Intra-host model of malaria infection: characterizing artesunate resistance

Sompob Saralamba

Mahidol-Oxford Tropical Medicine Research Unit
Faculty of Tropical Medicine, Mahidol University, Thailand

Joint International Tropical Medicine Meeting 2009

Outline

Mahidol Oxford

WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Question/Hypothesis from the team

The NEW ENGLAND JOURNAL of MEDICINE

Artemisinin Resistance in *Plasmodium falciparum* Malaria

Arjen M. Dondorp, M.D., François Nosten, M.D., Poravuth Yi, M.D., Debashish Das, M.D., Aung Phae Phyo, M.D., Joel Tarning, Ph.D., Khin Maung Lwin, M.D., Frederic Ariey, M.D., Warunee Hanpithakpong, Ph.D., Sue J. Lee, Ph.D., Pascal Ringwald, M.D., Kamolrat Silamut, Ph.D., Mallika Imwong, Ph.D., Kesinee Chotivanich, Ph.D., Pharath Lim, M.D., Trent Herdman, Ph.D., Sen Sam An, Shunmay Yeung, Ph.D., Pratap Singhasivanon, M.D., Nicholas P.J. Day, D.M., Niklas Lindegardh, Ph.D., Duong Socheat, M.D., and Nicholas J. White, F.R.S.

The New England Journal of Medicine

Volume 361:455-467 July 30, 2009 Number 5

Problem:

Increasing parasite clearance time in patients receiving artesunate monotherapy have been observed in Western Cambodia.

Hypothesis:

This phenomenon results from reduced drug efficacy on the ring stage of the parasite life cycle.

Mahidol Oxford

WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Question/Hypothesis from the team

Problem:

Increasing parasite clearance time in patients receiving artesunate monotherapy have been observed in Western Cambodia.

Hypothesis:

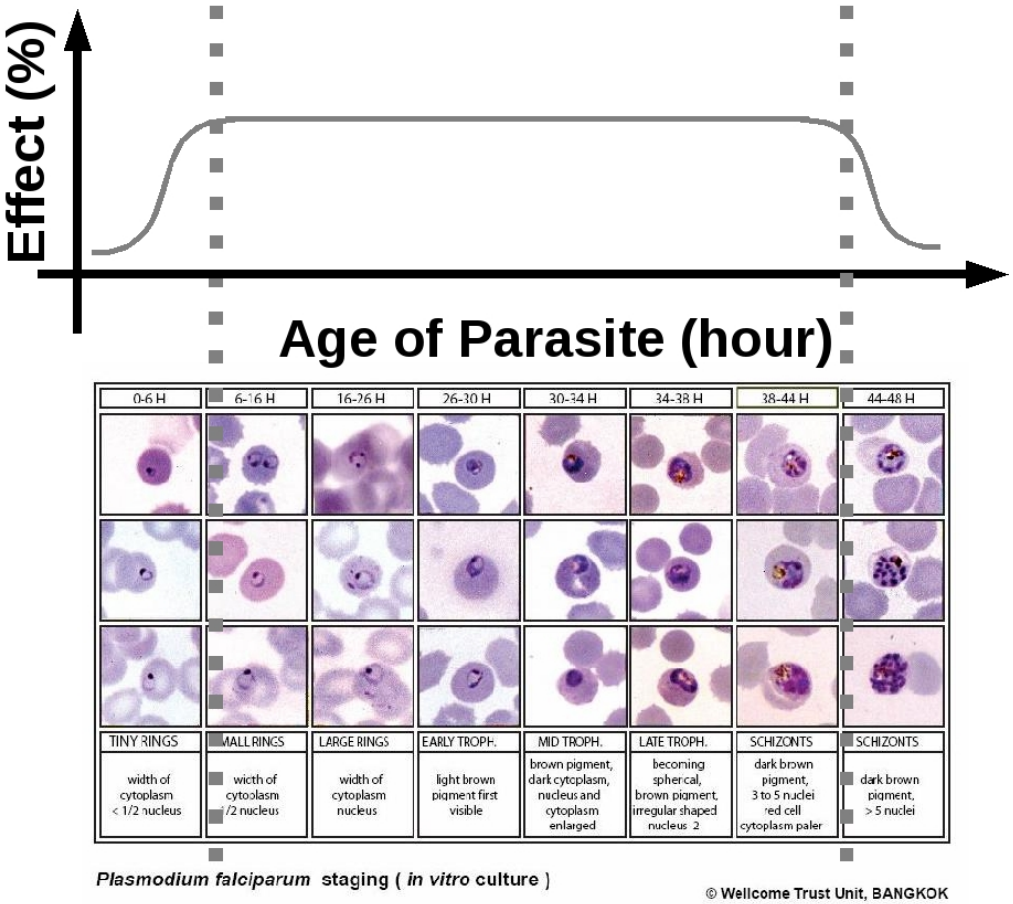
This phenomenon results from reduced drug efficacy on the ring stage of the parasite life cycle.

Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

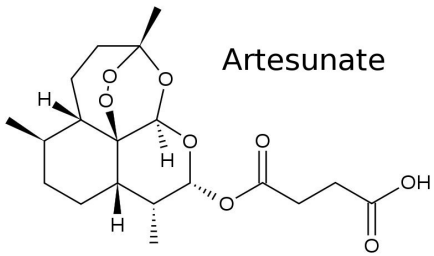
Possible causes of long clearance time

- Poor absorption of drug
- High initial load of parasites
- Reduced activity of drug

Parasite Staging& Artesunate



Plasmodium falciparum staging (*in vitro* culture)



