of days post emergence of parasites from the liver into the bloodstream. The inset depicts the first 50 days of infection; triangles above indicate the first day of fever. The black line is the level of detectability by microscopy (10 PRBC/μL). (B) Daily gametocytemias of the same six individuals. The gametocytemias are usually ~2 orders of magnitude less than the asexual parasitemias a few days prior. In the treated individuals, the asexual parasitemias and gametocytemias dropped rapidly after treatment. (C) Estimated probability of human-to-mosquito transmission given the gametocytemias illustrated in (B). The x-axis maximum is changed from 800 to 300, as none of the six individuals were predicted to remain infectious after day 152. The areas under the infectivity curves are 3.3, 5.7, and 3.9 days for treated and 19.3, 25.7, and 23.0 days for untreated individuals, respectively. Areas under the infectivity curves are equivalent to the number of fully infectious days. Drug treatment with an ACT rapidly reduces the probability of onward infection. Although the model predicts the persistence of long-lived low-level and sub-detectable infections (as observed in malaria therapy), (C) illustrates that these infections are usually not transmissible after the initial period of infection.

Figure 2. Modeled pharmacokinetics and pharmacodynamics of chloroquine, mefloquine and lumefantrine. (A) Modeled plasma concentrations of chloroquine (CQ) and mono-desethylchloroquine (mdCQ) (both in ng/ml) are given in blue. The green curve illustrates the
proportion of complete killing against asexual blood stage parasites as a function of plasma concentration. We estimated an *in vivo* EC$_{50}$ of 90 ng/ml and a Hill slope of 2.5. The horizontal dashed black line indicates the assumed threshold for gametocyte killing, calculated from 5× the *in vitro* EC$_{50}$ against drug-sensitive asexual parasites (40 ng/ml). (B) Modeled plasma concentrations of mefloquine (MFQ; ng/ml) are shown in blue. The green curve illustrates the proportion of complete killing against asexual parasites as a function of plasma concentration. We used the published *in vivo* EC$_{50}$ is 600 ng/ml and the Hill slope is 2.5 [49]. The gametocytemic threshold for MFQ is 322 ng/ml. (C) Modeled plasma concentrations for lumefantrine (LMF; in ng/ml); the blue line is the output of a two-compartmental model parameterized from field data. We calculated the *in vivo* IC$_{50}$ as 235 ng/ml and the Hill slope as 2.5. The 5× *in vitro* EC$_{50}$ is 174 ng/ml.

**Figure 3.** Effect sizes of various antimalarial therapies assuming early treatment. The mean effect sizes of various simulated treatment scenarios were calculated. (A) Distribution of net infectiousness of untreated individuals, as well as the infectiousness of treated (artesunate-mefloquine) individuals with admission characteristics roughly matching Thai patients. Effect sizes were calculated from simulated treatment of 100%, 80%, 60%, and 40% of symptomatic (S) individuals. (B) Treatment of 80% of the symptomatic simulated Thai population with different regimens: chloroquine (CQ); artesunate-mefloquine (AM); artesunate-mefloquine plus primaquine (AM+PQ); artesunate-mefloquine plus methylene blue (AM+MB); a non-gametocytocidal antimalarial (NG); and a transmission-blocking antimalarial (TB). All distributions are from 1000 runs.

**Figure 4.** Effect sizes from antimalarial prophylaxis. (A) Prophylactic effects of artesunate-mefloquine (AM) treatment. Individuals were assumed to have been infected and successfully
treated with AM. A secondary infection was simulated emerging into the bloodstream a variable number of days after treatment of the primary infection. (C) Comparison of the prophylactic effects of CQ, AM, AM+PQ, AM+MB, NG, or TB. Primary infections were assumed to have been successfully treated 10 days previously. Abbreviations: chloroquine (CQ); artesunate-mefloquine (AM); artesunate-mefloquine plus primaquine (AM+PQ); artesunate-mefloquine plus methylene blue (AM+MB); a non-gametocytocidal antimalarial (NG); and a transmission-blocking antimalarial (TB). All distributions are from 1000 runs.

Figure 5. Effect sizes of antimalarials as a function of treatment coverage and time of treatment. (A) Modeled effect sizes of various antimalarial treatments assuming early treatment. For early treatment, simulated individuals were matched to patients with admission characteristics of a Thai field study. Treatment occurred 15-16 days after emergence of parasites from liver (4-5 days after first fever). The shaded red region illustrates effect sizes that are achievable assuming treatment of nonsymptomatic individuals, assuming 87% of individuals are symptomatic. The blue region illustrates treatment of 80% of symptomatic individuals. (B) Modeled effect sizes of various antimalarial treatments assuming late treatment. For early treatment, simulated individuals were matched to patients with admission characteristics of a Myanmar field study. Treatment occurred 23 days after emergence of parasites from liver assuming that some individuals with low gametocytemias were not treated. Abbreviations: artesunate-mefloquine (AM). All effect sizes are the arithmetic mean of 1000 runs.
Figure 1

A

B

C
Figure 2

A

Gametocytemic threshold
Drug plasma density
Asexual-stage killing

B

Gametocytemic threshold
Drug plasma density
Asexual-stage killing

C

Gametocytemic threshold
Drug plasma density
Asexual-stage killing
Figure 3

A

B

C

D

Figure 3

Net Infectious Days

0 20 40 60 80 100 120 140

Net Infectious Days

0 100 200 300 400 500 600 700 800

Asexual parasite densities (parasitized red blood cells/μL)

Asexual parasite densities (parasitized red blood cells/μL)

Untreated All 80% SSympto 60% S 40% S

Untreated NG AM AM+MB AM+PQ TB CQ

Mean Infectivity

Effect Size

31.0 1.70 5.73 10.4 16.8 22.7 31.0 14.0 10.3 10.6 11.2 10.3 13.1 1.39 3.26 3.16 3.25 2.55 2.39 3.26 3.16 2.98 3.25 2.55

Thailand, 80% symptomatic treated
Figure 4

A

B

Secondary infection emerges 10 days after primary infection treatment.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean Infectivity</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>31.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NG</td>
<td>28.2</td>
<td>1.10</td>
</tr>
<tr>
<td>AM</td>
<td>28.3</td>
<td>1.09</td>
</tr>
<tr>
<td>AM+MB</td>
<td>27.8</td>
<td>1.11</td>
</tr>
<tr>
<td>AM+PQ</td>
<td>29.5</td>
<td>1.05</td>
</tr>
<tr>
<td>TB</td>
<td>28.0</td>
<td>1.11</td>
</tr>
<tr>
<td>CQ</td>
<td>7.00</td>
<td>4.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean Infectivity</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>28.2</td>
<td>1.00</td>
</tr>
<tr>
<td>NG</td>
<td>28.3</td>
<td>1.09</td>
</tr>
<tr>
<td>AM</td>
<td>27.8</td>
<td>1.11</td>
</tr>
<tr>
<td>AM+MB</td>
<td>29.5</td>
<td>1.05</td>
</tr>
<tr>
<td>AM+PQ</td>
<td>28.0</td>
<td>1.11</td>
</tr>
<tr>
<td>TB</td>
<td>7.00</td>
<td>4.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean Infectivity</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>28.2</td>
<td>1.00</td>
</tr>
<tr>
<td>NG</td>
<td>28.3</td>
<td>1.09</td>
</tr>
<tr>
<td>AM</td>
<td>27.8</td>
<td>1.11</td>
</tr>
<tr>
<td>AM+MB</td>
<td>29.5</td>
<td>1.05</td>
</tr>
<tr>
<td>AM+PQ</td>
<td>28.0</td>
<td>1.11</td>
</tr>
<tr>
<td>TB</td>
<td>7.00</td>
<td>4.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean Infectivity</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>28.2</td>
<td>1.00</td>
</tr>
<tr>
<td>NG</td>
<td>28.3</td>
<td>1.09</td>
</tr>
<tr>
<td>AM</td>
<td>27.8</td>
<td>1.11</td>
</tr>
<tr>
<td>AM+MB</td>
<td>29.5</td>
<td>1.05</td>
</tr>
<tr>
<td>AM+PQ</td>
<td>28.0</td>
<td>1.11</td>
</tr>
<tr>
<td>TB</td>
<td>7.00</td>
<td>4.43</td>
</tr>
</tbody>
</table>
Figure 5

A. Effect Sizes as Function of Treatment Coverage, early treatment

B. Effect Sizes as Function of Treatment Coverage, late treatment
### Table 1. Antimalarial Effect Sizes in Two Treatment-Seeking Contexts

<table>
<thead>
<tr>
<th>Simulated Location</th>
<th>Measure</th>
<th>Untreated</th>
<th>Chloroquine</th>
<th>Artesunate Mefloquine with gametocytocidal effect removed</th>
<th>Artesunate Mefloquine</th>
<th>Artesunate Mefloquine + Primaquine</th>
<th>Artesunate Mefloquine + Methylene blue</th>
<th>Full transmission blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southwestern Thailand (early treatment)</td>
<td>Area Under Infectivity Curve</td>
<td>31.02</td>
<td>4.69</td>
<td>5.81</td>
<td>1.77</td>
<td>0.33</td>
<td>0.62</td>
<td>0.04</td>
</tr>
<tr>
<td>Southwestern Thailand (early treatment)</td>
<td>Effect Size</td>
<td>N/A</td>
<td>6.61</td>
<td>5.34</td>
<td>17.49</td>
<td>92.97</td>
<td>50.15</td>
<td>745.62</td>
</tr>
<tr>
<td>Myanmar (late treatment)</td>
<td>Area Under Infectivity Curve</td>
<td>40.14</td>
<td>14.73</td>
<td>18.92</td>
<td>12.39</td>
<td>8.31</td>
<td>10.57</td>
<td>4.68</td>
</tr>
<tr>
<td>Myanmar (late treatment)</td>
<td>Effect Size</td>
<td>N/A</td>
<td>2.72</td>
<td>2.12</td>
<td>3.24</td>
<td>4.83</td>
<td>3.80</td>
<td>8.58</td>
</tr>
</tbody>
</table>

**Table 1. Antimalarial Effect Sizes in Two Treatment-Seeking Contexts.** The effect sizes of various artemisinin-based treatment regimens were simulated using the mechanistic within-host malaria model. Treatment-seeking behavior was assumed to match field patterns observed in either Southwestern Thailand (early treatment) or Myanmar (late treatment). The areas under the infectivity curves were computed for the various treatments listed in each of the two regions; reported values are the arithmetic means of 1000 simulated individuals. Effect sizes were calculated by taking the ratio of the mean infectiousness of untreated treatment-seeking individuals vs. the mean infectiousness of treatment-seeking individuals who received treatment.
Supplemental Text S1: Model equations and distributions

I) Pharmacokinetics
   a. Pharmacokinetics of artemisinins
   b. Pharmacokinetics of lumefantrine
   c. Pharmacokinetics of mefloquine
   d. Pharmacokinetics of methylene blue

II) Pharmacodynamics
   a. Pharmacodynamics of artemisinins, lumefantrine, mefloquine, and chloroquine against asexual parasites
   b. Pharmacodynamics of artemether and lumefantrine, and mefloquine against gametocytes

III) Fitting Thailand Gametocyte Carriage Data

IV) Fitting Treated Model to Field Data from Myanmar, Kenya, and Tanzania

V) Coding Specifications
I) Pharmacokinetics

   a. Pharmacokinetics of artemisinins
   For all ACTs we assume that artemisinin and/or artesunate and dihydroartemisinin are present at relevant concentrations for only the days on which they are consumed. This is due to the fact that artemether, artesunate, and dihydroartemisinin (the active metabolite of both), are rapidly absorbed [1] and very quickly eliminated ($t_{1/2}: .5-3$ hrs) [2,3]. We thus do not explicitly model the plasma concentrations of this component, as clearance of pharmacodynamically relevant concentrations occurs within the smallest time step of the model (1 day).

   b. Pharmacokinetics of lumefantrine
   The pharmacokinetics of lumefantrine differ from those of artemisinins. Clinical studies have found that lumefantrine pharmacokinetics follow a two compartment model with first-order absorption and a lag time of approximately two hours between ingestion and onset of absorption [2]. Lumefantrine is cleared much more slowly than the artemisinins (the mean terminal half-life ranges from 30 – 87 hours) [2]. Due to its much-longer half-life, we explicitly model lumefantrine plasma concentrations.

   The standard adult prescription regimen of AL is four doses of a fixed dose tablet containing 20 mg artemether and 120 mg of lumefantrine twice daily for three days; it is this regimen that we are interested in modeling. For the pharmacokinetics of lumefantrine, we rely upon a group of studies that were conducted in Thailand among patients with $P$ falciparum infections who were treated with AL [1,2,4]. In these studies, plasma concentrations of lumefantrine were tracked over time and a two-compartment model was fit to the population data. To simulate plasma concentrations for our model, we used the equations describing a two-compartment model and parameterized them with the parameters from Ezzet, van Vugt et al. [5];
the current dosage recommendations correspond to what they called ‘regimen B.’ For this regimen, 18 Thai patients hospitalized in Bankok were paired with 72 community-based patients from Mae La, and pharmacological modeling was performed to fit the plasma lumefantrine measurements. The C\textsubscript{max} achieved was approximately 7,000 ng/ml for the patients from Bankok and approximately 8,000 ng/ml for the patients from Mae La. Since the pharmacokinetic parameters differed slightly between the two populations, we utilized the values from Mae La.

This combination of parameters was found to fit day 7 plasma lumefantrine concentrations from a later study [6] quite well. At day 7, the PK model parameterized as described above yielded a prediction of 759.5 ng/ml for lumefantrine, compared to an observed mean value of 528 ng/ml (range 49-5175 ng/ml) in a study among Karen women in Thailand (the model predicts that 528 is reached between days 8 and 9) [6]. The two other Thai studies yielding plasma lumefantrine concentrations near these values are Ezzet et al. [1] (although this study examined a two day regimen rather than three) and van Vugt et al. [4] (with pharmacokinetic parameters as reported in White et al. [2]).

While these studies have similar pharmacokinetic profiles, some other studies report much higher levels of lumefantrine plasma concentrations. In one study, 219 patients with acute, uncomplicated \textit{P. falciparum} malaria in Thailand were treated with AL and lumefantrine concentrations were monitored during treatment [7]. In a second study, lumefantrine concentrations were monitored among 14 Western Europeans without malaria but treated with AL [8]. In both studies, the mean C\textsubscript{max} was much higher than in the set of trials above: \(\geq 25,700\) and 28,300 ng/ml, respectively. Further, the terminal half-life of the latter study was quite long (11.5 days vs 3.1 and 4.5 for two Thai studies) [2]. The higher values for the latter study were expected: lumefantrine levels are lower among individuals who are infected with \textit{P. falciparum},
and in this study the medications were taken with a standard diet (ensuring adequate lipid consumption). However, the former study provides a slight outlier, as these were infected Thai individuals; moreover, since their plasma concentrations were not followed after treatment, few pharmacodynamic implications can be drawn. Thus, for lumefantrine modeling we utilize the two compartment model parameterized from the Ezzet, van Vugt et al. [5] study.

To model pharmacokinetic variation, we multiply the bioavailability parameters ($F_1 - F_b$) by a randomly chosen constant, simulating the effects of increased or decreased absorption. We do not simulate the effects of parasite load on bioavailability, though high loads generally yield lower bioavailability [5].

c. Pharmacokinetics of mefloquine

For the pharmacokinetic profile of mefloquine, we utilized a recent study in which adults presenting with *P. falciparum* at the Hospital for Tropical Diseases, Bangkok, Thailand were treated with either a fixed dose ($n = 25$) or loose dose ($n = 25$) formulation of AM [9]. The fixed dose formulation consists of two fixed dose tablets containing 100 mg artesunate and 200 mg of mefloquine daily for 3 days. The loose dose formulation is dosed by weight and consists of non-fixed artesunate at 4 mg/kg of body weight/day for 3 days plus mefloquine at 15 mg/kg on day 1 and 10 mg/kg on day 2. These individuals’ mefloquine plasma concentrations were followed for 28 days.

We used a different study for estimation of the terminal half-life of mefloquine. In this study, 39 Peruvian adults infected with *P. falciparum* were treated with a loose dose formulation of AM and their mefloquine plasma concentrations monitored for 56 days [10]. The terminal half-life was calculated (utilizing concentrations at days 21, 28, 35, 42, and 56) as 347 hours, compared to 286 and 322 for the fixed and nonfixed regimens in Thailand. Mefloquine
pharmacodynamics are often described by a two component model, [11] and because the Thai half-life estimates come from earlier measurements, they might not represent the true terminal half-life but rather a combination of initial and terminal half-lives. In neither study was a compartmental model fit to the concentrations.

To model the plasma concentrations of both loose and fixed concentrations, we extracted the recorded mefloquine plasma concentrations data for days 0-28 from Krudsood et al. [9] using the DataThiefIII software [12]. To extrapolate plasma mefloquine concentrations past day 28, we utilized the terminal half-life from the Peruvian study [10]. This model allows for simulation of mefloquine concentrations at any day during and after treatment for both loose and fixed formulations and utilizes the most reliable data from both studies.

To model pharmacokinetic variation for mefloquine, because we do not have an analytic pharmacokinetic model, we simply multiply plasma concentrations by a random constant (taking the first 29 days of treatment) and then use the terminal half-life to extrapolate the remainder; we choose a value between $\frac{1}{3}$ and 3 (the upper and lower bounds are proportionally equal as mefloquine absorption is not as negatively affected by diet as lumefantrine).

d. **Methylene Blue**

Regarding the pharmacokinetic profile of methylene blue, all of its pharmacokinetic data are from uninfected individuals. In these individuals its oral and intravenous half-lives are short (5–6.5 hours) [13,14]. Further, its bioavailability via oral or intravenous administration is poor [15]. However, the bioavailability and half-life of methylene blue depend significantly on the mode of administration and are increased by aqueous delivery (fraction absorbed = 72.3±23.9%; terminal half-life = 18.4 hours) [15]. Efforts are ongoing to adjust its pharmacokinetic profile [15].
i. Equations Determining Pharmacokinetics

The plasma concentrations of lumefantrine in the central compartment are given by the equation

\[ C(t) = \begin{cases} 
\sum_{i=1}^{n-1} D_i \left[ A e^{-\alpha(t-t_{D_i}-Tlag)} + B e^{-\beta(t-t_{D_i}-Tlag)} - (A + B) e^{-ka(t-t_{D_i}-Tlag)} \right] & \text{if } t - t_{D_n} \leq Tlag \\
\sum_{i=1}^{n} D_i \left[ A e^{-\alpha(t-t_{D_i}-Tlag)} + B e^{-\beta(t-t_{D_i}-Tlag)} - (A + B) e^{-ka(t-t_{D_i}-Tlag)} \right] & \text{if not}
\end{cases} \]

The parameters for this model are given in [2,16]. For mefloquine and chloroquine, we did not use an explicit compartmental model, rather we use the measured plasma concentrations from published studies and interpolate among them. For time points where we could not interpolate plasma concentrations, we use the terminal plasma concentration half-lives to extrapolate.

II) Pharmacodynamics

a. Pharmacodynamics of artemisinins, lumefantrine, mefloquine, and chloroquine against asexual parasites

A variety of models have examined the pharmacodynamics of the artemisinins within the asexual blood stage cycle [17,18]. These models simulate the effects of artemisinins against the various developmental stages of the asexual stage (rings, mature trophozoites, schizonts). However, since the time step of this model is 2 days (1 complete asexual cycle), we can simulate the asexual effects of the artemisinins and their partner drugs as a bulk effect by decreasing the overall parasite densities by a given factor without regard to asexual stage-specificity.

The effectiveness of antimalarials on asexual parasitemias is quantified using a parasite reduction ratio (PRR), which is the ratio of the initial parasite concentration to the concentration of parasites remaining 48 hours after drug administration. For the artemisinins, the maximum
PRR \textit{in vivo} is $10^3 - 10^5$; for lumefantrine the maximum PRR \textit{in vivo} is $10 - 10^3$ [19]. For our model we have made the assumption that the maximum PRR of artemether is $10^4$ and that of lumefantrine is $10^2$. While the expected net maximum PRR would then be $10^4 \cdot 10^2 = 10^6$, because new parasites are being generated while the drugs are active, the net PRR is lower. In order to measure the net PRR for our model, we ran it 1000 times assuming treatment with AL according to the early treatment (Thailand) treatment seeking behavior (see below) and took the average daily interpolated asexual parasitemia.

The modeled net maximum PRR for this average parasitemia was $7.48 \cdot 10^5$ (the fold-change between days 2 and 4 after treatment). This value is biased however, as approximately 30% of individuals cleared their infections during this portion of treatment and their parasitemias were counted as having reached the model lower bound of $10^{-5}$ after clearance. When those individuals who cleared their infections during this portion of treatment are dropped, the modeled PRR falls to $2.01 \cdot 10^4$. The latter estimate of net maximum PRR for AL agrees with an estimate of $7.0 \cdot 10^4$ for the PRR of AL in treated Nigerian children [20]. Regarding mefloquine, because this drug is an arylamino alcohol along with lumefantrine, White et al. [2] speculated that the two drugs share similar modes of action (at least against asexual parasites) and so we set the maximum PRR of mefloquine to be $10^2$ as well.

While the above calculations determine the maximum asexual killing effect of the drugs, once the plasma concentrations begin to fall, this killing effect will decrease, and thus dose-response curves for these drugs must be calculated. Because the artemisinins are so rapidly cleared, we simulate the pharmacodynamics of the artemisinins as a binary model, such that the PRR is $10^4$ when the drug is present and 1 when absent. However, for the partner drugs lumefantrine and mefloquine, because the duration of partial activity is on the order of weeks and
spans many asexual cycles, we cannot assume that the PRR for these drugs is either maximal or unitary. We thus need to determine the in vivo dose-response relationships for the partner drugs.

While in vitro studies of drug effectiveness can be readily conducted so that dose-response relationships might be derived, determining in vivo dose-response relationships is more complicated. Indeed, the in vivo relationship between observed plasma concentrations and parasite response is dependent on the proportion of free (more active) to bound (less active) drug, a proportion that is difficult to determine as high performance liquid chromatography measures only total plasma concentrations and this proportion varies according to drug. Other sources of variation are the concomitant effects of the immune system, which varies from individual to individual, and the diversity of genotypes found in the field.

However, there have been field studies and mathematical modeling efforts at determining the dose-response relationships for mefloquine and lumefantrine. For mefloquine, two mathematical studies attempted to quantify the in vivo EC$_{50}$, here defined as the concentration at which the drug kills parasites at a rate of .5 times the PRR; this value was computed from the minimal inhibitory concentration (MIC), the concentration at which the within-host growth of parasites is exactly equal to the parasitical effect of the drug (approximately equal to the in vivo EC$_{90}$) [21,22]. Both studies utilized a MIC of approximately 500 ng/ml, a value that was determined from field studies among mildly resistant strains [23,24]. The study by Simpson et al. [21] calculated the EC$_{50}$ as 665.4 ng/ml for mildly resistant strains, whereas the study by Hoshen et al. [22] yielded two EC$_{50}$ values: 350 ng/ml for sensitive parasites and 1150 ng/ml for resistant strains.

After experimenting with the model developed by Simpson et al. [21] with their original data, we decided to use an EC$_{50}$ value of 600 ng/ml for mefloquine, a value that assumes mild-
moderate mefloquine resistance. For the entire dose-response curve for mefloquine, we utilized a 4-parameter sigmoidal curve with a Hill slope of 2.5, a minimum of 0, and a maximum of 1, as in Simpson et al. [21] (see equations below). For lumefantrine, less modeling work has been published; however, field studies have attempted to determine an in vivo MIC. These studies generally attempted to determine the day 7 lumefantrine plasma concentration that served as the best (greatest Youden index) predictor of treatment failure. The values from these studies ranged from 175-500 ng/ml, with 280 ng/ml often mentioned [2,6].

Given the relative paucity of published research regarding the in vivo pharmacodynamics for lumefantrine and chloroquine, and the extensive modeling work done for mefloquine, for our study here we relied upon the mefloquine modeling work as our baseline and assumed that the EC$_{50}$ for mefloquine was 600 ng/ml; for lumefantrine and chloroquine we multiplied this quantity by the ratio of their in vitro IC$_{50}$ values to that of mefloquine. We assumed that the IC$_{50}$ of chloroquine was 25 nM (8 ng/ml) [25], that the IC$_{50}$ of lumefantrine was 66 nM (34.9 ng/ml) [26], and that the IC$_{50}$ of mefloquine was 170.4 nM (64.5 ng/ml) [27]. Thus, the ratio of in vitro IC$_{50}$ values for chloroquine and mefloquine is 25:170 = .147, and the ratio for lumefantrine and mefloquine is 66:170 = .387.

If we multiply these conversion factors to the assumed EC$_{50}$ value for mefloquine (600 ng/ml), we get that the corresponding values for chloroquine and lumefantrine are 88 ng/ml and 232 ng/ml, which we round to 90 ng/ml and 235 ng/ml, respectively. These latter values are the EC$_{50}$ values that we use for chloroquine and lumefantrine herein.

### i. Equations Determining Pharmacodynamics Against Asexual Parasites

We assume that the antimalarial pharmacodynamic dose-response curves against asexual parasites (excepting the artemisinins) follow the form:
\[
PRR \cdot K(x) = PRR \cdot \left( \frac{a - b}{1 + (\frac{x}{c})^d} + b \right)
\]

where \(a\) is fixed to be 0, \(b\) is fixed to be 1, \(c\) is what we term the `EC_{50},` \(d\) is the Hill slope which we set to be 2.5, \(x\) is the plasma concentration of the drug, and \(PRR\) is the square root of the \(\log_{10}\) of the 48 hour maximum parasite reduction ratio for the drug. To determine asexual parasite densities during treatment, we subtract the quantity \(PRR \cdot K(x)\) from the \(\log_{10}\) asexual parasite densities during drug treatment. If multiple drugs are present at a given time point we assume that the effects are strictly additive (i.e. no synergism).

b. Pharmacodynamics of artemisinins, lumefantrine, and mefloquine against gametocytes

One beneficial aspect of modeling gametocytes with a 1-day time step is that this granularity allows for simulation of the effects of antimalarial drugs over small time scales. Indeed, we can model the stage-specific effects of drugs on gametocytes, since we can track gametocytes as they age. These effects are very important to determining infectivity, as the duration of gametocytes within the host after drug treatment and clearance of asexuals determines how effective a drug is at blocking transmission. However, until recently, there was little data with which to parameterize such an effect. With the recent release of data reporting the stage-specific in vitro effect of various antimalarials on gametocytes [26], we can now parameterize the stage-specific gametocytocidal effects for many drugs.

For this paper, we begin with two sets of assumptions. The first set, which we call the ‘simplified’ assumptions, assumes that the artemisinins killed gametocytes that were up to 7 days old at the time of treatment, reduced gametocytes by 50% per day for days 8-10, and had no effect on late-stages. This assumption is consistent with prior evidence that the artemisinins are
effective at killing immature gametocytes, but that they do not affect mature gametocytes very strongly; see discussion in Bousema et al. [28] as well as the in vitro study by Kumar and Zheng [29]. For the partner drugs, before Adjalley et al. [26] lumefantrine alone was not known to have a gametocidal effect on *P. falciparum*, thought it has been shown to kill/disable gametocytes in both *P. yoelii* and *P. berghei* [30,31]. Regarding mefloquine, a recent study indicates that mefloquine is at best a very weak gametocytocidal drug against later stages (III-IV) [32]. Thus for our simplified assumptions, we assume that lumefantrine and mefloquine are not gametocytocidal.

The second set of assumptions is that antimalarials decrease gametocyte populations proportionally by stage-specific constants. From the in vitro data [26], we assume that the artemisinins kill 45.3%, 17.4%, 18.8%, and 16.9% of stage I-II, III, IV, and V gametocytes per day, respectively. In the case of lumefantrine, the effects were more ambiguous; if we do not assume a delayed action phenotype, then these proportions are 22.4%, 0%, 0%, and 0%; if we assume delayed action, then we have reductions of 22.4%, 20.9%, 16.2%, and 11.1%, respectively [26]. We assume that lumefantrine acts without delayed action. Mefloquine was not included in [26]; however, data indicates that mefloquine is at best a very weak gametocytocidal drug against later stages (III-IV) [32]. Because mefloquine and lumefantrine share chemical similarities [2] and agree in their gametocytocidal effects in the limited data available, our default model assumption is that mefloquine and lumefantrine have identical gametocytocidal properties.

The gender of gametocytes is not specifically modeled (although mature gametocytes are often found in male:female ratio of 1:4, and so gender could be predicted if needed) [33].
III) Fitting Thailand Gametocyte Carriage Data

In order to test the treated model, we needed to select a dataset against which to validate the model. For our first analysis we utilized a dataset generated from patients treated in western Thailand [34]. Malaria transmission in this region is low and seasonal [35]. In this study, data from 1,175 patients treated with an ACT (artesunate-mefloquine and artemisinin-piperaquine) were pooled and analyzed [34]. No significant differences in gametocyte clearance were observed between the various ACTs used [34]. The proportion of individuals who were gametocytemic was tracked and monitored over time. Gametocytemic patients differed wildly in their admission characteristics in terms of age (2-62 years), duration of fever before seeking treatment (0-60 days), and intensity of infection at presentation; a variety of these individuals’ malariometric indices are reported in (Table S1). The daily percentage of Thai individuals who were gametocytemic at treatment and post treatment is shown in Figure S1 [34].

The Thai study provides significant amounts of detail about the infections of individuals before and during treatment. In order to validate that the treated model infections cleared similarly to those from the field, we first needed to match the treatment seeking behavior of modeled individuals to those observed in Thailand. This would provide a simulated population resembling the Thai population at admission so that we could compare the two populations’ responses to treatment. The most important parameter to vary was the delay in treatment (assumed to be AM): a shorter delay meant a higher pre-treatment parasitemia, and a longer delay meant a lower parasitemia.

After experimentation, we found a good fit assuming that individuals were treated on days 15-16 after emergence of parasites from the liver (Table S1). This assumption yielded a median number of fever days before treatment of 3.8 days, assuming fever every other day [36],
in line with the field-reported value of a median of 5 days of fever before treatment [34] (it is unknown how this value was calculated in the field). Further, the model-generated pre-admission geometric mean and median asexual parasitemias are roughly the same (though lightly lower) as those observed in the field (Table S1). Once the treatment seeking behavior and admission characteristics were matched, we then compared the geometric mean of the maximum gametocytemia observed before and during treatment as well as the rates of gametocyte clearance.

We simulated treatment of the synthetic Thai population with AM. We varied the gametocytic properties of AM in three ways in order to compare model response to field data. For the first set of simulations we utilized the ‘simplified’ assumptions. The second set of assumptions utilized the observed in vitro stage-specific killing proportions determined in Adjalley et al. [26]. For the third set of assumptions, we adjusted the various in vitro stage-specific killing proportions for the artemisinins and their partner until a reasonable fit was achieved.

The model using the simplified assumptions as well as the in vitro adjusted assumptions underpredicted maximum gametocyte levels, indicating greater than observed gametocyte killing, while the in vitro assumptions overpredicted the gametocyte levels (Table S1). This metric did not provide much useful information to discriminate among models other than to confirm that the maximum simulate gametocytemias were relatively close to those observed in the field.