Stage-specific

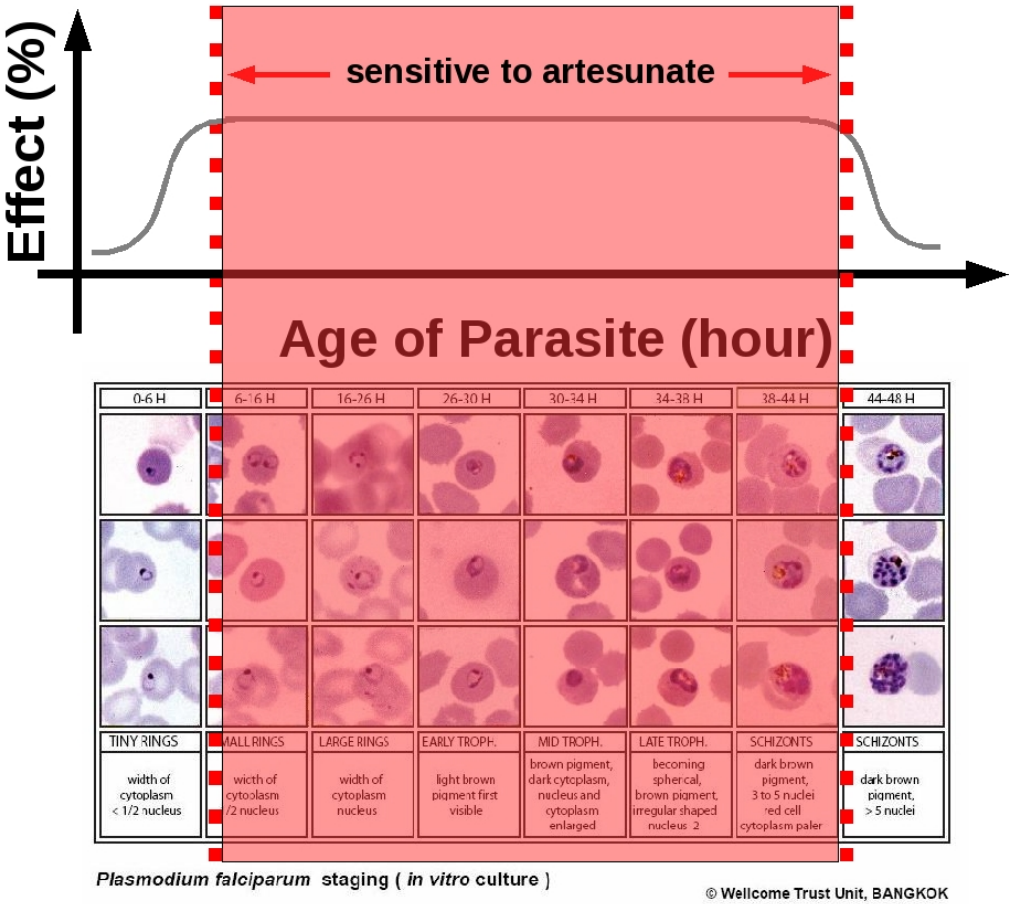
Very young rings (< 6 hrs) and

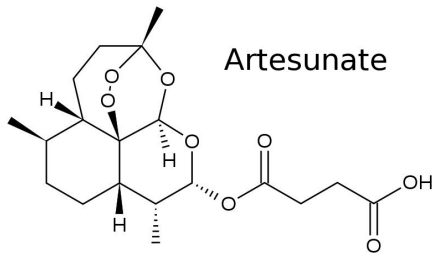
very mature schizonts (> 44 hrs)

are insensitive to the drug.

Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Parasite Staging& Artesunate





Stage-specific

Very young rings (< 6 hrs) and

very mature schizonts (> 44 hrs)

are insensitive to the drug.

Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

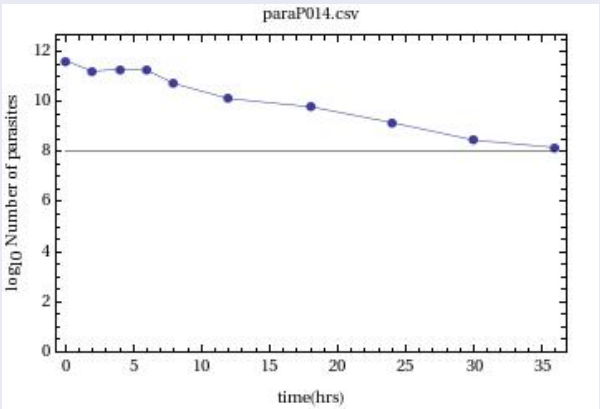
Data for the model

In each study sites, the patients were divided into two groups for different dose regimens, AS7 and MAS3.

- AS7 - 2mg/kg/day of artesunate for 7 days.
- MAS3 - artesunate 4mg/kg/day for 3 days followed by mefloquine 15/mg/kg on day 3 and 10 mg/kg on day 4.

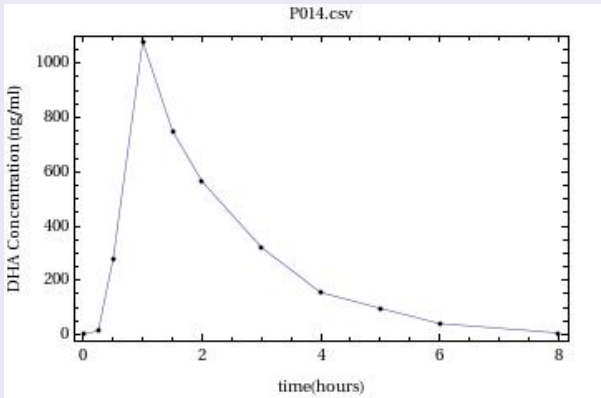
Parasite count

Parasitemia was assessed on admission, 4, 8 and 12 hours after start of antimalarial treatment and then every 6 hours until 2 consecutive slides were negative.

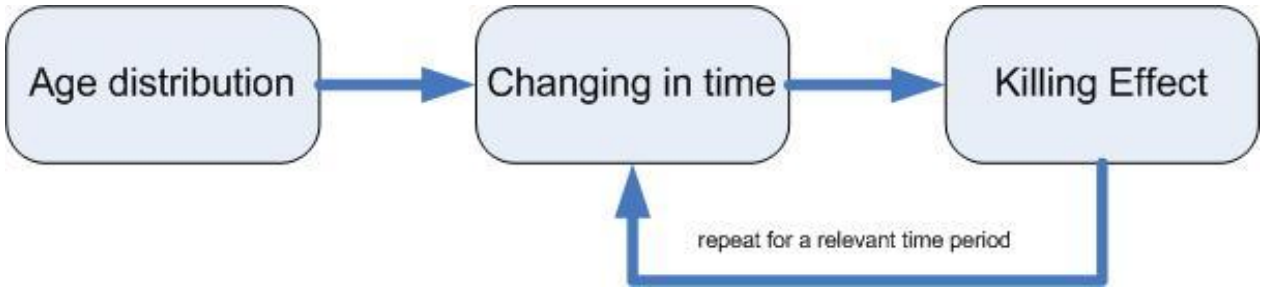


Dihydroartemisinin(DHA) concentration

Plasma concentrations of DHA were taken at 15 and 30 minutes, 1, 1.5, 2, 3, 4, 5, 6, 8 and 12 hours after the first artesunate dose.



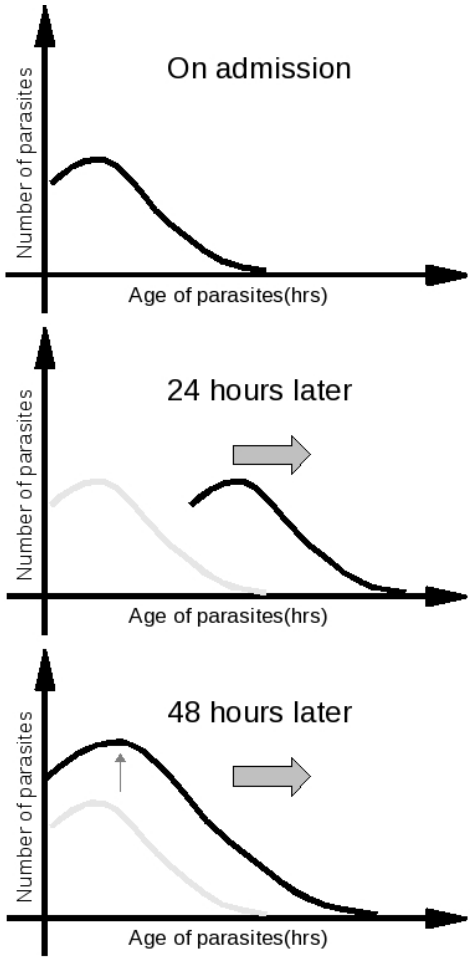
Model



Our models can be divided into three main parts:

1. initial age distribution
2. changing in time
3. killing effect

Parasite age distribution & Changing in time



Initial age distribution

The number of parasites distribute over their lifetime follow the normal distribution.The life-cycle is fixed at 48 hours.