However, the daily percentage of individuals who were gametocytemic proved much more useful. In (Figure S2A), the model was run with untreated individuals and the daily percentage gametocytemic was plotted. The percentage gametocytemic is seen rise rapidly to
approximately 75% and then decay slowly over time due to killing of asexuals and gametocyte clearance by the immune system. We then treated this synthetic population with AM using the ‘simplified’ and \textit{in vitro} gametocytocidal assumptions and compared the results to the untreated model output as well as the Thai field data and output assuming that the antimalarials were not gametocytocidal. \textbf{Figure S2B} shows the gametocyte clearance data for these treatment types normalized to day 0.

The simplified assumptions matched the observed data quite well; however, gametocytes were cleared faster in the model than observed in the field, possibly indicating that ACTs do not completely kill early stage gametocytes. The \textit{in vitro} assumptions do not fit as well: the gametocytemia levels are too high, indicating that the \textit{in vivo} gametocytocidal effects of AM are significantly greater than those observed \textit{in vitro}. After some experimentation, an approximate best fit of model output to field data was found using the parameters in \textbf{Table S2}. In fact, our fitting process was more complicated than this, because we also used other data sets to fit the stage-specific gametocytocidal factors (see below). Indeed, the parameters of \textbf{Table S2} are those parameters that best fitted the Thai clearance data as well as the clearance data from Myanmar, Kenya, and Tanzania (see below); in this section we only describe the Thailand target data and model fits to this set. From \textbf{Table S2} we see that more gametocytes died per day after exposure \textit{in vivo} than would have been expected from the \textit{in vitro} data. Because the Thai data came from quite varied individuals, a semi-quantitative fit is all that can realistically be achieved.

The discrepancy between the field data and model outputs with the \textit{in vitro} assumptions may have multiple causes; we examine two of them here. First, the difference may be due to the fact that the drugs \textit{in vitro} were impairing the functionality of gametocytes without entirely killing them; these gametocytes might have been able to produce luciferin, but were otherwise impaired.
These gametocytes might possibly have been more easily cleared by the immune system and so the effects of drugs *in vivo* would be greater than those observed *in vitro*. Second, the effects of immunity on the proportion of individuals who were gametocytemic might be greater than assumed in the model. Although the majority (57.9%) of gametocytemic individuals in the Thai study were experiencing their first episode, and *P. falciparum* infections occurred only once every two years on average [34], some individuals might have developed anti-parasitic immunity beyond that observed in the malaria therapy data. It is difficult to identify how much of the difference is due to either cause because we cannot easily monitor the natural progression of gametocytemia in field settings (due to the ethical imperative to treat individuals on presentation), and our conversion factors necessarily incorporate both of these effects.

IV) Fitting Treated Model to Field Data from Myanmar, Kenya, and Tanzania

We used three other data sets as target data to match model outputs. The first data that we consider comes from Myanma children and adults treated with ACTs whose gametocytemias were monitored over time [37]. As for the Thai data, we first matched the gametocyte levels of patients at admission (7900 gametocytes per µL). We adjusted the modeled treatment seeking behavior so that individuals did not receive treatment until 23 days after emergence of parasites from the liver. However, even with this delay we were not able to replicate such high pretreatment gametocytemias, and we had to assume that only individuals with gametocyte densities in the top 20% were treated. Why these gametocytemias were so high even accepting for the delay in treatment is a matter of speculation, and we cannot decide the question here other than to note its importance.
Once the model treatment seeking behavior was set and individuals with high gametocytremias were selected, the model was run assuming various treatment types (Figure S2C). Since the Myanmar data included treatment with AM+PQ, we were also able to calibrate the modeled effects of primaquine; our best-fit parameters are provided in Table S2. We disaggregated the effects of PQ from those of AM by taking the normalized fraction of individuals gametocyte positive over time after treatment with AM and AM+PQ treatment and subtracting; these data for both model and field settings are shown in Figure S2 D.

In addition to the percentage of individuals who were gametocyte positive over time, we also utilized the gametocyte densities of treated children from two sites as target data. In these studies, Kenyan and Tanzanian children treated with antimalarials had gametocytemia levels monitored over time using microscopy and polymerase chain reaction (PCR) [28]. Figure S3A shows the natural log of the gametocyte densities per microliter in the study sites, as well as the gametocytremias in individuals treated according to the Thai treatment seeking behavior (15-16 days after emergence of parasites from the liver). These trends are taken from the daily geometric means of individuals registering gametocyte positive by PCR (the threshold for detection by PCR is assumed to be .02 gametocytes per µL).

Gametocytemia levels in modeled individuals continue to rise after treatment as more gametocytes emerge into the bloodstream because individuals were assumed to have been treated early and there is a large bolus of sequestered gametocytes that emerge during treatment. All fits shown in Figure 3 assume the stage-specific killing parameters from Table S2. In Figure S3B these same data are plotted after normalizing by dividing each trend by its value at day 0. Normalization helps to illustrate differences in clearance that are unrelated to the pretreatment gametocyte densities. Modeled clearance rates are slower than those observed in the field;
however, this is true even for the Kenya non-ACT treatment set versus the modeled chloroquine data. Neither of these treatments should have significant impacts on gametocytemia over time other than to arrest their production from asexual parasites and thus both trends should overlap. The differences between the two trends may be related to the effects of immunity, both to the single infection and preimmunity from earlier infections, as well as to other complications. In order to identify the gametocytocidal effects of treatments from these other confounding factors, we normalized model trends by subtracting the modeled chloroquine trend from the other trends. Likewise, we subtracted the Kenyan non-ACT treatment trend from the observed field clearance trends; these data are plotted in Figure S3C.

This figure illustrates that, after these two normalizations, the ACT+PQ treatment trend matches the Tanzania ACT+PQ trend quite well, even reproducing an upward slope in the last few timepoints observed in the field. This upward slope may be due to the fact that the distribution of gametocytemias among individuals is nonlinear (this is due to the fact that the distribution of maximum asexual parasitemias among individuals is log-linear). As individuals with fewer gametocytes have their infections clear, the remaining individuals have disproportionately higher gametocyte densities, and so the censored geometric means rise over time due. Figure S3C also illustrates that the modeled AM treatment trend approximately splits the difference between the Kenya ACT treatment trend and the Tanzania ACT treatment trend without PQ, as would be expected.

Figure S3 (D-F) illustrates the same data as Figure S3 (A-C) with the exception that modeled treatment is assumed to occur according to the treatment seeking behavior observed in Myanmar (23 days after emergence from liver including only individuals with gametocytemia densities in the top ~20%). The starting gametocytemia densities are much higher than those
observed in the Thai scenario among modeled individuals (field trends are identical); however, after normalization the same qualitative trends are observed as above, with the exception of the peak in gametocytemia midway through the doubly normalized trends (Figure S3F). This peak is due to the fact that chloroquine clears parasites much faster in the model than observed in the Kenyan non-ACT treated data set (Figure S3E). The reason for this is that treatment in the Myanmar setting occurs at the absolute peak of observable gametocytemia, whereas in the field settings gametocytes are still presumably emerging from the liver in increasing numbers at the onset of treatment, and thus gametocyte levels post-treatment in the chloroquine-treated model scenario fall faster than those in the field (the reverse of the effect noted in the Thai model treatment). Because we do not know how far into their infections these children were treated, we cannot know which of the two treatment-seeking behaviors best match the field data.

V) Coding Specifications

The model was built in MATLAB (Mathworks, Version R2011a). The model outputs include the random number generator seed values so that runs may be replicated exactly.
References


Figure S2

A

B

C

D

Proportion of Day 0 Percent Gametocyte Positive

Days Post Treatment

Proportion of Day 0 Percent Gametocyte Positive

Days Post Treatment

Difference in Gametocytomas, Normalized to Day 0

Days Post Treatment

Untreated

non-gametocytocidal

in vitro

scaled in vitro

simplified

Thai, ACT (AM, DHA+PPQ)

Untreated

Thai, ACT (AM, DHA+PPQ)

in vitro

non-gametocytocidal

simplified

scaled in vitro

Thai, ACT (AM, DHA+PPQ)

Myanmar AM

CQ

Myanmar AM+PQ

AM

AM+MB

Untreated, thinned

NG

AM+PQ

Model
Figure S3

A, B, C, D, E, F: Natural Log Gametocytes per Microliter vs. Days Post Treatment for different treatments.
Mechanistic Malaria Model 1.0

- Number of runs
- Probability infection is treated [0-1]
- Treatment delay in days (if treated)
- Choice of partner drug
- Residual partner drug from earlier infection
- Number of days ago previous treatment was received (if previous infection occurred)
- Gametocyte pharmacodynamics (Adjalley et al.)
  - For MEF as partner, 'LMF' is recommended
- Percent survival per 48 hours, DHA (1/Parasite Reduction Ratio)
  - For Pailin-type DHA resistance, use 0.415%
- Percent survival per 48 hours, partner drug (1/Parasite Reduction Ratio)
- Stochastic pharmacokinetics
- Partner drug in vivo IC50 (ng/ml)
  - Default is 900 ng/ml for MEF
  - Default is 241 ng/ml for LMF
- Assume that the partner drug completely blocks transmission
- Save data
  - Model outputs are saved as:
    - Asexuals.txt
    - Gametocytemias.txt
    - Daily_Infectivities.txt
    - Net_Infectivities.txt
- Note: Each run takes approximately 10 seconds
Table S1. Treated Model Validation

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Measure</th>
<th>Thai Patient Data(^1) (N = 1,175)</th>
<th>Model, Simplified Gametocyte Assumptions (N = 1,000)</th>
<th>Model, in vitro Assumptions (N = 1,000)</th>
<th>Model, Modified in vitro Assumptions (N = 1,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Fever Prior to Admission (Days)</td>
<td>Median</td>
<td>5.0</td>
<td>3.5(^1)</td>
<td>4(^1)</td>
<td>3.5(^1)</td>
</tr>
<tr>
<td>Asexual Parasitemia At Presentation (PRBC/µL)</td>
<td>Geometric mean</td>
<td>5363</td>
<td>4240</td>
<td>4845</td>
<td>4311</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>8720</td>
<td>7023</td>
<td>7820</td>
<td>6815</td>
</tr>
<tr>
<td>Gametocytemia At Presentation and During treatment (GAM/µL)</td>
<td>Minimum</td>
<td>1.0</td>
<td>10.0(^1)</td>
<td>10.1(^1)</td>
<td>10.0(^1)</td>
</tr>
<tr>
<td></td>
<td>Geometric mean</td>
<td>209.7</td>
<td>125.8</td>
<td>236.8</td>
<td>115.0</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>20370</td>
<td>25628</td>
<td>62305</td>
<td>14715</td>
</tr>
</tbody>
</table>


Fever duration calculated assuming fever once every two days.

Minimum gametocytemia in model is 10 gametocytes per µL, given the assumption of detection by smear.

Table S1 | Treated Model Validation. In order to determine how well model behavior matches field data post treatment, the model was calibrated to reproduce the characteristics of the Thai study population. The malariometric indices of infected, ACT-treated individuals are shown in the first data column. The model fitting was performed to give the optimal match to the malariometric indices in the field. Once the model parameters were set, the model was run assuming treatment with one of three sets of assumptions: the 'simplified' assumptions, the in vitro gametocytocidal data from Adjalley et al., or the modified stage-specific gametocytocidal drug effects (described in text).
Table S2. Daily Proportion of Gametocytes Killed by Drug Treatment.
The proportion of gametocytes killed per day as a function of gametocyte age. The model killing fractions were determined using the in vitro data (Adjalley et al., 2011) as a starting point and then curve fitting treated model gametocyte clearance to field data. All in vitro data is from (Adjalley et al., 2011). The ratio model:in vitro provides the scaling factors needed to translate in vitro killing to observed decreases in gametocytemia after treatment in the field. Mean scaling factors for each drug are provided at right. MFQ = mefloquine; LMF = lumefantrine; CQ = chloroquine; DHA = dihydroartemisinin; MB = methylene blue; PMQ = primaquine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFQ</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>LMF</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>CQ</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DHA</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MB</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PMQ</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table S2. Daily Proportion of Gametocytes Killed by Drug Treatment. The proportion of gametocytes killed per day as a function of gametocyte age. The model killing fractions were determined using the in vitro data (Adjalley et al., 2011) as a starting point and then curve fitting treated model gametocyte clearance to field data. All in vitro data is from (Adjalley et al., 2011). The ratio model:in vitro provides the scaling factors needed to translate in vitro killing to observed decreases in gametocytemia after treatment in the field. Mean scaling factors for each drug are provided at right. MFQ = mefloquine; LMF = lumefantrine; CQ = chloroquine; DHA = dihydroartemisinin; MB = methylene blue; PMQ = primaquine.
Supplemental Text S2

User manual for the graphical user interface to the mechanistic malaria model (M³), Version 1.0

I) Getting Started

0. Terms of use
   a. Redistribution, modification, and/or utilization for commercial purposes
   b. Citations

1. Installation
   a. On a Windows computer
   b. On a Macintosh computer

II) Running the Model

III) Saving the Outputs

IV) For Researchers
I) Getting Started

0. Terms of use

You agree to all of the following terms of use when redistributing/modifying/using this software in any way, including use of its output and/or any part of its computer code.

a. Redistribution, modification, and/or utilization for commercial purposes

The Mechanistic Malaria Model (M3) Simulator. Copyright © 2011 Geoffrey Johnston.

This program is free software: you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation, version 3.

This program is distributed in the hope that it will be useful, but without any warranty; without even the implied warranty of merchantability or fitness for a particular purpose. See the GNU General Public License for more details.

For a copy of the GNU General Public License see http://www.gnu.org/licenses/.

b. Citations

Please cite this software in any publications that benefit from its use, including (but not limited to) its output or any of its computer code.

1. Installation

a. On a Windows computer

In order to install and run the software, you will need to download two files.

The first file, called a runtime library, allows the model software to run on any computer, whether or not MATLAB is installed. To download this file, go to the Fidock Lab website and download the Windows MATLAB runtime installer.
Once downloaded, double click on the file *MCRInstaller.exe*; this file installs the MATLAB runtime compiler which allows MATLAB programs to be run on any computer even if MATLAB is not installed. Follow all of the installation directions; the ‘Typical’ installation is recommended.

The second file that you will need is the model file itself (*model.exe*); this file is found in the supporting information. Place this file in the same directory as the *MCRInstaller*.

To run the M³ software, simply double click on *model.exe*; this should open the graphical user interface to the model.

b. **On a Macintosh computer**

In order to install and run the software, you will need to download two files.

The first file, called a runtime library, allows the model software to run on any computer, whether or not MATLAB is installed. To download this file, go to the Fidock Lab website and download the Macintosh MATLAB runtime installer.

Once downloaded, double click on the file *MCRInstaller.dmg*; this file installs the MATLAB runtime compiler which allows MATLAB programs to be run on any computer even if MATLAB is not installed. Follow all of the installation directions; the ‘Typical’ installation is recommended.

The second file that you will need is the model file itself (*model.exe*); this file is found in the supporting information. Place this file in the same directory as the *MCRInstaller*.

To run the M³ software, simply double click on *model.exe*; this should open the graphical user interface to the model.

**II) Running the Model**
In the User Manual we will assume that the model is being run from the graphical user interface (GUI). The GUI appears as shown in Figure 1. The GUI allows users to change model parameters, examine output in real time, and save their results.

![Figure 1: The Graphical User Interface](image)

There are seven windows that are displayed on the GUI. The three windows on the left display the daily $\log_{10}$ asexual parasitemia per $\mu$L, the $\log_{10}$ gametocytemia per $\mu$L, the daily probability of human to mosquito transmission. These are termed the ‘Parasitological Responses.’ The top two windows in the middle of the GUI depict the modeled daily plasma concentrations of the partner drug used in treatment as well as the pharmacodynamics of the partner against asexual parasites (these windows remain blank if untreated).