Changing in time

Every one hour the distribution graph is moved to the right. Number of parasites at the age 48 hours will be multiplied with a multiplication rate for being the number of new parasites at the age 1 hour.

WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Parasite age distribution & Changing in time

without drug

Initial age distribution

The number of parasites distribute over their lifetime follow the normal distribution.The life-cycle is fixed at 48 hours.

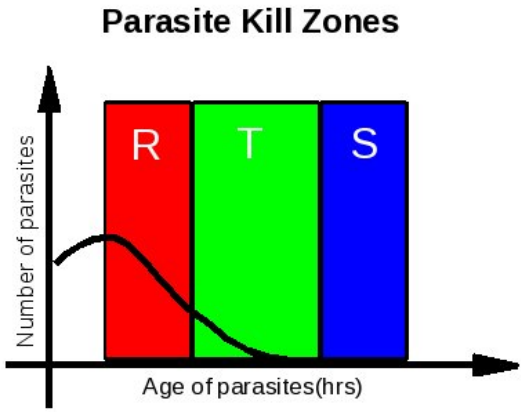
Changing in time

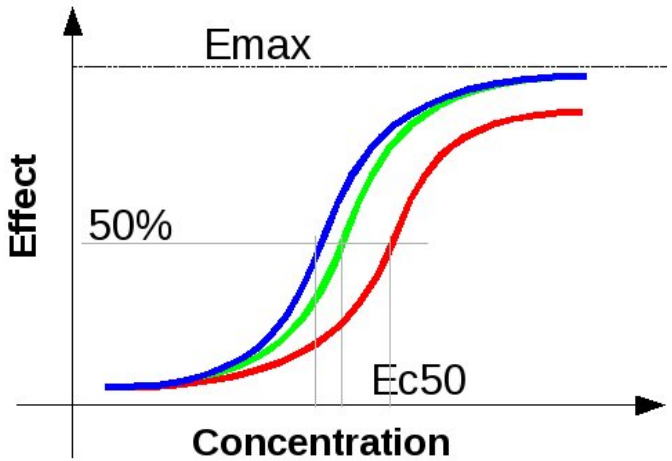
Every one hour the distribution graph is moved to the right. Number of parasites at the age 48 hours will be multiplied with a multiplication rate for being the number of new parasites at the age 1 hour.

Mahidol Oxford

WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Killing effect





Killing Effect

The parasites will be killed or removed from the system if they have the ages in the kill zones. The drug concentrations are used in the calculation of the killing percentage in each kill zone.

Parasite Kill Zones

The parasites ages from young rings to schizonts are divided into threes parts and in each part has its own concentration-effect relationship.

Killing effect

with drug

Killing Effect

The parasites will be killed or removed from the system if they have the ages in the kill zones. The drug concentrations are used in the calculation of the killing percentage in each kill zone.

Parasite Kill Zones

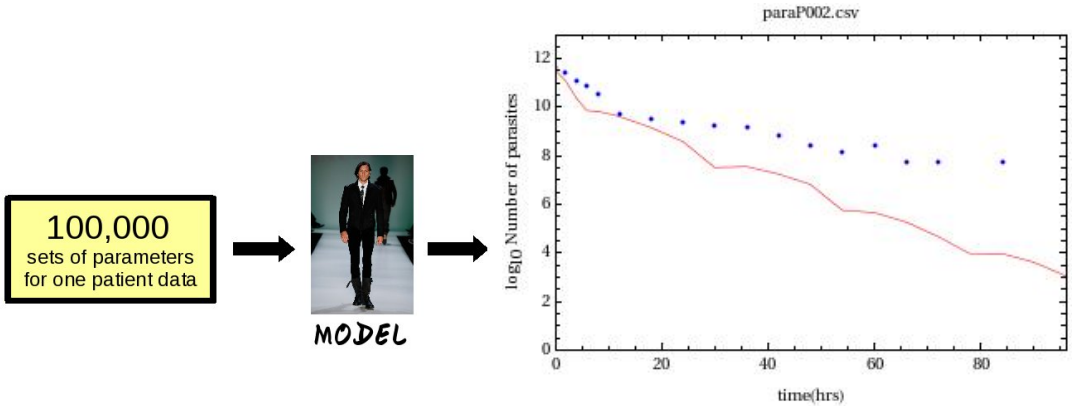
The parasites ages from young rings to schizonts are divided into threes parts and in each part has its own concentration-effect relationship.

Model parameters

Parameters	Symbols	Sampling ranges
Initial load of parasites	N_0	$10^{10} - 10^{12}$
Mean age of parasites (hrs)	μ	[4, 16]
Standard deviation (hrs)	σ	[2, 8]
Parasite multiplication rate	PMR	[6, 12]
Parasite lifespan (hrs)	LC	48
Slope of the sigmoid curve	γ	[1.5, 9.5]
50% effect concentration (ng/ml)	EC_{50}	[0, 100]
Maximum killing effect (%)	E_{max}	[0,99.9999]
The speed of killing (1/hr)	α	[3, 12]
begin/end kill zone (Rings)	$KZRb$, $KZRe$	$KZRb = 6$, $KZRe \in [20, 30]$
begin/end kill zone (Trophozoites)	$KZTb$, $KZTe$	$KZTb = KZRe + 1$, $KZTe = 38$
begin/end kill zone (Schizonts)	$KZSb$, $KZSe$	$KZSb = 39$, $KZSe = 44$


Fitting Model

The main output of the model is the parasitemia over time.



Root Mean Square Deviation

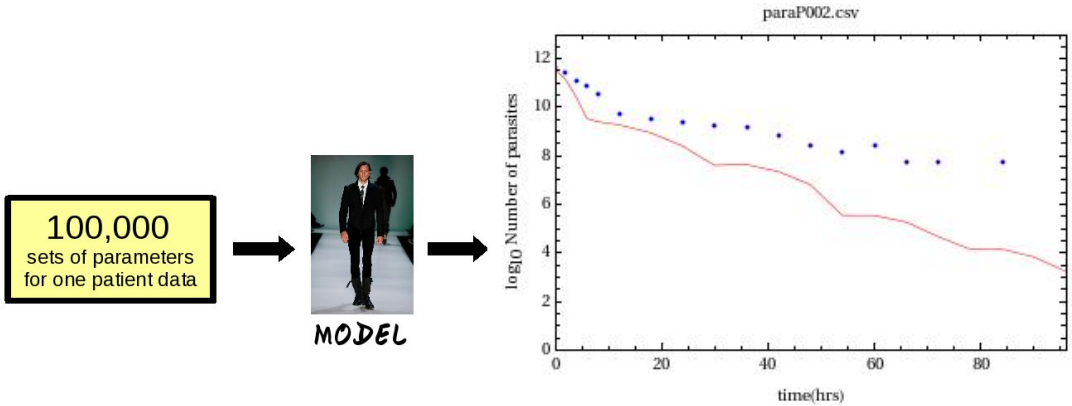
$$RMSD = \sqrt{\frac{\sum (Xobs_i - Xmod_i)^2}{m}}$$

where m is the number of points in each patient data.

Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Fitting Model

The main output of the model is the parasitemia over time.



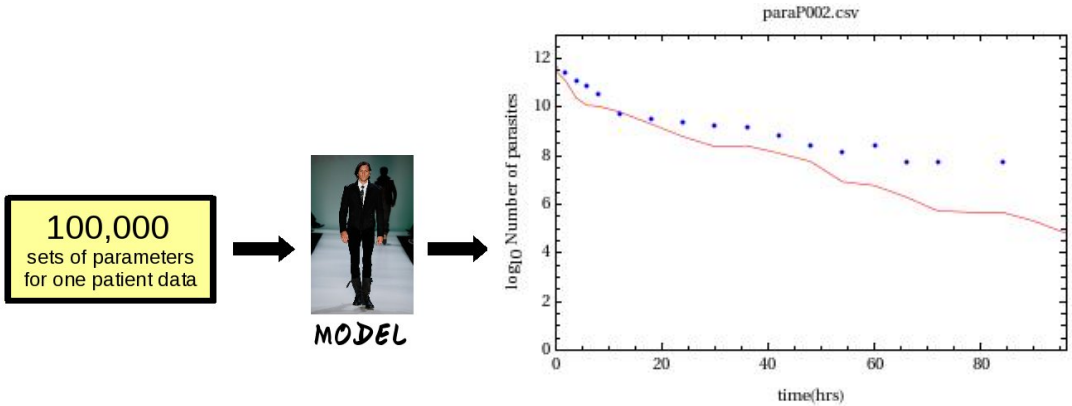
Root Mean Square Deviation

$$RMSD = \sqrt{\frac{\sum (Xobs_i - Xmod_i)^2}{m}}$$

where m is the number of points in each patient data.

Fitting Model

The main output of the model is the parasitemia over time.



Root Mean Square Deviation

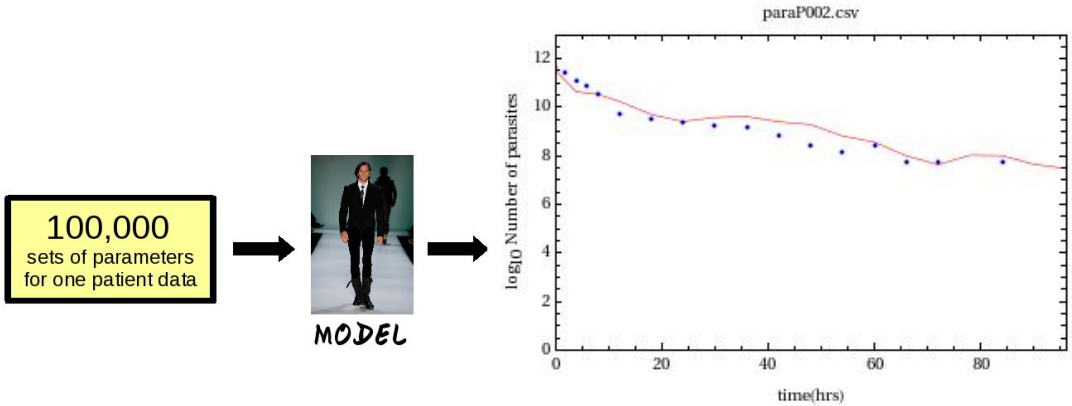
$$RMSD = \sqrt{\frac{\sum (Xobs_i - Xmod_i)^2}{m}}$$

where m is the number of points in each patient data.

Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Fitting Model

The main output of the model is the parasitemia over time.



Root Mean Square Deviation

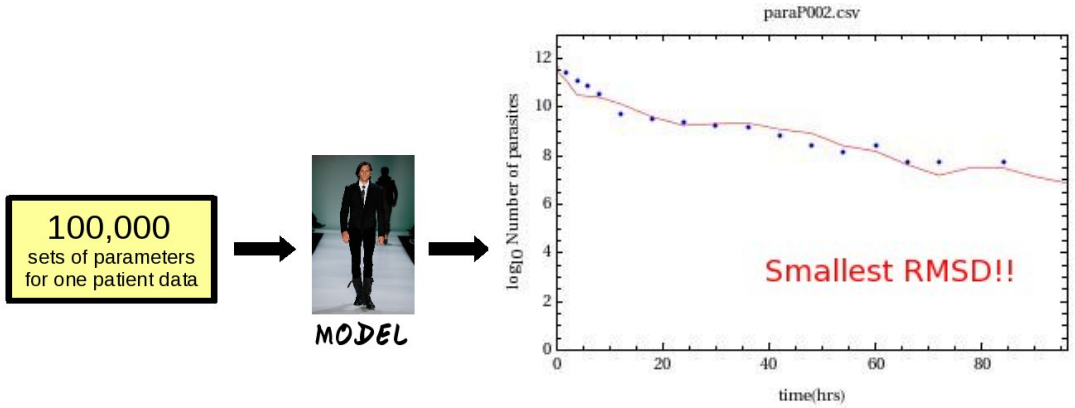
$$RMSD = \sqrt{\frac{\sum (Xobs_i - Xmod_i)^2}{m}}$$

where m is the number of points in each patient data.

Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Fitting Model

The main output of the model is the parasitemia over time.

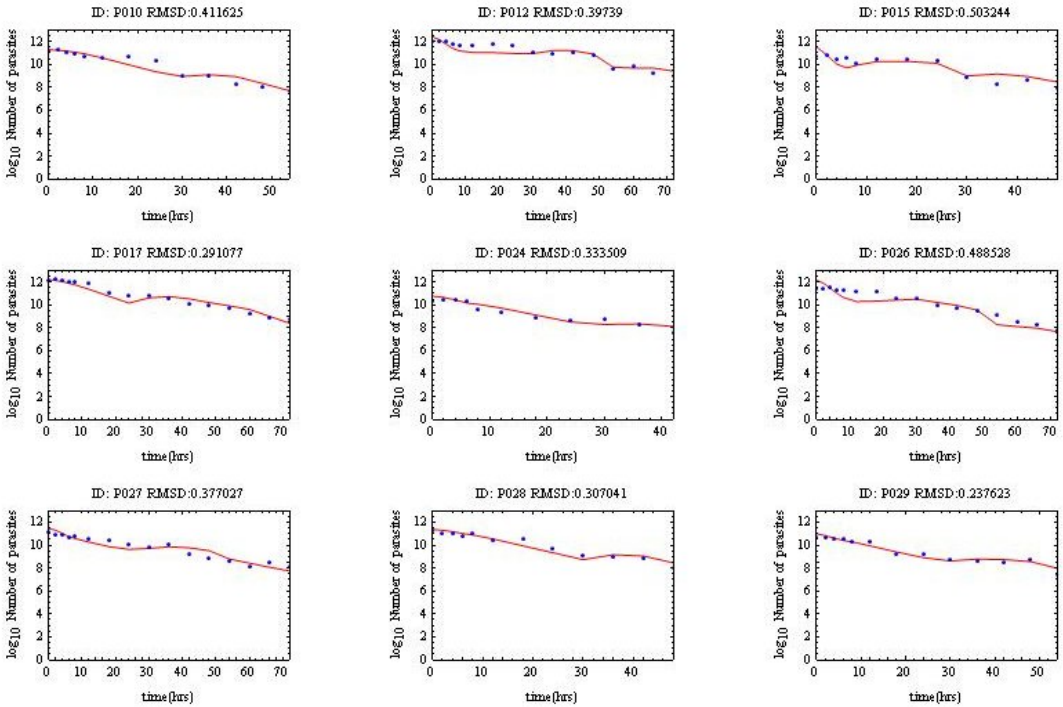


Root Mean Square Deviation

$$RMSD = \sqrt{\frac{\sum (Xobs_i - Xmod_i)^2}{m}}$$

where m is the number of points in each patient data.

Fitting model with the individual data



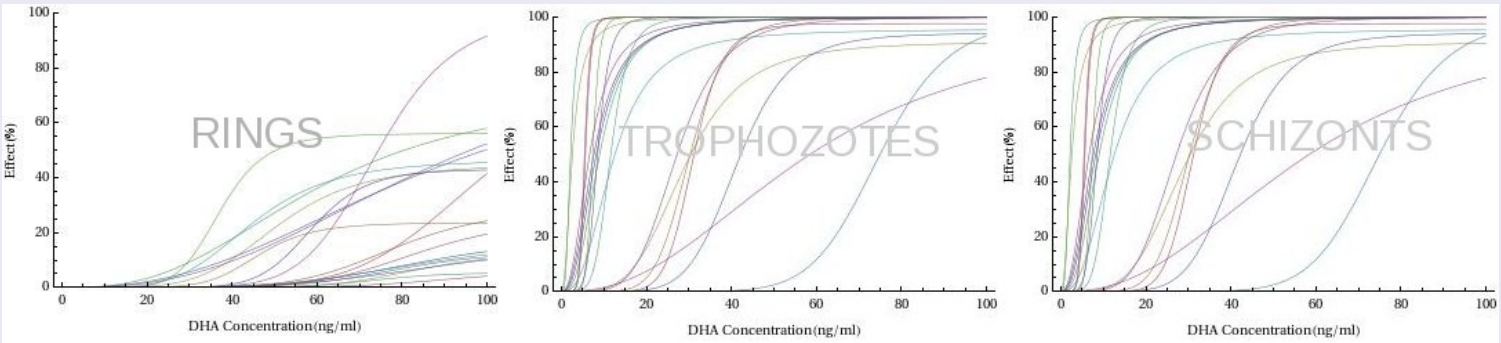
Study Site/Regimen	RMSD(in log10)
Wang Pha/ AS7	0.34(n=20)
Pailin/ AS7	0.45(n=19)
Wang Pha/ MAS3	0.26(n=20)
Pailin/ MAS3	0.39(n=20)

** n = number of patients

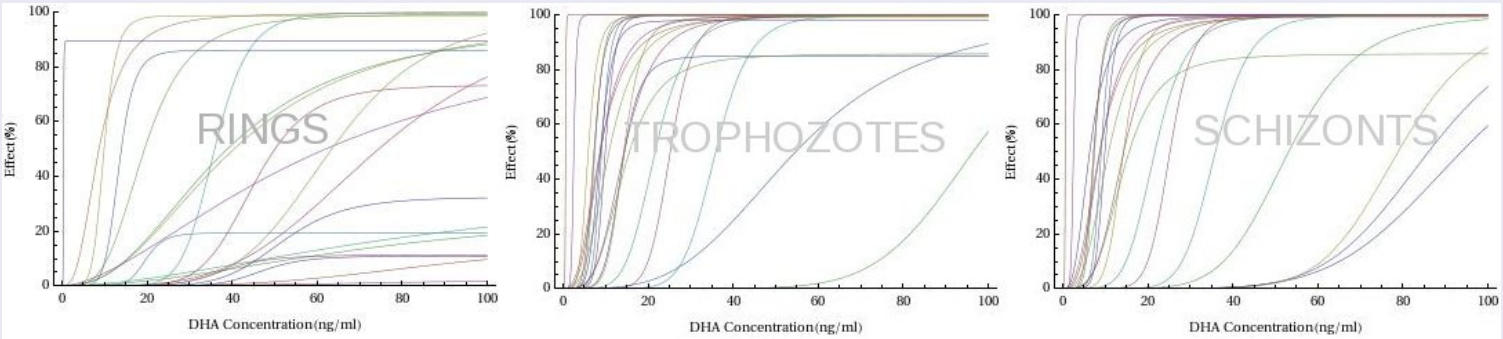
Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