The bottom two windows depict the pharmacodynamics of the partner drug against gametocytes. The daily percentage of gametocytes surviving after drug exposures are shown (at left is the effects of dihydroartemisinin, at right is the partner of choice). The x-axis is the age of the gametocyte: in general, antimalarials have greater effects on young gametocytes than old ones. The higher the daily value for a given gametocyte age, the greater the proportion of gametocytes of that age that survive exposure. These windows display the pharmacodynamic
profiles against gametocytes regardless of treatment (but have no effect if untreated). These four windows are termed the ‘**Host Responses**.’

The parameters that can be adjusted in the model are found in the upper right hand section of the GUI. (Not all model parameters are user-adjustable in Version 1.0.) We describe these parameters here:

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Default Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Runs</td>
<td>1</td>
<td>The number of runs the model will perform. Each run takes approximately 10 seconds on a Windows laptop with an Intel Core 2 Duo processor; faster computers will take significantly less time per run. Range: ([1 – \infty)).</td>
</tr>
<tr>
<td>Probability infection is treated</td>
<td>0</td>
<td>The probability that an infection will be treated with an ACT; the parameters determining the efficacy of this treatment are defined below. Set this value to 1 to insure individuals are treated. Range: ([0 – 1]).</td>
</tr>
<tr>
<td>Treatment delay in days</td>
<td>2</td>
<td>The number of days after the first fever that an individual will receive treatment. Set this value to 0 to have an individual treated on their first fever day. Range: ([0 – \infty)).</td>
</tr>
<tr>
<td>Choice of partner drug</td>
<td>LMF</td>
<td>The partner drug that will be utilized in the treatment. This parameter determines the pharmacokinetics of the partner, as well as setting the</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td>Description</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>-------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>in vivo IC50. The IC50 and other parameters can be adjusted separately.</td>
<td></td>
<td>There are currently three possible selections: ‘LMF’ - Lumefantrine; ‘MEF fixed’ - Mefloquine, fixed dose; ‘MEF loose’ - Mefloquine, loose dose.</td>
</tr>
<tr>
<td>Residual partner from earlier infection</td>
<td>No</td>
<td>Determines whether an earlier infection was treated. If ‘Yes,’ then the model simulates residual partner drug being present. The number of days previous the treatment was assumed to occur is set by a parameter below; the default value is 20 days.</td>
</tr>
<tr>
<td>Number of days ago previous treatment was received (if infection occurred)</td>
<td>N/A</td>
<td>This parameter determines the number of days previous that an infection was treated. The model assumes that only the partner drug is present in the system (and that the dihydroartemisinin component is no longer present). The default value is 20 days. This parameter has an effect only if the ‘Residual partner from an earlier infection’ parameter is set to ‘Yes.’ Range: [0 – ∞).</td>
</tr>
<tr>
<td>Gametocyte pharmacodynamics (Adjalley et al.)</td>
<td>LMF</td>
<td>This parameter determines the stage-specific effects of the partner drug on gametocytes. The x-axis indicates the age of the gametocyte and the y-axis indicates the proportion of gametocytes that survive one day of drug treatment. These curves were taken</td>
</tr>
</tbody>
</table>
from the Adjalley et al. paper. The ‘simplified’ assumptions are described in Text S1 and affect the pharmacokinetics of both dihydroartemisinin and its partner.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent survival per 48 hours, DHA</td>
<td>0.01</td>
<td>This is the percentage of parasites surviving treatment with dihydroartemisinin after 48 hours. This parameter can be adjusted in order to simulate the resistance observed in Pailin, Western Cambodia, by changing the value to 0.415. This parameter does not determine the net parasite reduction ratio per 48 hours; that is determined by a variety of factors (including this parameter). Range: [0 – 100].</td>
</tr>
<tr>
<td>Percent survival per 48 hours, partner drug</td>
<td>1</td>
<td>This is the minimum percentage of parasites surviving treatment with the partner drug after 48 hours. The effectiveness of the partner is modified by its plasma concentration; this parameter determines its maximum effectiveness. This parameter does not determine the net parasite reduction ratio per 48 hours; that is determined by a variety of factors (including this parameter). Range: [0 – 100].</td>
</tr>
<tr>
<td>Stochastic pharmacokinetics</td>
<td>No</td>
<td>This parameter determines whether the</td>
</tr>
</tbody>
</table>
pharmacokinetics of the chosen partner drug are stochastic or not. If set to ‘Yes,’ the standard pharmacokinetic properties of the partner are modified as described in **Text S1**.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partner drug <em>in vivo</em> IC50</td>
<td>241</td>
<td>This parameter determines the plasma concentration at which the partner drug exerts half of the killing effects of its maximum parasite reduction ratio against asexual parasites. In other words, when the plasma concentration of the partner drug reaches this value, the 48 hour percent survival is half of its maximum value. The default value for mefloquine is 900 ng/ml; for lumefantrine, it is 241 ng/ml.</td>
</tr>
<tr>
<td>Assume that the partner drug completely blocks transmission</td>
<td>No</td>
<td>This parameter determines whether the partner drug is perfectly transmission blocking. If set to ‘Yes,’ upon treatment the partner drug immediately kills all gametocytes that were produced and does not allow any gametocytes to be produced in the future.</td>
</tr>
<tr>
<td>Save data</td>
<td>No</td>
<td>This parameter determines whether some of the output data is saved. If set to ‘Yes,’ the daily asexual parasitemias, gametocytemias, infectivities, and net infectivities are saved. See Section III) ‘Saving the Outputs’ below for more information.</td>
</tr>
</tbody>
</table>
III) Saving the Outputs

When saving the model outputs, first, set all of the parameters to their desired values. The ‘Save data’ parameter needs to be set to ‘Yes’ before the model is run; you cannot save data by adjusting this parameter once the model has started. Assuming that ‘Save data’ is correctly set, the model will save the following outputs: the daily asexual parasitemias, the daily gametocyttemias, the daily infectivities, and net infectivities for each run. Each of these four sets of data is saved to a separate ASCII text file. The file names are ‘Asexuals.txt,’ ‘Gametocyttemias.txt,’ ‘Daily_Infectivities.txt,’ and ‘Net_Infectivities.txt.’ On Windows computers, these files should appear in the directory in which the model is run.

IV) For Researchers

Although the model GUI provides significant functionality to the end-user, researchers may desire additional capabilities. For example, researchers may desire to modify parameters beyond those included in the GUI or run very large numbers of simulations.

There are two options to accommodate these needs. The first option is that researchers could modify the source code as they see fit and run multiple instances of the model from the command line or a modified GUI. The second option is to utilize the computing structure already developed by the authors to run massively parallel computations. To perform the simulations in the paper, as well as ongoing work, the authors use a high-performance computing cluster known as Hotfoot, based at Columbia University. This cluster runs Linux and utilizes the Maui/Torque job scheduler to allocate computing resources. To access this cluster, the authors wrote a script for Maui/Torque that allows for parallel execution of the model and modified the MATLAB code so that the code can be called by this script. This cluster allows for hundreds of
simultaneous runs, such that the time needed to run simulations is reduced approximately 50-fold versus single-instance simulation. If researchers are interested in accessing this resource to run their own simulations, please email the corresponding authors for more information.
Chapter 3: Epidemiological and Economic Considerations for Worldwide Deployment of Rapid Diagnostic Testing and Antimalarial Subsidies for Malaria Control
Epidemiological and Economic Considerations for Worldwide Deployment of Rapid Diagnostic Testing and Antimalarial Subsidies for Malaria Control

Geoffrey L. Johnston¹,², Eili Y. Klein³, David A. Fidock,¹,⁴¶ Ramanan Laxminarayan⁵,⁶,⁷, and David L. Smith⁸¶

¹Department of Microbiology and Immunology, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
²School of International and Public Affairs, Columbia University, New York, NY 10027, USA
³Department of Emergency Medicine, John Hopkins University, Baltimore, MD 21287, USA
⁴Division of Infectious Diseases, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
⁵Center for Disease Dynamics, Economics & Policy, Washington, DC 20036
⁶Princeton Environmental Institute, Princeton University, Princeton, NJ 08544
⁷Public Health Foundation of India, New Delhi, India 110070
⁸Bloomberg School of Public Health, John Hopkins University, Baltimore, MD 21205, USA

¶To whom correspondence should be addressed. E-mail: df2260@columbia.edu, Tel: +1 212-305-0816, FAX: +1 212-305-4038; E-mail: dlsmith@jhsph.edu, Tel: +1 410-502-3377, FAX: +1 410-502-5884
Abstract

Malaria is a leading cause of childhood morbidity and mortality throughout the developing world, particularly in Africa. Artemisinin-based combination therapies (ACTs) are currently the recommended first-line therapy for treating malaria and are in use worldwide. However, given their relatively high cost, these therapies are currently heavily subsidized in both the public and private sectors in many parts of Africa and Southeast Asia. The private sector subsidy mechanism, known as the Affordable Medicines Facility for malaria, is now under review. To help evaluate the benefits of antimalarial subsidization, we fitted semi-mechanistic models to malaria mortality data from 1980-2010. We simulated the impacts of first-line antimalarial therapy and drug resistance as well as insecticide treated bednets, ACTs, and rapid diagnostic tests (RDTs) on mortality during this period. We also projected future trends in mortality and assessed the benefits of treatment by the metrics of lives saved and dollars per disability life year (DALY) averted. First, we find that ACTs are cost-effective at reducing mortality over time: our model predicts that ACTs average approximately $13 per DALY averted, well below the $50 threshold for an intervention to be considered ‘very cost-effective’ [1]. Second, we find that the risk-averse decision planner will only employ RDTs in a limited number of settings. Third, our model predicts an 8-10 year window after emergence of resistance in Africa before resistance necessitates a change in therapy for Africa as a whole. ACT subsidization is a highly cost-effective tool even if resistance is not averted for a significant period of time. However, if resistance can be averted for even three years, we predict that ~1.3 million lives will be saved by 2040, assuming emergence of resistance in Africa in 2015.
Introduction

Malaria is a significant cause of morbidity and mortality throughout the developing world. Though estimates of the death toll during the past three decades vary substantially because of real underlying uncertainty, the general trends are well documented. During 1980-2000 a tide of mortality swept through Africa after chloroquine (CQ) and sulphadoxine-pyrimethamine (SP), the two previous first-line treatments, failed due to drug resistance [2,3]. The situation has improved recently due to improved treatments and large-scale deployment of long-lasting insecticide treated bednets (LLINs) in Africa [4].

The recommended first-line therapy for malaria is now artemisinin-combination therapy (ACT) [5]. Artemisinin is a fast-acting, potent antimalarial with exceptional biological properties. Artemisinin-based combination therapies combine an artemisinin with a partner antimalarial in order to improve the clinical efficacy of artemisinin and delay the evolution of resistance [6-8].

Given the importance of artemisinin to global malaria control, it is imperative to protect it from resistance to the extent possible while ensuring access to all who are in need. In 2002, an Institute of Medicine committee was convened to address concerns that resistance to artemisinins would evolve. The committee’s report, issued in 2005, advocated “a sustained global subsidy of artemisinin co-formulated with other antimalarial drugs in order to reduce malaria mortality (‘saving lives’) and delay resistance (‘buying time’) until new categories of antimalarials could be developed [6]. This subsidy would save lives by lowering the costs of ACTs and thereby increasing access; it would also buy time by ensuring that ACTs were more affordable than artemisinin monotherapies, thus crowding out the monotherapies from the market [6].

Large-scale uptake of ACTs began in 2005, and the Affordable Medicines Facility for malaria (AMFm) was launched in the fall of 2009 in nine countries to subsidize the private-sector purchase of ACTs and has been successful at lowering the retail price of ACTs [9-12]. In 2011 the commitments toward AMFm amounted to $316 million from various partners [13]. A distinct advantage of the policy was pooled procurement, which allowed the AMFm to purchase ACTs at the cost of $1 per curative dose.

The pilot AMFm program is now being evaluated for renewal [13]. In this paper we develop a semi-mechanistic model to evaluate the benefits of ACT subsidization. We examine trends in imputed malaria mortality over time from multiple sources and develop an
intermediate, ‘consensus’ set of mortality data. To this mortality data we fit a model of the effects of drug resistance, deployment of ACTs, and scaling-up of LLIN usage. We also include a model of the effects of rapid diagnostic testing (RDT) on ACT efficacy, given that RDTs are another mechanism by which resistance might be averted.

We then use this model to project what the effects of future ACT resistance might be, assuming similarity to the spread of resistance in 1980-2000. We evaluate subsidization from the perspective of numbers of lives saved as well as dollars per disability-adjusted life year, a common measure of program cost-effectiveness [14]. Given the difficulties involved in predicting to what extent subsidization will delay resistance, we conduct a sensitivity analysis of the effects of ‘buying time’ on these metrics.

These studies lead to three main conclusions. First, we find that ACTs are cost-effective at reducing mortality over time: our model predicts that ACTs average approximately $13 per DALY averted. Second, we find that rapid diagnostic testing (RDT) can reduce ACT overuse by as much as 15-33% at high coverage levels, though costs are increased by 25-30% if RDT prices remain constant. Third, we predict that if ACT resistance patterns follow those of CQ, then the 10% clinical treatment failure threshold is not likely to be reached in Africa until 8-10 years after the emergence there of resistance, providing a short window for the development and deployment of new pharmaceutical compounds.

Methods
Most mathematical models for the evolution of anti-malarial resistance, whether population genetic or population dynamic models, have several common properties [15,16]. Models must make some assumption about the initial frequency of resistance. These initial conditions can include a lag, called a “honeymoon period,” between the introduction of the drug and the emergence of resistance somewhere. Then, after resistance has emerged or been imported from elsewhere, some other model for the spread of resistance considers the initial condition and determines increases in the frequency of resistance in some set of populations. Models for spread predict that, if there is net positive selection for resistance because of drug use, the frequency of resistance will increase over time and that these changes in a single population will have a sigmoidal shape. Selection is related to factors that may vary from place to place, including the intensity of malaria transmission, the rates of use of antimalarial drugs, and the mathematical
assumptions of the model. There is also some uncertainty about the asymptotic frequency of resistance, which may be related to superinfection, the cost of resistance, and the effects of chemoprophylaxis [17,18]. In the end, though, key uncertainties can be reduced to four parameters: 1) the length of the honeymoon period; 2) the rate of increase of drug resistance over time; 3) the asymptotic frequency of resistance; and 4) the clinical consequences of resistance. Here, we have adopted a semi-mechanistic approach in which we take the sigmoidal shape without making any explicit underlying assumptions about the underlying biological (e.g. parasitological, pharmacological, epidemiological, clinical, behavioral, immunological) causes. Values of these three parameters are chosen to make the model consistent with existing data.

Model Specification
We used estimates of worldwide malaria mortality to parameterize a model of the spread of first-line antimalarial resistance and subsequent reductions in mortality using LLINs, ACTs, and RDTs. The malaria mortality data came from three sources, which served as the basis upon which our semi-mechanistic model was built. The first data are the estimated worldwide malaria deaths from the Global Burden of Disease (GBD) Project [4]; these data estimate mortality from 1980-2010. The second data are from the 2011 World Malaria Report, which provides the WHO estimates of malaria mortality from 2000-2010 [2]. These two data sets differ considerably in their estimates of mortality over time; as an intermediate scenario we took the arithmetic mean of the two datasets. This approach results in deaths for 2000-2010; the mean estimate for 2000 matches the estimate for 2000 reported in [19] adapted from the WHO World Health Report, 2002. For those years in which only one data set was available (1980-1999), we took the data from the GBD and scaled it linearly.