Concentration-Effect curves of each stage

Pailin (MAS3)-Western Cambodia

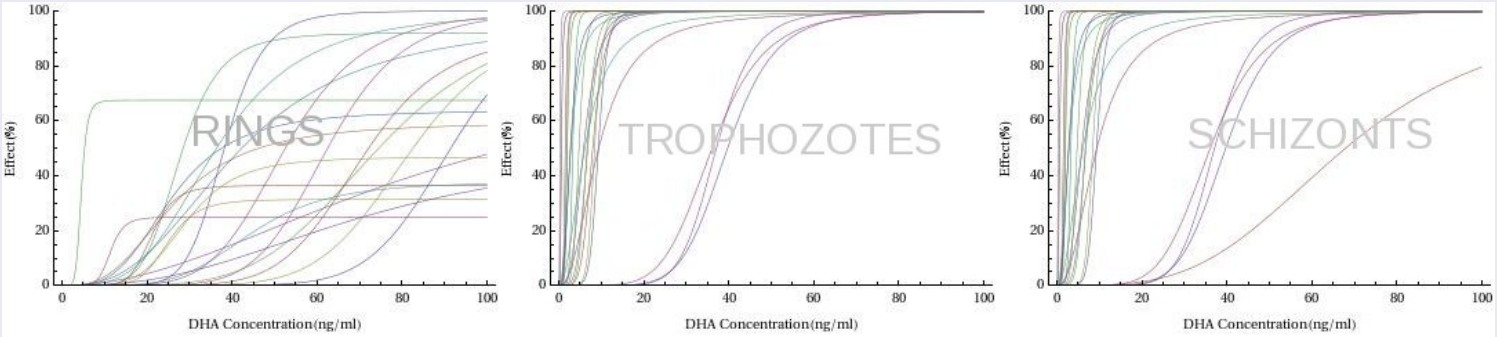


Wang Pha (MAS3)-Western Thailand

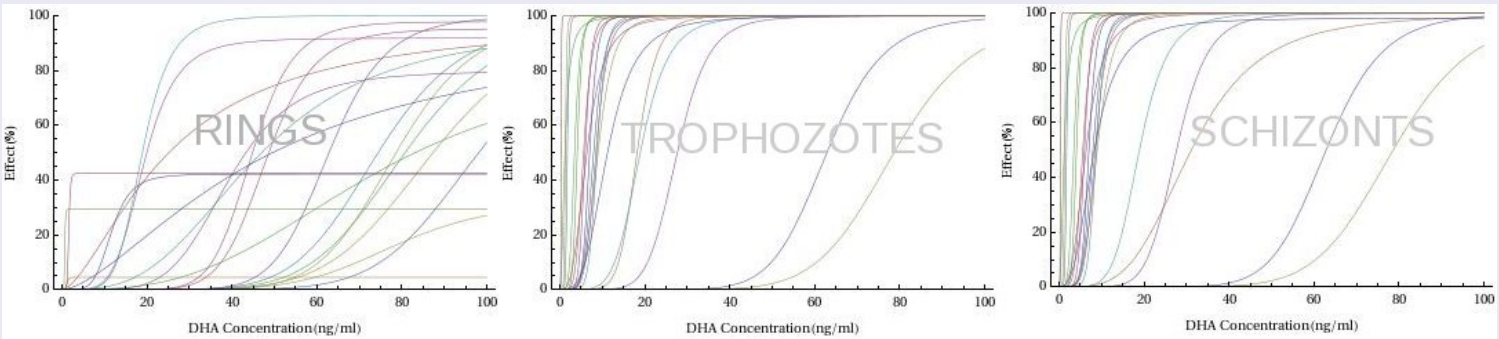


Concentration-Effect curves of each stage

Pailin (AS7)-Western Cambodia



Wang Pha (AS7)-Western Thailand

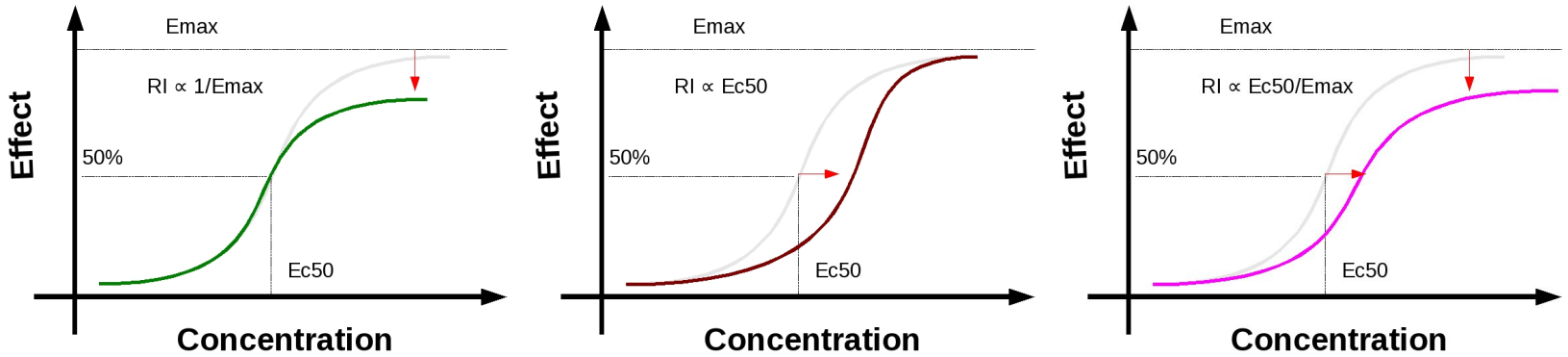


Resistance Index

Resistance Index

$$\textit{Resistance Index(RI)} = \frac{EC_{50}}{E_{max} \times \gamma \times \alpha}$$

The higher the resistance index the more resistant that stage of that parasite is to the drug.



Comparing RI between stages and sites

The first 10 best fit parameters of each patient were used in the calculation of RI for every stages. These RIs were compared using the mixed-effect model.

Multilevel mixed-effect model

$$\mathbf{y}_{ij} = \mathbf{X}_{ij}\beta + u_i^{(1)} + u_{ij}^{(2)} + \epsilon_{ij}$$

$\mathbf{y}_{ij}$-the logarithms of RI for $n_{ij} = 10$, $\mathbf{X}_{ij}$- a set of regressors(stage, site), $u_i^{(1)}$-a random intercept at the patient level, $u_{ij}^{(2)}$-a random intercept at the model level in each patient.(i=1 Wang Pha, 2 Pailin, j=1 Rings, 2=Trophozoites, 3=Schizonts)


Comparison RI between stages and sites

Using a two-level model mixed-effects linear regression, it shows that

- RI of rings was significantly higher than trophozoites and schizonts regardless of location.
- RI for trophozoite stage parasites was not significantly different to RI of schizonts regardless of location.
- RI for rings in western Cambodia was significantly higher than in western Thailand.


Conclusion from the model

- The model can reproduce the observed data.
- **The results from the model support the hypothesis that increasing of parasite clearance time results from reduced efficacy of the drug at the ring stage of parasite life-cycle.**
- The model can be used for designing alternative dosing regimens.

Acknowledgement

MORU Mathematical & Statistical Modelling Team

- Lisa J. White
- Wirichada Pongtavornpinyo
- Richard J. Maude
- Sue J. Lee
- Kasia Stępniewska
- Sompob Saralamba

MORU Clinical Team

- Arjen M. Dondorp
- Nicholas PJ. Day
- Nicholas J. White
- François H. Nosten
- Kesinee Chotivanich

MORU Pharmacology Team

- Joel Tärning
- Niklas Lindergårdh

National Malaria Center (CNM), Cambodia

- Duong Socheat

Mahidol Oxford MORU
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

ขอบคุณครับ
köszönöm תודה! děkuji
mahalo 고맙습니다
thank you
merci 谢谢 danke
Ευχαριστώ شكرا
どうもありがとう gracias


Age distribution

If the initial number of parasites is N_0 then the initial number of parasites at age a is

$$N(a,0) = \frac{N_0}{\sum_{a=1}^{L} p(a)} p(a) \tag{1}$$

where $a = 1, 2, 3 \ldots L$ and $p(a)$ - is the probability density function at time interval a,

$$p(a) = \frac{\exp \frac{(a-\mu)^2}{2\sigma^2}}{\sqrt{2\pi}\sigma} \tag{2}$$

Parasite Killing

For each stage,the concentration-effect relationship is

$$E(c(t)) = E_{max} \frac{c(t)^{\gamma}}{c(t)^{\gamma} + EC_{50}^{\gamma}} \tag{3}$$

In the kill zone, the decrease in parasite number per time step can be described as:

$$N(a+1, t+1) = N(a, t) \exp -k_j(t) \tag{4}$$

where $N(a, t)$ is the number of parasites with the age a at time and $k_j(t)$ is the decay constant at time t of the zone j (j=1 Rings,2= Trophozoites, 3= Schizonts).

$$k_j(t) = \alpha \ln \left(\frac{100}{100 - E_j(c(t))} \right) \tag{5}$$

Parasite counts

Peripheral blood parasite counts (as number of parasites/μL) were converted into total body parasite biomass by assuming a total blood volume of 80 ml/kg.

$$\text{total biomass} = \text{peripheral blood parasitemia} \times \Psi \tag{6}$$

with $\Psi = 80,000 \times \text{body weight}$.