The shape of the sigmoidal curves were scaled so that deaths in 1980 were 20% higher than what would have resulted if we had taken the GBD 1980-2000 mortality data and simply shifted it down to meet the mean estimate of 2000. The reason for this scaling is two-fold. First, since the trends are so prominent in the GBD data, if we had used the un-scaled trends then there would have been an unrealistically low number of deaths in 1980. Second, the effect of this scaling is that the increase in deaths during 1980-2000 is shallower than if the non-scaled data had been used; we wanted to produce a more conservative estimate of the spread of CQ resistance than would have resulted from a translation of the GBD data. The results of this
estimate are shown in Figure 1, where the red line indicates the intermediate scenario of deaths used in the modeling and the shaded area provides the difference between GBD and WHO estimates where both exist.

Once the mortality data had been generated, we then fitted a semi-mechanistic model to these data based on the following assumptions. First, we assumed that in the absence of any treatment, malaria mortality would be increasing linearly over time. This assumption was justified on the basis that the rural African population has been increasing linearly over the past 30 years (linear fit $R^2 = .998$; data from World Bank DataBank; http://databank.worldbank.org) and that approximately ~90% of all malaria deaths are from Africa [2]. The second assumption was that first-line malaria therapy was relatively constant over the period 1980-2000 and that the increase in deaths was due to increasing resistance and declining efficacy of these antimalarials. Specifically, we assumed that resistance over time $R(t)$ was described by the function $R(t) = 1 - (1/(1 + t/a)^b)$. This model assumes that resistance had no significant effect on mortality until 1980; up to that point, resistance had been most prevalent in Southeast Asia, where the global share of deaths is low. The virtue of this model is simplicity: although the spread of first-line antimalarial resistance is a complicated process involving appearance, emergence, and spread in multiple locations [16], the trend of resistance worldwide will appear sigmoidal because the different rates of spread in various areas will be averaged over time.

To account for the contributions of LLINs and ACTs to malaria reductions, we included the uptake of these control measures over time [2] in our model. We did not include any effects of spraying on reductions in mortality, as LLINs and ACTs were the likely first-order drivers of mortality over the past decade, given that spraying coverage in Africa is still low even after recent growth (~11% in 2010 [2]), and that spraying may not be as effective in areas of very high LLIN coverage [20]. For LLINs, we assumed a linear relationship between African household coverage (for 2000-2010 [2]) and deaths averted. For ACTs we also assumed a linear relationship between worldwide public and subsidized private sector purchases (2005-2010 [2]; we ignore unsubsidized purchases given their small share of total supply) and decreases in mortality. However, the effects of ACTs are also augmented by the use of RDTs, which increase the efficacy of antimalarial treatment by increasing the proportion of malarious individuals treated as a proportion of all treatment-seeking individuals.
To parameterize the effects of RDTs on improving ACT efficiency, we developed a model to predict how much RDTs increase the efficacy of ACTs and reduce overtreatment; we then applied this model assuming the RDT uptake trends from [2]. Consider the case of ACT usage in the absence of RDTs. Individuals with fever visit either public or private health care providers to purchase/receive treatment. Most of these individuals will have a febrile illness other than malaria but will receive malaria treatment nevertheless. The proportion of febrile individuals who have malaria (\(prop_{mal}\)) will vary with the levels of malaria endemicity in an area. However, WHO model estimates predict that \(prop_{mal}\) will be 38% on average under current LLIN coverage levels in 2015; this is the value that we use here [2]. In absence of diagnosis, \(prop_{mal}\) is the proportion of individuals who will be cured by treatment. A much smaller fraction of \(prop_{mal}\) will avert death, and the remaining proportion \((1 - prop_{mal})\) of individuals will be treated improperly.

In the presence of RDTs, however, the probability that an individual will be properly treated for malaria increases. Thus, the probability that a given ACT will cure an individual or save a life will increase, and a given pool of drug will save more lives than in the absence of RDT usage. The probability that a febrile treatment-seeking individual will receive treatment for malaria (the true positivity rate or the sensitivity of treatment) is a function of six variables in the presence of RDTs: \(prop_{mal}\); the proportion of individuals receiving RDTs (\(RDT_p\)); the probability that an individual will receive treatment given a positive RDT (\(c_pos\)); the probability that an individual will not receive treatment given a negative result (\(c_neg\)); the false negative rate of the RDT (\(false_neg\)); and the false positive rate of the RDT (\(false_pos\)).

Given the function for the specificity of ACTs in the presence of RDTs, we can calculate by what factor specificity is increased versus the case with no RDT usage; we call this function \(sensitivity_{mult}\) and is defined as \(sensitivity/prop_{mal}\). These parameters vary significantly depending on the treatment-seeking behavior of the population; for example, non-compliance with a negative RDT (1-\(c_neg\)) varies from <16% to 85% depending on age, season, training, etc. [21-23]. We assume very good compliance with RDTs: specifically, we assume compliance with a positive result is 95%, compliance with a negative result is 60%, the false negative rate of the RDT is 5% and the false positive rate is 20%.

Given these assumptions, the specification for our model is (where mortality is the dependent variable, \(\beta\) is the vector of parameters to be estimated, \(\rho\) is the vector of assumed
parameters for the RDT specificity function, $RDT(t)$ is RDT usage over time, $ACT(t)$ is ACT usage over time, and $LLIN(t)$ is LLIN usage over time):

$$Y(t) = (\beta_1 + \beta_2 t) - \beta_3 \left[ 1 - \left( 1 + \frac{1}{\beta_4} \right)^{\beta_5} \right] - \beta_6 \cdot \text{specificity\_mult}(p, RDT(t)) \cdot ACT(t) - \beta_7 \cdot LLIN(t)$$

To estimate $\beta$ we used the lsqnonlin function (MATLAB 2012a; MathWorks), which finds the best-fit estimates using least-squares (to minimize the sum of squares cost function) among a set of constrained parameters. We found that meaningful parameter identification with the full set of parameters was not possible, even though we specified functional forms for the effect of each of the contributors to mortality over time and we had differential patterns of uptake among the various contributors. We thus manually set the probability of an ACT saving a life in such a way as to be consistent with the data (see below); the rest of the parameters were then fitted to the data. We can also use this statistical model to explore parameter sensitivity apart from any mortality estimation. Table 1 provides the parameter estimates with this specification from the mortality data.

Our assumption of linear returns to control measures is most applicable in areas of medium endemicity. In areas of high endemicity, interventions might have lower returns to control measures given the high levels of incidence. In low endemicity areas, interventions may be sufficient to interrupt transmission. We thus assume that malaria transmission is not reduced sufficiently on a worldwide scale such that elimination is achieved in many areas. In such a case the returns to ACTs will be higher than modeled here. We also do not consider any synergies among treatments (ACTs being more effective in the presence of LLINs, for example).

We also assume that LLIN usage after 2011 is constant at 2011 levels with approximately 50% coverage of African households with at least one bednet [2]. For RDT uptake, we used African data and assumed that if individuals seek treatment in a public health facility that provides RDTs, then the case is first tested by an RDT. In the public sector RDT coverage has risen rapidly over the past few years; in 2010 level, 45.7% of suspected malaria cases attending public facilities were in a facility that used RDTs [2]. However, only 29% of individuals are estimated to seek care in a public facility in Africa [2], and we assumed that there is no uptake outside the public sector; thus our coverage level was estimated at 13.25% of cases for 2010 and
afterwards. We also scaled RDT efficacy over 2007-2010 according to the panel detection scores reported in [2]. It is very likely that this proportion will rise over time\(^1\); however, our baseline estimates are for the effect of ACTs only, not in concert with other interventions.

For our costing analysis and calculation of DALYs averted, we assumed that the price of ACTs fell from $3 per dose to $1 per dose over the period of 2005-2012 and then stayed constant at this level going forward [13]; the price of $1 per dose is the approximate price cap negotiated by the Clinton Health Access Initiative (CHAI) and reported by the Global Fund.\(^2\) We assumed that RDTs cost $0.67 per test and that this price is also constant.\(^3\) Our baseline assumptions thus consider the benefits of ACT subsidization _ceteris partibus_. For the cost of the first-line therapies we assumed a cost of $0.085 per dose, which is the mean cost per treatment episode for an adult in 1990 [24]. Note that the cost of SP in 1990 was not much higher, at $0.13 per dose, and that the cost of CQ dosing for children would be lower (the relative proportions of each are described below).

The DALYs averted for each treatment are derived from two sources: mortality and morbidity. For mortality, we use the age distribution of clinical malaria cases from [4] and the life tables for estimated African life expectancy in each age class from WHO estimates.\(^4\) For morbidity, we assume that the proportions of fevers that are malarious are cured for each treatment, accounting for resistance, and that these avert 3/52 of a DALY (i.e. each infection causes 3 weeks of morbidity). We do not consider prophylactic effects of treatment.

One caveat to our analysis of resistance is that increasing ACT treatment levels past a certain point might increase the spread of resistance. Because there may be more selection for resistance when the number of treatments increases, resistance might spread faster. We do not consider such thresholds or functional responses here but do note that further research is needed to help understand the relationship between treatment, overtreatment, and spread of resistance. Also, we do not consider cases where ACT subsidization slows the spread of resistance versus the CQ case, which is also possible given the crowding out effect. Rather, because little is known

\(^1\) See [www.theglobalfund.org/documents/amfm/AMFm_AffordableMedicinesFacilityMalaria_FAQ_en](http://www.theglobalfund.org/documents/amfm/AMFm_AffordableMedicinesFacilityMalaria_FAQ_en).
\(^3\) This estimate was taken from the RDT cost-effectiveness estimator provided by the WHO: [http://www2.wpro.who.int/sites/rdt/using_rdts/assessing_cost_effectiveness.htm](http://www2.wpro.who.int/sites/rdt/using_rdts/assessing_cost_effectiveness.htm).
\(^4\) See Annex Table 2, [http://www.who.int/healthinfo/paper09.pdf](http://www.who.int/healthinfo/paper09.pdf); average of males and females in AFR D and AFR E regions.
about such processes on a global level we simply assume that ACT resistance and CQ resistance spread at the same rates, and examine what happens to the sensitivity of our results if the waiting time to resistance is varied.

**Consistency of Model Outputs with Prior Studies**

Once our statistical model was specified and fitted to data, we examined the quality of the fit; Figure 2 shows the model-predicted mortality as well as the estimated mortality over time, along with the estimated fractions of lives saved from first-line therapies, LLINs, and ACTs aided by RDT usage. The first point to note is that the model predicts that first-line therapies prevented ~793,000 deaths or 55% ($\beta_3/\beta_1$) of total malaria mortality in 1980. By 1999, however, the model predicts that resistance to these therapies had reduced efficacy by approximately one-half.

We consulted the literature to examine whether we could derive some estimates of these parameters as a basis for comparison with our estimates. We found few estimates of the total levels of first-line antimalarial usage during 1980-2000; however, one study estimated that in 1989 national malaria control programs distributed the equivalent of 134 million adult doses of chloroquine (CQ); 17.7 million adult doses of sulphadoxine-pyrimethamine (SP); and 2.0 million doses of adult quinine (QN) [25]. Thus, the vast majority of first-line therapies (87%) provided through the public sector were CQ. Further, the paper reports that approximately 20% of treatments were through national malaria control programs, implying a total number of 769 million adult doses of antimalarials consumed worldwide in 1989. We assume that private sector drug shares were similar to those of the public sector.

We can use our numbers of lives saved from first-line antimalarial use to generate a very rough estimate of the number of doses received. We can assume that, at a first pass, the number of lives saved ($\beta_3$) by first-line therapies in absence of resistance equals the number of doses ($x$) times the proportion of fevers that are malarious ($prop\_mal$) times the probability that an ACT averts a death ($\beta_6$) in absence of an RDT. Thus $x \approx \beta_3 \cdot prop\_mal^{-1} \cdot \beta_6^{-1}$; if we use our estimates from above, we predict a total of 1.40 billion treatments, or 1.8 times more treatments that estimated in [25]. One reason that our estimates differ is that our estimate is of total doses, whereas [25] estimates adult dose equivalents. The total recommended adult dose of CQ for acute malaria is 2.5 g; for children it is 25 mg/kg. If a child weighs an average of 25 kg (55 lb), this would represent 0.625 kg of CQ (1/4 of the adult dose). Thus, if two-thirds of the doses
estimated in [25] were for adults and the rest for children (at a 4:1 ratio of dosage), the two total dosage estimates would be approximately equal. This calculation assumes that the probability of saving a life given CQ or an ACT is equal (in the absence of resistance).

Regarding the spread of first-line therapy resistance, the vast bulk of treatments during 1980-2000 and onward were CQ. According to [26], “most African countries only abandoned chloroquine between 2004 and 2008.” Thus malaria drug resistance and mortality kinetics should roughly follow those of CQ. According to a contemporaneous study in three rural populations in Senegal [27], “from 1984 to 1995 … the emergence of chloroquine resistance has been associated with a dramatic increase in malaria mortality … (mortality in 0-9 year olds) was multiplied by 2.1, 2.5, and 5.5 times, respectively.” A recent retrospective analysis confirms that CQ resistance was causing substantial increases in mortality in Uganda through 1997-1998 [28]. There can be no doubt that mortality was increasing during 1980-2000 due to CQ resistance.

Chapter 21 of [29] provides WHO estimates of chloroquine treatment failure rates throughout Africa, from 1997-2002. Failure rates range from approximately 70% in Ethiopia to as low as 0-15% in Nigeria (where a considerable number of deaths are concentrated). Thus there was substantial room for increases in mortality due to CQ resistance as late as 2002. The spread of resistance was slow throughout Africa during 1980-2005, likely due in part to the fitness cost of CQ resistance [30] in an environment of high multiplicity of infection. Indeed, CQ resistance was first discovered in Cambodia in 1957 [31] but was not documented in Africa until 1978.

We chose the parameter $\beta_6$ so that relative effects of LLINs and ACTs on mortality are approximately equal (Figure 2). (The proportion of lives saved by ACTs from 2006-2010 varies from 48-54%.) This is in some conflict with the study by Murray et al. [4], which attributes the bulk of changes in mortality over 1980-2010 to drug uptake and resistance. However, we have seen that our estimate of $\beta_6$ is roughly consistent with available data. Further, LLINs and ACTs have both been found to be contributors to reduced morbidity and mortality in field settings [5,26,32].

Analytic Description of the Effects of RDTs for Malarial and non-Malarial Diseases

There are many benefits to using RDTs to diagnose febrile treatment-seeking individuals [33]. First, RDTs reduce the level of ACT overtreatment, i.e., the treatment of individuals without malaria. By reducing ACT usage, RDTs will reduce the appearance, emergence, and spread of
ACT partner drug resistance and thus increase the waiting time to appearance of ACT resistance. Although the precise functional relationship between overtreatment and resistance is not known, there is evidence for the existence of such a relationship [34]. We do not consider the benefits of multiple first-line therapies here, although control strategies incorporating these might be able to preserve the effective lifetime of artemisinin [18]. Another benefit of RDTs is that individuals who are shown to be RDT negative can seek treatment for a disease other than malaria. If malaria-negative individuals are presumptively treated for malaria, either by themselves or a health-care provider, these individuals may delay seeking treatment for the true cause of morbidity because of the mistaken antimalarial use.

The extent to which malaria overtreatment interferes with rational treatment of other causes of morbidity is difficult to determine; however, we try to provide an estimate here. Figure S1A provides our estimate of the distribution of febrile illnesses in Africa; the proportions of each cause of fever are shown, including the fraction of comorbidity with malaria. The proportion solely attributed to malaria (purple) has fractions of comorbidities with other diseases, but these cannot be determined from the data. Text S1 I provides an explanation of our estimation. To breakdown how those causes might benefit from treatment, we apportioned the various causes of fever into categories. Text S1 II provides an explanation of the treatment options for each type of febrile illness. Figure S1B illustrates the assumed proportion of malaria mortality as a function of prop_mal; the remaining fraction of mortality is assigned proportionally according to the proportions in Figure S1A.

In order to sort out the complex effects of RDTs on treatment outcomes for malaria and non-malarial diseases, we can quantify the effects of RDTs as follows. We focus on the benefits of RDT information for improved antibiotic targeting in our analyses here. Let $a$ be the increase in the extensive margin of treatment, i.e., the encouragement or discouragement of individuals to treatment in the presence of an RDT subsidy. Let $b$ be the change in intensive margin in targeting, i.e., the increased proportion of individuals receiving proper antimalarial treatment. Let $c$ be the change in extensive margin of RDTs for non-malarial disease, and let $d$ be the change in intensive margin for non-malarial disease, considering only diseases that benefit from antibiotic usage. Thus, with RDTs, $a \cdot \text{prop\_mal}$ is the percentage of individuals receiving treatment for malaria that actually have malaria, and $a \cdot \text{prop\_mal} \cdot b$ is the percentage receiving proper treatment, i.e., ACTs. Let $\text{prop\_antibio}$ be the proportion of individuals receiving antibiotics in
the absence of RDTs who have a disease that is cured by antibiotics, then $c \cdot prop\_antibio \cdot d$ is the percentage in the presence of RDT subsidies.

These percentages will differ depending on whether individuals seek treatment in the public sector or the private sector. Thus we have that the effect of RDTs can be broken down as follows: $a_{pri} \cdot prop\_mal_{pri} \cdot b_{pri}$ is the percentage of individuals with malaria seeking treatment in private sector; $c_{pri} \cdot prop\_antibio_{pri} \cdot d_{pri}$ is the percentage in the presence of RDT subsidies in the private sector; the quantities $a_{pub} \cdot prop\_mal_{pri} \cdot b_{pub}$ and $c_{pub} \cdot prop\_antibio_{pub} \cdot d_{pub}$ are the same percentages seeking treatment in the public sector.

Using this framework, we can analyze the complex data resulting from field studies. First, $a_{pri}/a_{pub}$ and $c_{pri}/c_{pub}$ represents ‘crowding out’; we assume that there is some crowding out and set $a_{pri}/a_{pub} = c_{pri}/c_{pub} = 1.5$, i.e., more people seek treatment in the private versus the public sector and that RDTs increase this proportion [35]. In the absence of RDTs we assume 29% public 71% private so this ratio is 2.44; under RDTs we assume the proportions are 40% and 60%. We assume that RDTs do not affect the total extensive margins ($a_{pri} + a_{pub} = 1$ and $c_{pri} + c_{pub} = 1$.

1. Effects on Malarial Diseases
For the effects on the intensive margin, we assume that RDTs actually slightly decrease the proportion of treatment-seeking individuals with malaria receiving of ACT treatment ($b_{pri} = b_{pub} < 1$), which we generate using our RDT model. In the case of presumptive treatment, the number of DALYs averted is proportional to $prop\_mal$. Once test-and-treat protocols are implemented, however, some individuals with malaria do not receive treatment because of failures of compliance and/or testing. Thus, as RDT coverage increases, the average number of DALYs averted per treatment-seeking individual decreases. This is one of the costs of RDTs; we call this ‘treatment discouragement.’

Corresponding to the quantities above, we can also consider the effects of RDTs on overtreatment outcomes. Let $\alpha \cdot (1 - prop\_mal)$ be the percentage of individuals receiving treatment for malaria that do not have malaria under RDT subsidies and let $\alpha \cdot (1 - prop\_mal) \cdot \beta$ be the percentage of individuals without malaria seeking treatment and receiving ACTs under
RDT subsidization (‘overtreatment’). The proportion of individuals being overtreated is shown in Figure S2 which we generate using our RDT model; we assume that $\beta_{\text{pub}} = \beta_{\text{pri}}$.

Using our model of the effectiveness of RDTs, we find that antimalarial overtreatment can be reduced by 17.1% at 80% RDT coverage, assuming ‘very good’ compliance and by 33% at 93% treatment coverage, assuming ‘excellent’ compliance. (For ‘very good compliance’ we assumed 95% compliance with a positive RDT and 60% compliance with a negative RDT; for ‘excellent compliance,’ 95% and 80%, respectively.) A recent report finds that overtreatment can be reduced by 58%; however, in this case the ACT subsidy was also reduced to discourage over consumption in addition to RDT subsidies [35]. Once the effects of the lowered ACT subsidies are controlled for, the authors find that, “(s)omewhat surprisingly, (our analysis) does not reveal many significant impacts of RDTs on ACT targeting” [35]. They attribute this lack of effect of RDTs on reducing overtreatment to the fact that “noncompliance in our population was high,” thus illustrating the importance of encouraging compliance among RDT negative individuals. However, other studies report a nearly complete reduction in overtreatment [36], illustrating the sensitivity of antimalarial overtreatment outcomes to compliance, test sensitivity and specificity, RDT coverage, the proportion malarious among treatment-seekers, etc.

2. Effects on non-Malarial Diseases

Let $\kappa \cdot (1 - \text{prop}_\text{antibio}) \cdot \delta$ be the percentage of individuals who have diseases that do not respond to antibiotics but receive antibiotics. Both of these quantities may be differentiated by public and private sectors as above. We assume that $\alpha_{\text{pri}} / \alpha_{\text{pub}} = \kappa_{\text{pri}} / \kappa_{\text{pub}} = 1.5$ and $\alpha_{\text{pri}} + \alpha_{\text{pub}} = 1$ and $\kappa_{\text{pri}} + \kappa_{\text{pub}} = 1$, i.e., RDTs crowd out antibiotics at the same level as antimalarials and do not alter the extensive margins. For the change in the percentage receiving antibiotics, we assume that in the absence of RDTs, 25% of individuals with febrile illnesses receive them.

The effect of RDTs on antibiotics, $d$, depends on how individuals and health care practitioners use both RDT positive and RDT negative results. Let us assume that in the public sector, if antibiotics are prescribed to $x\%$ of individuals that are RDT positive that they are prescribed to $(x + 24)\%$ of RDT negative individuals (in line with field estimates [22,37]); this is the benefit of RDT targeting for non-malarial diseases. In order for the antibiotic prescription to be revenue neutral, that is, for there to be the same quantities of antibiotics prescribed before and
after RDT subsidization, antibiotics need to be prescribed to \((1 + .24 \times RDT_{pos})\) percent of RDT positive individuals, where \(RDT_{pos}\) is the percentage of RDT positive individuals.

Using our RDT model, we predicted what proportion of RDT positive and RDT negative individuals had illnesses that were curable with antibiotics (Text SI III). Further, given our assumption of revenue neutrality and the 24% benefit of RDTs to targeting of antibiotics, we can calculate what percentage of individuals is cured of disease with antibiotics as a function of RDT coverage. The quantity \(d\) as a function of RDT coverage and \(prop_{mal}\) in the presence of RDT subsidies is shown in Figure 3A, assuming RDTs have a false positive rate of 20% and a false negative rate of 5%.

As illustrated in Figure 3A there is a threshold region such that antibiotic targeting using RDTs saves more lives than no antibiotic targeting, because if most individuals do not have malaria, RDT negatives predominate and using RDT information improves treatment outcomes. However, outside of this region there are few RDT negatives and so RDT information should be discarded and presumptive antibiotics given. For simplicity we assume that \(c_{pri} \cdot prop_{antibio}_{pri} \cdot d_{pri} = c_{pub} \cdot prop_{antibio}_{pub} \cdot d_{pub}\), i.e., that individuals target antibiotics in the private sector at the same proportion as the public sector. Note that we have assumed a fixed targeting scheme with a ~24% difference. If antibiotic supplies are limited then preference should always be given to RDT negative individuals, i.e., there should be a 100% difference. Indeed, the greater the targeting, the larger the region of RDT coverage and \(prop_{mal}\) in which it is optimal to use RDT information.

Regarding the levels of antibiotic overuse, in the presence of RDTs overtreatment with antibiotics is reduced. However, in the absence of an RDT for bacterial febrile illnesses, these individuals cannot be said to be overtreated in a practical sense, since it is likely difficult to distinguish among individuals who will benefit from antibiotics among febrile treatment-seekers. We do not simulate the effects of antibiotic overtreatment on antibiotic resistance here, although this could become a significant factor as RDT usage increases; in this paper, we assume that RDTs do not affect the extensive margins for antibiotic usage.

3. Combining Effects of RDTs on Malarial and non-Malarial Diseases

Once we calculated the benefits of RDTs in terms of non-malarial deaths averted, we incorporated the effects of decreasing intensive margins for ACT treatment among treatment
seeking individuals \(b_{pri} = b_{pub} < 1\); Fig S1) to get a fuller picture at the costs and benefits of RDTs. The resulting change in mortality averted per RDT used is shown in Figure 3B; the z-axis illustrates the change in probability of averting mortality with antibiotics and antimalarials among individuals who would have died. Once the treatment discouragement effect was incorporated, RDTs caused a net increase in mortality over the a large range of RDT coverage and prop_mal values, because the loss of malaria positive individuals being treated with ACTs outweighed the effect of improved antibiotic targeting.

However, three points must be noted. First, we have implicitly assumed that ACT usage is not supply constrained. That is, we have assumed that at baseline every treatment-seeking individual can purchase ACTs at subsidized prices. If this is not the case, i.e., if ACTs are rationed, then the benefits of RDTs increase (see example in Text SI IV). Figure 3C illustrates the net effect of using RDTs to target antibiotics and antimalarials. This figure illustrates that RDTs result in improved outcomes for a larger region of RDT coverage levels and prop_mal values. Second, we have assumed that RDTs do not affect the external margins of antibiotic and antimalarial treatment. Thus, the subsidized price of RDTs must be so low as to not affect the purchasing decisions of these other therapies, or individuals have a high willingness-to-pay for RDTs. If RDTs reduce external margins, then their benefits will be reduced.

Third, we have only considered the direct effects on mortality of RDT usage. We have not considered the positive and negative externalities that result from RDT targeting, namely, the benefits of decreased antimalarial usage on increasing the waiting time to ACT resistance. In the Results section below we determine how ACT resistance will result in a significant increase in malaria-related morbidity and mortality, and so our estimates here represent the lower bound for the benefits of RDTs.

**Effects of ACT Subsidization for non-Malarial Diseases**

Regarding the effects of ACT subsidization alone on treatment choice, there is some evidence that ACT subsidization alone (independent of RDTs) discourages antibiotic use even among individuals treating in the private sector [35], because individuals treat malaria with antibiotics if those are cheaper than antimalarials (‘lowest cost first’ purchasing behavior) [35]. Thus, ACT subsidization will affect the extensive and intensive margins of both antimalarial and antibiotic usage. Second, subsidization of private sector ACTs may crowd out public sector use; we
discussed the effects of crowding out in the context of RDTs above. We do not examine these complex externalities on non-malarial diseases other than to note that further research is needed.

**Results**

Once the model had been fitted and compared with other estimates, we used the model to forecast malaria mortality under various scenarios. **Figure 4** shows the four scenarios considered here: (I) expansion of AMFm over time to reach approximately 384 million doses of ACTs delivered per year; (II) maintenance of AMFm at the current level of approximately 291 million doses per year; (III) discontinuance of AMFm to approximately 193 million doses per year; (IV) and the counterfactual scenario of AMFm having never occurred. These scenarios do not model where the expansion of AMFm occurs, i.e. whether in high or low endemicity settings, or the age structure of the expansion. Rather, we predict the effects of worldwide increases/decreases in uptake assuming that the probability of averting mortality and morbidity in the future is governed by the same probabilities as was fitted to the 1980-2010. It is possible that by targeting the subsidy to certain groups the subsidy would be more effective going forward, though such benefits might be reduced by leakage. We thus might consider the present estimates conservative in this sense.

A sample model projection is provided in **Figure 5**; this figure assumes that AMFm usage going forward is maintained at a constant level (Scenario III). We see that the increase in ACTs provided through AMFm causes a large decrease in mortality in 2011 and beyond (green line). After reaching a post-resistance minimum of ~639,000 in 2011, deaths rise over time due to increases in the rural African population size. However, ACTs provide a substantial reduction in mortality; in 2011 onward, at current levels of public and private subsidization ACTs are predicted to save ~450,000 lives per year.

While the green line assumes constant ACT efficacy, we might also consider the effects of ACT resistance on deaths. Currently there have been no documented cases of clinical treatment failures with ACTs, which is what we refer to as ‘ACT resistance’ [38]. However, it is likely only a matter of when, rather than if, treatment failures to ACTs will appear. ACT resistance will eventually occur due to the therapeutic failure of artemisinin; we refer to artemisinin resistance and ACT resistance interchangeably since much of the therapeutic efficacy of ACTs relies upon the action of artemisinin.
We can simulate the effects of failing artemisinin and ACTs on mortality using our model. From the 1980-2010 mortality data we were able to estimate the shape of the curve describing the spread and effects of CQ resistance. This curve provides a baseline by which we might project mortality going forward if ACT resistance spreads as CQ resistance earlier did. It is not possible to know whether ACT resistance will spread faster or slower than CQ resistance at this point. However, using CQ resistance as a model for ART resistance might provide an illuminating reference point for further analysis. Further, the patterns of resistance between CQ and artemisinin might be similar for biological reasons as well. In the case of CQ resistance, at least four point mutations were required for CQ-resistant parasites, and mutants with 4-8 point mutations also occurred [39,40]. Given the nonspecific mode of action of artemisinin, it is likely that multiple mutations will also be necessary for the development of artemisinin resistance [41], possibly incurring fitness costs in the same way that CQ mutations engendered a fitness costs against wild-type strains.

To simulate the spread of ACT resistance we used the same sigmoidal function as fitted for CQ and applied it to the efficacy of ACTs over time. It is not known how resistance for a combination might spread versus for a single drug; however, a degree of resistance to most of the ACT partner drugs has been documented (with the notable exceptions of piperaquine, where there is insufficient evidence, and lumefantrine, where there is limited evidence of high-level resistance), and so the spread of ACT resistance is likely to be governed by artemisinin failures [42]. For the purposes of modeling we assume here that ACT resistance will emerge in Africa in 2015. Recall that there was a period of 21 years before appearance of CQ resistance in SE Asia and appearance in Africa. Our assumption of emergence happening early in Africa is thus a worst-case scenario.

Given the assumption of a similar temporal progress of resistance for CQ and artemisinin and a date of 2015 for emergence in Africa, the projected mortality curve is shown in Figure 5A in red. The shaded red area illustrates the difference between the sensitive and resistant cases. The 10% treatment failure rate threshold (at which the WHO recommends that a therapy be replaced) would be reached at 2023 with 50% treatment failures worldwide by 2033. There would thus be a period of approximately eight years after emergence of resistance in Africa before the resistance would reach levels at which the WHO recommends switching therapies.

One caveat to our analysis of resistance is that we do not consider the possibility of
reversion, i.e. that the proportion of resistant to sensitive alleles decreases, rather than increases over time. Certainly this phenomenon has been observed over time, for example the increase in CQ sensitivity in Malawi over time after cessation of treatment [43]. However, as noted in [17], “the complexity of malaria infections (in Malawi) suggests that drug-resistant parasites may still be present at low frequencies and that the reintroduction of CQ may be followed by a rapid resurgence in resistant infections.” Thus, we consider the general shape of the logistic resistance curve as areas where drug resistant alleles are present and where reintroduction would be met with rapid clinical failures.

**Figure 5B** illustrates the number of lives saved using ACTs per year under each of the four scenarios. We can see that if the subsidy is expanded, then ~600,000 lives would be saved per year at maximum; 450,000 lives saved if AMFm were maintained, and 300,000 lives saved per year if subsidies go only to public providers. In each of the four scenarios we modeled the effects of resistance, which reduces the number of lives saved per dose with increased mortality coming rapidly around year 2025.