Parasite counts

The relationship between the observed peripheral blood parasitemia (circulating parasites) and the corresponding total body parasite biomass at time t was described as:

$$N_{obs}(t) = \sum_{a=1}^{L} N(a,t)g(a) \tag{7}$$

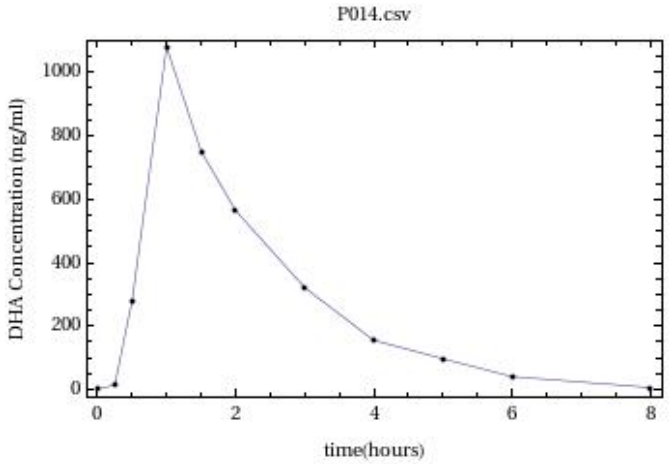
where $N_{obs}(t)$-the circulating parasitemia, $g(a)$-the probability function of seeing the parasite age a hours from the blood sample

$$g(a) = \begin{cases} 1 & \text{for } a < 11 \\ \exp \ln(0.5)(a-11)/3 & \text{for } a \geq 11 \end{cases} \tag{8}$$

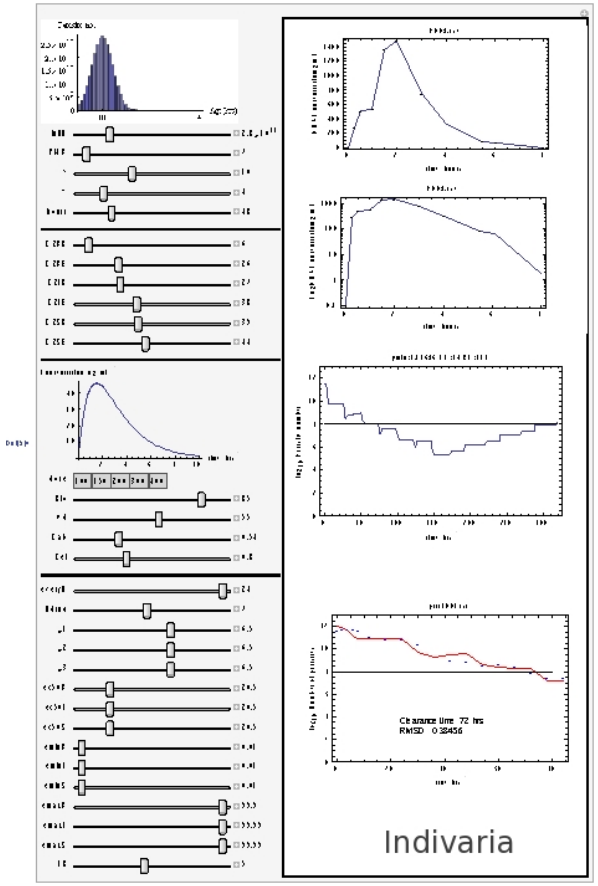

Drug concentration

The concentration data of each patient were divided into an absorption phase (from the first point to maximum concentration point) and an elimination phase (from the maximum concentration point to the last point).

$$c(t) = \begin{cases} qt & \text{absorption} \\ r \exp -st & \text{elimination} \end{cases} \tag{9}$$



Indivaria



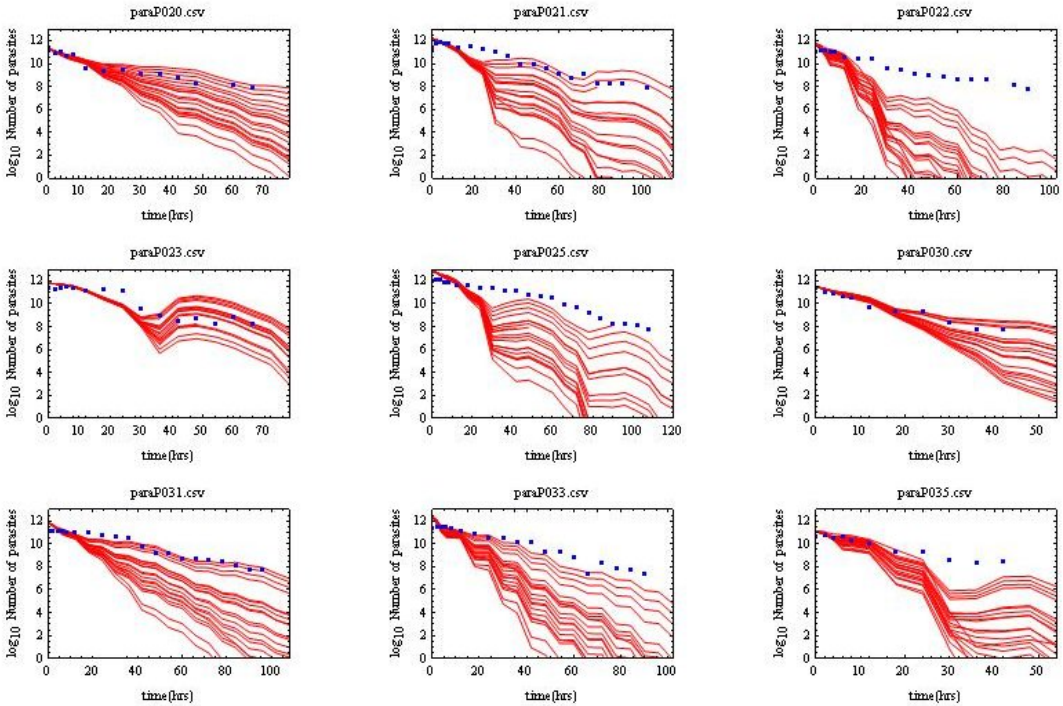
Indivaria = Individual + Malaria

Indivaria is a *Mathematica* package developed by MORU Mathematical modelling team. It is the package for doing within host models of malaria infections.

Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

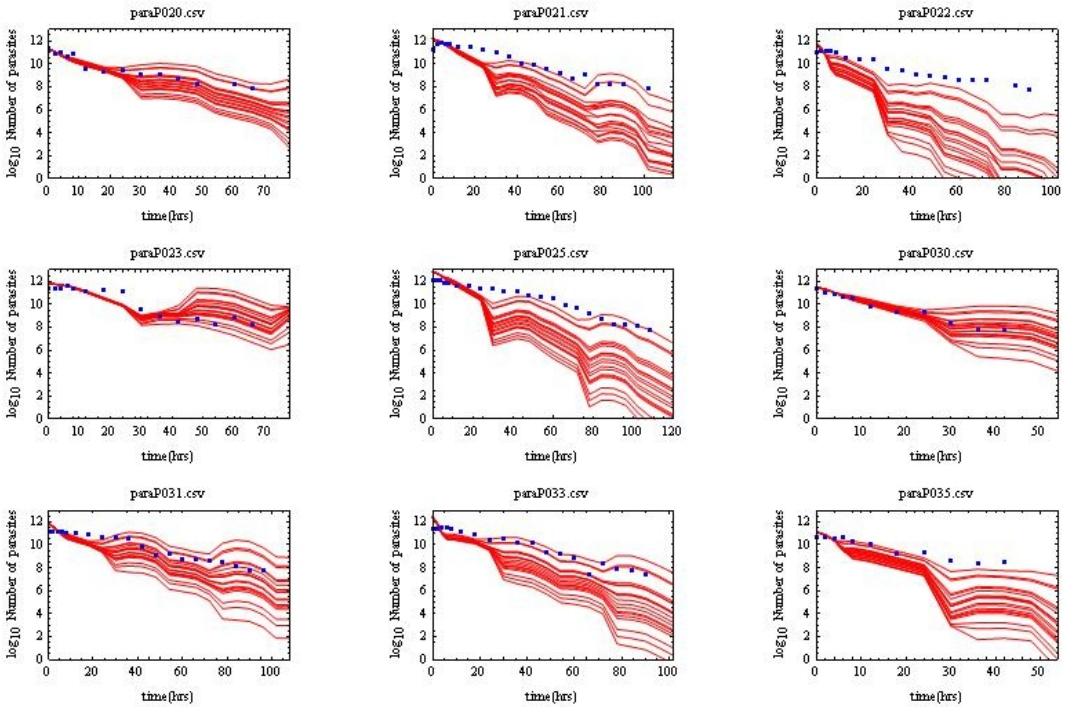
Splitting Dose

Giving artesunate twice a day for 7 days.



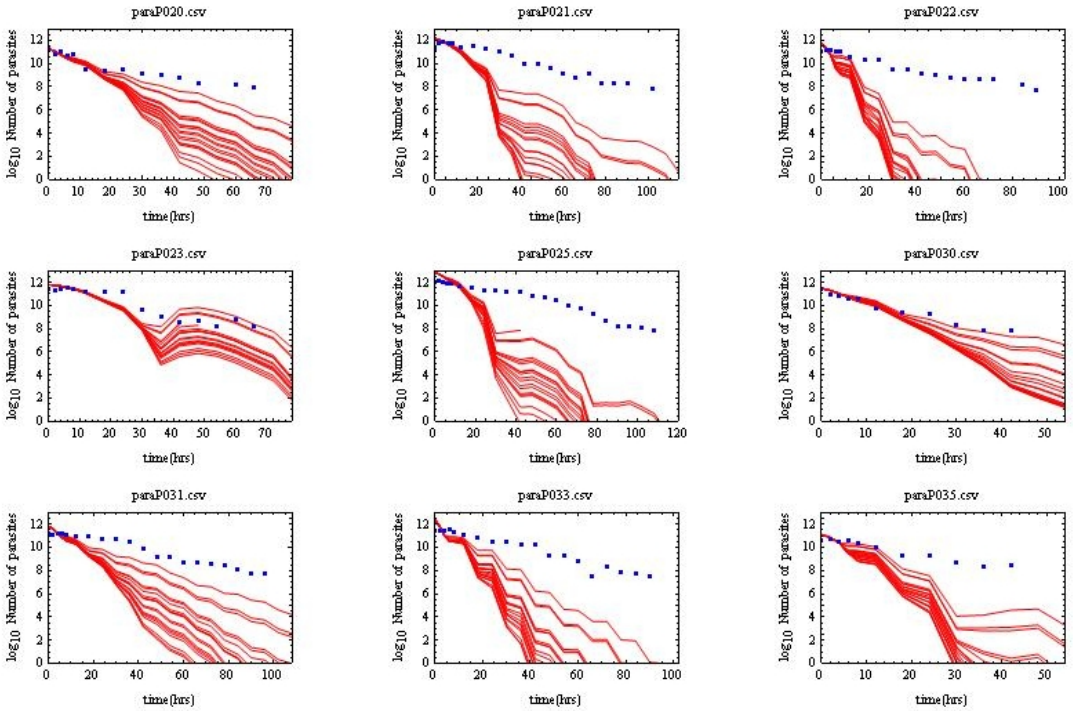
Double Dose

Give artesunate 4 mg/kg for 7 days (Using DHA concentration of MAS3 in AS7 patients).



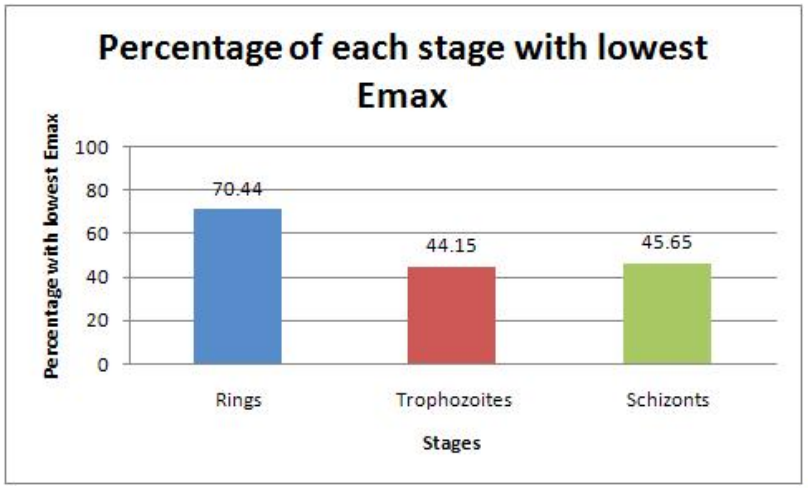
Split & Double Dose

Give artesunate 4 mg/kg for 7 days.



Lowest E_{max} at Ring stage

The E_{max} of each stage from the sets of parameters that have rmsd < 1.0 in each patient were compared for the minimum.



Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD TROPICAL MEDICINE RESEARCH PROGRAMME