As discussed previously, part of the justification for AMFm is that by crowding out monotherapies, resistance may be averted for a time. We simulated this effect by increasing the waiting time to appearance of resistance in Africa by 3 years until 2018 for the expanded subsidy levels. We see from **Figure 5B** that the result is to shift rightward the mortality curve such that more lives are saved per year in the ‘buying time’ case; the total difference over the depicted period is ~1,324,000 lives saved due to the three year shift. Thus, there are substantial returns to preserving the artemisinins in terms of lives saved, even by as short a time as a few years.

We also examined the cost-effectiveness of presumptive treatment (also called ‘presumptive’ or ‘empirical’ treatment of febrile individuals) with CQ and ACTs over time, including the effects of resistance. **Figure 6** shows the estimated number of dollars spent on each symptomatic therapy to avert one DALY. The dashed line illustrates the $50 threshold for an intervention to be considered ‘very cost-effective’ [1]. Due to the very low cost of CQ, dollars per DALY averted is low for presumptive treatment during the 1980s through the mid 1990s, though there is some cost of treatment of febrile patients who do not have malaria. However, resistance slowly increases the costs of treatment because of increased numbers of treatment failures, and by ~2022 the costs of treating individuals is no longer very cost effective. This might seem counter-intuitive, as resistance has rendered CQ almost useless at this point (96%
treatment failure rate). However, given the extremely low cost of CQ, even at this low probability of cure, it would still make financial sense to use CQ up to this point had no other therapies been available. In terms of the human costs in morbidity and mortality, however, as we have seen the therapy should have been supplanted many years ago (~1991). Our costing analysis for CQ resistance does not take into account the very considerable drug development costs to find replacement therapies, and so the costs of resistance are even higher than reported here.

As regards ACTs, these are cost-effective even at the $3 per dose level, but much more so at the CHAI-negotiated price of $1 per dose. Our modeled treatment scenario for ACTs also includes the effects of RDTs; we call treatment with ACTs conditional upon a positive RDT a ‘test-and-treat’ protocol. At current RDT coverage levels the vast majority of treatment is presumptive. From Figure 6 we see that ACTs became more cost-effective than presumptive treatment with CQ around 2010 as the prices for ACTs dropped. However, if we include the effects of resistance on ACT treatment outcomes, ACTs rapidly become less and less cost-effective; whereas it took 42 years for presumptive treatment with CQ to fail from a cost-effectiveness perspective, it only takes 25 years for the ACTs to fail.

**Discussion**

The costs of malaria over the past few decades have been great in terms of lives lost and morbidity incurred. In this paper we used a simple statistical model to examine the effects of CQ usage and resistance on overall malaria mortality over time. We also incorporated the effects of LLINs and ACTs on mortality, including the benefits of RDTs.

We found that our model could replicate the mortality trends averaged over three studies for the period of 1980-2010 using a set of parsimonious assumptions. We also corroborated our estimates from a variety of other published sources. The effects of CQ resistance during the period of 1980-2004 were dire: our model estimates that 5.7 million deaths are attributable to CQ resistance over this period. We also found that the 10% threshold of treatment failures worldwide was reached ~8-10 years after emergence of CQ resistance in Africa. If we compare this period to the time it takes to bring a new malaria drug or drug combination to market, it took approximately twenty years before ACTs were widely deployed after they were first reviewed in the literature [44]. Thus, our findings imply that the spread of resistance is faster than the spread
of drug development even if that resistance incurs a substantial fitness cost, unless urgency is brought toward development and clinical trials.

Our results have important implications for the preservation of ACTs. Regarding the extension of AMFm, we find that ACT subsidization saves lives relatively cheaply. Presumptive treatment with ACTs costs well below the level of ‘very cost-effective’ interventions in terms of dollars per DALY. Thus, even if ACT subsidization does not buy a substantial amount of time against resistance, there are very strong arguments in favor of subsidization from the perspective of both lives saved and dollars per DALY. Second, we found that delaying resistance can avert many deaths in the medium-term. If we assume ACT and CQ resistance spread similarly, pushing back resistance for only three years would save approximately 1,324,000 lives by 2040, in the absence of new antimalarials.

The main mechanism by which ACT subsidization would ‘buy time’ would be by crowding out monotherapies. Given that the pilot phase of AMFm began only in 2010, there are few studies of the effectiveness of subsidization at reducing monotherapy use. In Tanzania, artemisinin monotherapy use was quite low at baseline (< 1%) and so ACTs did not crowd out monotherapy so much as replace older treatments and increase treatment coverage [45]. In rural Cambodia, in the absence of any ACT subsidy, ACT coverage was low (11%) and artemisinin monotherapy coverage rates were quite high (~40% in 2002) [46]. The artemisinin monotherapy coverage rates at baseline in Cambodia were so much higher than in Tanzania likely because of proximity to producers in China, higher incomes, and familiarity with artemisinin. In areas where ACTs were provided at low or no charge, coverage rates increased (23-52%) and artemisinin monotherapy rates declined (5-13%).

The extent to which the subsidy might buy time by crowding out monotherapies is unknown, but it surely depends upon context and there is some evidence for a benefit. Further, a perverse outcome is also possible if AMFm is discontinued. Because CHAI and the Global Fund have encouraged widespread artemisinin cultivation, if the subsidy is reduced it is possible that those producers will sell the artemisinins as monotherapies, thus increasing the supply of monotherapy above levels that would have prevailed had the subsidies never existed. It is also possible that increasing levels of antimalarial subsidization will actually decrease the time until onset of antimalarial resistance and/or increase the rate of spread of resistance because of the increase in selection pressure. Given these uncertainties, it is not immediately clear whether or
not antimalarial subsidization will delay or speed the onset of resistance, and thus we simply
point out these conflicting factors and note that further research is needed.

Another mechanism by which resistance might be averted is through decreasing the
overtreatment of ACTs by increasing the proportion of malarious individuals among treatment-
seekers. Overtreatment can be reduced using RDTs, and here we have analyzed the relationships
between overtreatment, cost-effectiveness, and RDT usage. Our first result regarding RDTs was
that RDTs have a net benefit on mortality outcomes for only a small range of RDT coverage and
\( prop_{\text{mal}} \) values.

However, our analysis did not include the benefits of reduced overtreatment. If
compliance is very good to excellent and coverage is increased to a high level (~70-90%), then
overtreatment can be reduced by as much as 15-33% versus the purely presumptive treatment
case (Figure S2). Thus, RDT usage is faced with a tradeoff of a decreased proportion of
malarious individuals treated versus decreased overtreatment levels.

In terms of a tradeoff, we predicted above that increasing the lifetime of ACTs by three
years would save ~1,324,000 lives by 2040, or 9.23% of the total number of lives saved at
baseline. Thus, each year of preserved ACTs results in 441,000 lives saved (3.1% of total until
2040). We can use this result to quantify the positive externalities of reduced overtreatment.

Let us assume that a 17.1% reduction in overtreatment (80% RDT coverage) at the
expanded AMFm level preserves 1.5 years of effective ACT lifetime over five years of RDT
coverage. Then \( 384,000,000 \times 5 \times 0.8 = 1,536,000,000 \) RDTs would save 663,000 lives, i.e.,
each RDT increases the probability that an individual treated with an ACT would survive by
0.043%. To consider the magnitude of this effect in context, in Text S1 we calculate that every
treatment-seeking individual has a 0.66% chance of dying. The magnitude of change from the
effects of RDTs on targeting is on the order of ~1-5% (Figure 3); the effects are small because
the improvements in targeting of antibiotics and the effects of ‘treatment discouragement’ are
small.

Thus, the changes shown in Figure 3 will affect on the order of 0.01% of treatment
seeking individuals in the optimal case, which is smaller than the scale of changes due to delayed
resistance. From this perspective, RDTs are a net benefit, as the benefits of increased lifetimes of
ACTs outweigh the individual costs. However, RDTs are a net benefit from the perspective of
the social planner. Individuals who are profit-maximizing would not internalize the benefits due
increased waiting time to resistance. Such individuals and would use the results from Figs 3B for their decision process, and thus RDT usage would be low because there is a narrow range of RDT coverage and prop_mal values for which RDTs provide a direct individual benefit.

Further, the analysis considering the benefits of delayed resistance has a considerable level of uncertainty associated with it. It is possible that RDTs will delay the onset of resistance, but it is also possible that they will not (or will delay the spread, rather than the onset). Because the levels of uncertainty around delaying resistance are not quantifiable at present, this may reduce our confidence in using such analyses in determining RDT policy. In this case, the results from Figure 3C should be used to determine the optimal levels of RDT coverage, as these effects pertain to individuals directly (or if ACT supplies are not limited, to Figure 3B). Thus, for the risk-averse social planner, RDTs should only be employed in limited circumstances with the assumption above, though as the risk-aversion decreases, they might be employed more widely.

We have laid out a framework for analyzing the effects of fever etiology, antibiotic and antimalarial targeting, RDT compliance, and RDT quality, as well as ACT resistance on the decision whether or not to adopt RDTs. Further research will probe those parameter sets that yield positive or negative contributions from RDTs. However, regardless of the costs and benefits of diagnostic testing, we have shown that subsidization of ACTs is very cost-effective in terms of the metrics of dollars per DALY, even in the absence of buying time, as well as total lives saved. Further, we have shown that the costs of malaria drug resistance are very high. Thus, subsidization programs like AMFm provide a strong return on public health investment dollars, as long as patterns of resistance are monitored over time.
Acknowledgements
There are no acknowledgements.

Financial Disclosure
GLCJ received funding from the National Science Foundation Graduate Research Fellowship Program. DLS acknowledges funding from NIH/NIAID (U19AI089674), the Bill & Melinda Gates Foundation (49446), and the RAPIDD program of the Science and Technology Directorate, Department of Homeland Security, and the Fogarty International Center, National Institutes of Health. DAF acknowledges funding from the Bill and Melinda Gates Foundation (OPP1040399) and the National Institutes of Health (grant R01 AI079709). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions
GLCJ, DLS, RL, DAF conceived of the research; GLCJ developed the mathematical model, with support from DLS; GLCJ wrote the paper, with input from DLS, DAF, RF, EYK.

Competing Interests
All authors have declared that no competing interests exist.

Abbreviations
Artemisinin-based combination therapy, ACT; Chloroquine, CQ; Long-lasting insecticide treated bed net, LLIN; Rapid diagnostic test, RDT; Sulphadoxine-pyrimethamine, SP.

Supporting Information
Figure S1. Etiology of fever and malaria mortality attribution.
Figure S2. Effects of rapid diagnostic testing on overtreatment treatment with ACTs.
Figure Legends

Figure 1. Malaria-attributable mortality estimates, 1980-2010. Malaria mortality data were collected from two sources and averaged to form an intermediate estimate. The dashed blue line on top is the estimated malaria-attributable mortality from the Global Burden of Disease (GBD) estimates [4]; the dashed orange line on bottom is the estimate from the 2011 World Malaria Report [2]. For the estimate used here, we took the mean of the two data series for 2000-2010. For the period 1980-2000, we applied a linear scaling to the GBD trend such that our estimated mortality differed at baseline from the GBD trend by 80% of the mean difference in 2000, scaling to 100% of the difference by 2000. The effects of the scaling are shown by the difference of the red trend line from the two vertical black lines at 1980 and 2000. The green cross provides the estimated year 2000 worldwide malaria-attributable mortality from the 2002 WHO World Health Report for comparison [19].

Figure 2. Relative shares of failing first-line therapies, bed nets, and ACTs on modeled mortality. We fitted a simple statistical model to the estimated worldwide malaria mortality data from 1980-2010. The relative contributions of artemisinin-based combination therapies (ACTs) and bednets were set to have approximately equal shares of mortality reductions. The model then assigned the total levels of reductions from failing first-line therapies (mostly chloroquine, CQ) as well as the trend of mortality over time using a least-squares fitting procedure. The dotted black line at top gives the predicted trend in mortality in absence of interventions, whereas the dotted orange line gives the estimated mortality assuming only first-line therapies. The orange line converges to meet the top line due to the spread of resistance. The modeled effects of bednets and ACTs are shown in blue and green, respectively. The red line provides the estimated mortality over time.

Figure 3. Effects of RDT targeting of antibiotics and antimalarials on mortality. The RDT model was run to determine the effects of RDT usage on mortality. (A) The fold-change in mortality from antibiotics usage. The baseline treatment scenario assumes 25% coverage with antibiotics and no RDT targeting; the fold-change using RDTs results from targeting antibiotics to RDT negative individuals. The green region indicates the region of net improvement in outcomes; prop_mal is the proportion malarious among treatment-seeking individuals. (B) The
net change in probability of saving a treatment-seeking individual that would otherwise have
died using RDT targeting for antibiotics and antimalarials. The benefits of RDTs for targeting
antibiotics are diminished by including the effects of ‘treatment discouragement’ on antimalarial
treatment outcomes. (C) The net change in probability of saving a treatment-seeking individual
that would otherwise have died using RDT targeting, assuming that ACTs are supply-
constrained. The optimal region for RDT usage increases relative to (B) given the direct benefits
of averted overtreatment.

**Figure 4. Four scenarios for private sector ACT subsidization.** We modeled the effects of
four different subsidization levels on future ACT uptake; these four Scenarios served as the bases
of our prospective analyses. In all four Scenarios we assume that public sector subsidization
levels remain constant; the change in artemisinin-based combination therapy uptake levels are
the result of changes in private sector subsidization (through the Affordable Medicines Facility
for malaria; AMFm). Scenario I represents an expansion of the AMFm such that ~384 million
doses are provided on average through both private and public sectors (dotted purple line).
Scenario II represents maintenance of AMFm at current levels (291 million doses). Scenario III
represents discontinuance of AMFm in 2012 (193 million doses through various public sector
donors from 2013 onwards). Scenario IV represents the counterfactual of never having had
AMFm. The blue crosses provide the total public sector subsidization of ACTs over time; the red
cross gives the total number of ACT doses provided in 2011, including AMFm; the black cross
gives the number of subsidized doses in the public sector only in 2011. We do not include private
sector purchases of ACTs at unsubsidized market prices in our analysis, as these represent a
small fraction of total doses.

**Figure 5. Malaria mortality averted by ACTs per year, including resistance.** The statistical
model was used to simulate the effects of artemisinin-based combination therapy (ACT) on
mortality over time. (A) ACT subsidization levels remain constant at 2011 levels (Scenario II).
The dotted black line gives the trend in malaria-attributable mortality in absence of intervention;
the orange line is mortality using first-line therapies (chloroquine, CQ) only; the blue line is
mortality with CQ and bed nets; the green line is mortality with CQ, bed nets, and artemisinin-
based combination therapies (ACTs). To simulate the effects of ACT resistance on mortality, we
assumed that resistance will emerge in Africa in 2015 (triangle) and that ACT resistance follows the same kinetics of temporal and spatial progression as those observed for CQ. The red line gives mortality with ACT treatment and resistance. The shaded red region is the difference between ACT resistance and fully sensitive scenarios. (B) Estimates of malaria-attributable mortality averted through ACTs over time. The model was run under the four ACT uptake scenarios to simulate mortality averted over time. For Scenario I, the expansion of the Affordable Medicines Facility, malaria (AMFm), two different resistance patterns were simulated: resistance emerged either in 2015 (blue) or 2018 (red). The effects of ‘buying time’ by prolonging the effective clinical life of artemisinin is observed by examining the difference between the red and blue lines; the difference in area between the curves is ~1,324,000 lives by 2040. Maintenance, discontinuance, and the counterfactual scenario of no AMFm are shown in magenta, orange, and green, respectively; AMFm is already predicted to have saved ~305,000 lives from 2011-2012 (difference between orange and green lines).

**Figure 6: Cost-effectiveness of antimalarials and effectiveness of RDTs.** The semi-mechanistic model describing malaria mortality was run to simulate the effects of antimalarial treatment over time and the number of disability-adjusted life years (DALYs) averted was calculated from life tables and the age-structure of mortality. Effects of treatment on morbidity were inferred from the overall levels of drug usage and the proportion of febrile illnesses attributable to malaria worldwide. The cost-effectiveness of presumptive treatment using first-line therapies (modeled as chloroquine, CQ) was then calculated over time including the effects of resistance (purple). The cost-effectiveness with ACTs in terms of dollars per DALY averted was also calculated (green; no resistance), including usage of rapid diagnostic tests (RDTs). ACT resistance was modeled to emerge in Africa either in 2015 (blue) or 2018 (red), and follow the same resistance kinetics as CQ.
References

37. Ansah EK, Narh-Bana S, Epokor M, Akanpigbiam S, Quartey AA, et al. (2010) Rapid testing for malaria in settings where microscopy is available and peripheral clinics
where only presumptive treatment is available: a randomised controlled trial in Ghana. BMJ 340: c930.


Table 1: Semi-Mechanistic Model Parameter Estimates from Malaria Mortality Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_1$</td>
<td>Number of malaria-caused deaths, 1980</td>
<td>1.43E+06</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>Increase in number of deaths, per year, no intervention</td>
<td>6.06E+04</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>Number of deaths averted by first-line therapies, per year, no resistance</td>
<td>7.93E+05</td>
</tr>
<tr>
<td>$\beta_4$</td>
<td>Time of 50% first-line treatment failures (years post 1980)</td>
<td>18.36</td>
</tr>
<tr>
<td>$\beta_5$</td>
<td>Hill-slope of increase in treatment failures</td>
<td>3.90</td>
</tr>
<tr>
<td>$\beta_6$</td>
<td>Probability that an ACT will avert a malaria-caused death, no RDT</td>
<td>1.50E-03</td>
</tr>
<tr>
<td>$\beta_7$</td>
<td>Probability that an LLIN in a household will prevent a malaria-caused death</td>
<td>5.31E-03</td>
</tr>
</tbody>
</table>

Table 1: Semi-mechanistic Model Parameter Estimates from Malaria Mortality Data. The semi-mechanistic model predicting malaria mortality from first-line therapy use, long-lasting insecticide-treated nets, artemisinin-based combination therapy use, and rapid diagnostic test uptake was fitted to malaria mortality data from 1980-2010. The fitting procedure utilized a constrained nonlinear least squares curve-fitting algorithm.
Figure 1

Number of Worldwide Deaths Due to Malaria Mortality

- Malaria Deaths, GBD Estimate, 1980-2010
- Malaria Deaths, WHO World Malaria Report 2011 Estimate, 2000-2010
- Estimated Mean Deaths Using GBD and WHO Estimates, 1980–2010
Figure 2

Number of Worldwide Deaths Due to Malaria Mortality

- Modeled malaria mortality assuming no intervention
- Malaria mortality including failing first-line therapy
- Malaria mortality including first-line therapy and LLINs
- Malaria mortality including first-line therapy, LLINs, and ACTs
- Estimated mortality from GBD and WHO estimates
Figure 3

A

B

C
Figure 4

- Scenario I: Long-term expansion of AMFm
- Scenario II: Maintenance of AMFm at current level
- Scenario III: Discontinuance of AMFm
- Scenario IV: No AMFm

Global Subsidized ACTs Purchased (2005−2010)
Global Subsidized ACTs (2011), Including AMFm
Global Subsidized ACTs (2011), Excluding AMFm
Figure 5

A. Modeled malaria mortality assuming no intervention
- Malaria mortality including failing first-line therapy
- Malaria mortality including first-line therapy and LLINs
- Malaria mortality including first-line therapy, LLINs, and ACTs
- Malaria mortality including first-line therapy, LLINs, ACTs w/ resistance
- Assumed first widespread emergence of clinical treatment failures (2015)

B. Malaria mortality averted by ACTs per year
- AMFm expanded, resistance 2018
- AMFm expanded, resistance 2015
- AMFm maintained, resistance 2015
- AMFm discontinued, resistance 2015
- No AMFm, resistance 2015
Figure 6

Dollars per DALY Averted for First-Line Therapies and ACTs

- Cost-effectiveness of first-line therapy (CQ)
- Cost-effectiveness of ACT, no resistance
- Cost-effectiveness of ACT, resistance 2018
- Cost-effectiveness of ACT, resistance 2015
Text S1: Causes and Treatments of Febrile Illnesses in Africa

I) Simple African Fever Etiology

To determine the causes of fever generally, we first took the attribution of mortality among African children aged 1-59 months of age from [1]. We then redistributed the causes proportionally after excluding those causes that are not associated with fever (injury and other non-communicable diseases were excluded). This analysis provides a simple etiology of fever, assuming that the proportions of morbidity presenting with fever correspond 1:1 to the causes of death by febrile illnesses. If we compare the proportion of malaria-caused fevers in this analysis to that predicted by the WHO for 2015 (prop_mal), we find the predicted value of 38% is much greater than the 24% attribution from mortality. Part of this difference (~14%) is due to difference of timing and analysis, but much of the difference also might relate to comorbidity. In the study Black et al. [1], mortality is classified by its primary cause so that diseases are not double-counted. However, some individuals are likely infected with malaria and bacterial infections, malaria and AIDS, etc., such that at time of presentation they are malaria positive, but their cause of death is attributed to another disease.

To account for comorbidity, we took the difference of prop_mal and our reattribution from [1] (14%) and shared this difference among the various causes of fever proportionally. Figure S1A provides the resulting distributions of fever in Africa. The proportions of each cause of fever are shown, including the fraction of comorbidity with malaria. The proportion solely attributed to malaria (purple) certainly has fractions of comorbidities with other diseases; this is simply the portion of malaria fevers attributable by the mortality data alone. If we assume that the attribution of causes of death is cause-neutral, then we would expect that of the 24% of deaths attributed to malaria, 14% in total (or 58% of malaria cases) would also have comorbidities with other diseases. We assume the cause-neutrality of death attributions here and thus assume that 58% of malaria cases have comorbidity; however, for this 24%, malaria is the cause of death and not the comorbidity.

If we consider regional variation rather than averages, we cannot use the assumption that malaria causes ~24% of all deaths among febrile treatment-seeking individuals; after all, many areas have much less than 23% malaria prevalence. To account for those areas with low or high malaria, we created a function to describe the relationship between the proportion of deaths
caused by malaria and the proportion of treatment-seeking individuals that are malaria positive. The function $f$ needed to satisfy the constraints that at $f(0)=0$, that $f(38) = 24$, and that the slope of $f$ is decreasing, given that malaria is less fatal on a per treatment basis the higher the prevalence, due to the protective effects of immunity. Our choice of function is illustrated in Figure S1B.

II) Treatment of Various Febrile-Illness Causes

For pneumonia, many of the cases are caused by bacteria, some by viruses, and others are of unknown origin [2]; we assume that case-management and antibiotic use might prevent 36% of pneumonia deaths [3,4].

For diarrhea, there are a variety of methods used for prevention (vitamin A, rotavirus vaccine, breastfeeding as well as the water, sanitation, and hygiene (WASH) protocols); for treatment, oral rehydration salts (ORS), zinc supplementation, and antibiotics [5]. The non-WASH interventions are estimated to cost $0.80 per capita per year [5]. Regarding effectiveness, Walker et al. [5] estimates that ORS is effective at averting 93% of all non-dysentery diarrheal deaths, antibiotics 99% effective at preventing dysentery diarrheal deaths, with zinc adding an additional 23% effectiveness to both. Thus, the vast majority of diarrheal deaths are preventable; we assume 99% are treatable.

For measles, two doses of vitamin A supplementation has been shown to reduce mortality by 62% [6,7]; two capsules cost $0.04\(^1\), though the cost including delivery is higher.

For bacterial ($N\ meningitidis$) meningitis, intramuscular injection of ceftriaxone has been shown to reduce morality rates by approximately 50% [8,9], and most epidemic meningitis in Africa is bacterial [10].

For AIDS, highly active antiretroviral therapy has been shown to reduce mortality in rural Africa by 87% versus antibiotic prophylaxis, at a cost of ~$1,400 per person per year [11].

For pertussis, treatment can address either the infection or the symptoms; regarding for the former, “Although antibiotics were effective in eliminating $B. pertussis$, they did not alter the subsequent clinical course of the illness” [12]; for symptomatic treatment, “Much of the (pertussis) morbidity is due to the effects of the paroxysmal cough… insufficient evidence exists to draw conclusions about the effects of any intervention for the cough in whooping cough” [13].

\(^1\) As reported by UNICEF: http://www.unicef.org/immunization/files/Vitamin_A_Supplementation.pdf
Thus, we cannot evaluate the effectiveness of interventions to prevent pertussis mortality once infection has presented.

In this paper we focus on the benefits of antibiotic treatment targeting using RDT data. There are other ways to bundle treatments using RDTs, by, say, pairing RDT negative diagnosis is delivered with either some other form of diagnostic or some type of catch-all treatment (antibiotic, vitamin A, ORS supplement). However, we do not consider those types of interventions here.

If we take the proportion of individuals whose cause of death is preventable upon presentation weighted by the proportion of febrile presentations (non-malarial causes only), we find that 48.7% of non-malarial febrile deaths were readily treatable (excluding AIDS, pertussis, and ‘other infections’) and that at least 12.5% of non-malarial febrile infections were easily curable by antibiotics (9.55% of febrile infections including malaria; thus $\text{prop}_{\text{antibio}} = 0.0955$), though this estimate does not include benefits from reductions in ‘other infections.’

However, an RDT only tells the patient whether they have malaria or not; it does not distinguish among the non-malarial causes. For those patients who are RDT negative, the RDT does not distinguish among diarrhea, measles, meningitis, or pneumonia (or whether bacterial or viral infection). As we have seen, the treatments for these cases differ. In clinical settings, RDT negative results have been shown to improve antibiotic targeting [14,15]. Indeed, antibiotics prescriptions increased from 19% in the RDT negative group to 45% in the RDT positive group in Zanzibar [22], and from 13.4% to 34.5% in Ghana [48]. This ~24% increase in antibiotic prescriptions likely significantly reduces mortality among the ~13% of infections that benefit from antibiotics; indeed, patients seek less retreatment after test-and-treat versus clinical diagnoses [22].

However, these results are valid for clinical settings, where treatment is adjusted conditional on a negative RDT. For individuals receiving care in the private sector, the benefits of a negative RDT may be more ambiguous, because these individuals are self-diagnosing, and because there are costs to seeking treatment multiple times, i.e., retreatment might be discouraged. Further, there is evidence that subsidizing ACTs may encourage antimalarial usage outside of the public sector, a phenomenon known as ‘crowding out’ [16].

III) Determining Total Average Levels of Mortality
Assuming a revenue-neutral antibiotic prescription protocol and a 24% antibiotic targeting level, that RDTs save lives for a large range of RDT coverage and prop_mal pairs (Figure 3B). To determine the number of DALYs saved through usage of RDTs, we can take the results from Figure 3B, multiplied by the probability that antibiotics will save a life; if we divide the cost of an RDT by this probability, we thus compute the benefit in dollars per DALY for RDTs from non-malarial illnesses.

To determine the actual probability that treatment saves a life, we need to know the probability of death in general. From our analysis, $\beta_6 = 0.0015$ (Table 1), that is, treatment with an ACT has a 0.15% chance of saving a life in the absence of RDT usage. If treatment with ACTs saves a life that would have otherwise been lost with a 95% probability, then among febrile treatment seeking individuals, the probability of death from malaria is 0.158%. Using the estimate of malaria as a percentage of all deaths from febrile illnesses (24%), we have that the chance of death among all febrile treatment seekers from combined worldwide and African data is thus approximately 0.66% on average. Assuming independence of probabilities of receiving antibiotics and having a febrile illness that responds readily to them, there is thus a $0.66 \cdot 0.76 \cdot 0.125 = 0.66 \cdot 0.955 = 0.063\%$ chance that an antibiotic will save the life of a person treated with an antibiotic for a febrile illness.

To determine these probabilities for RDT negative and positive individuals, we can use the febrile illness attribution modeling described in Section I above. We find that 3.8% of true malaria positive and 12.5% of true malaria negative individuals who would have died otherwise can avert mortality if antibiotics are given. Thus the probability that an antibiotic saves a life among true malaria positives and malaria negatives is $0.66 \cdot 0.038$ and $0.66 \cdot 0.12$, respectively.

To determine the number of DALYs saved through antibiotic targeting using RDTs, we can take the results from Figure 3B, multiplied by the probability that antibiotics will save a life; if we divide the cost of an RDT by this probability, we thus compute the benefit in dollars per DALY for RDTs from non-malarial illnesses.

IV) Analysis of RDTs Assuming Constrained ACT Supplies
To examine the effects of RDTs on malaria mortality assuming constrained ACT supplies, consider the following example. Assume that there are 1 million doses of ACT available for a given area, and that in the absence of RDTs, the specificity of treatment (treatment of malaria
positives) is 25%: without RDTs, all million doses are used, 25% of individuals are cured, and 75% of treatment is wasted. Now assume that, using RDTs, overtreatment is reduced by 20% and that 24% of treatment seekers are cured (24% < 25% because of reduction of intensive margins); then overtreatment is 60%; and $75 \cdot 0.2 = 15\%$ of treatments are left over.

These remaining treatments are then given out to the next round of patients at the same 60:24 ratio, and $0.15 \cdot (24) = 3.6\%$ of patients are cured, with $0.15^2$ or 2.25% remaining for a third round. The total percentage of patients cured can be expressed as $\sum_{i=0}^{\infty} 24 \cdot 0.15^i = 24 \cdot \left( \frac{1}{1 - 0.15} \right) \sim 28.2\%$, i.e., the total number of cures with 1 million treatments increases from 250,000 to 282,000 with RDTs. The number of cures per RDT is thus $(32,000/1,000,000 = 0.032)$.

Following the same logic, we adjusted the effectiveness of ACTs by incorporating the benefits of RDTs in terms of reduced overuse under supply constraints for the entire range of RDT coverage and $prop_{mal}$ values in Figure 3C.
References

Figure S1

**Figure S1. Etiology of fever and malaria mortality attribution.** (A) Estimate of the distribution of febrile illnesses in Africa; the proportions of each cause of fever are shown, including the fraction of comorbidity with malaria. The proportion solely attributed to malaria (purple) has fractions of comorbidities with other diseases, but this proportion of febrile illness represents that proportion of mortality attributable to malaria. (B) Assumed proportion of malaria mortality as a function of prop_mal; non-malarial mortality is assigned proportionally according to the proportions in (A). The black circle illustrates the average proportion of malaria mortality in Africa at the average level of prop_mal.
Figure S2: Effects of rapid diagnostic testing on overtreatment with ACTs. We predicted the effects of rapid diagnostic testing (RDT) on the levels of overtreatment with artemisinin-based combination therapies (ACTs). We examined three compliance scenarios: ‘very good compliance’ (95% compliance with a positive RDT and 60% compliance with a negative RDT, top surface); ‘excellent compliance,’ (95% and 80%, middle surface); and ‘perfect compliance’ (100% and 100%, bottom surface). The x-axis is the total coverage level of test-and-treat treatment with RDTs and ACTs; the proportion not covered is assumed to be treated presumptively. The y-axis is the proportion of treatment-seeking individuals with malaria (prop_mal). The z-axis represents the fold change in overtreatment, i.e., treatment of individuals with ACTs who do not have malaria, versus purely presumptive treatment. Contour lines are provided for the top surface and projected to the z=0 axis for clarity. The blue dot represents the effects of 80% coverage assuming the mean worldwide value of prop_mal used in the paper (~38%). The larger the fold decrease, the more overtreatment being averted and thus the better the outcome from a drug resistance perspective.