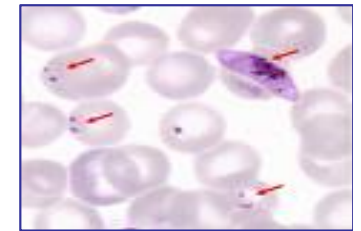




# Artemisinin Resistance :

## in vivo studies



**Debashish Das**

**Mahidol Oxford Research Unit, Bangkok, Thailand**



**Mahidol Oxford**



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# Artemisia annua (qinghao)

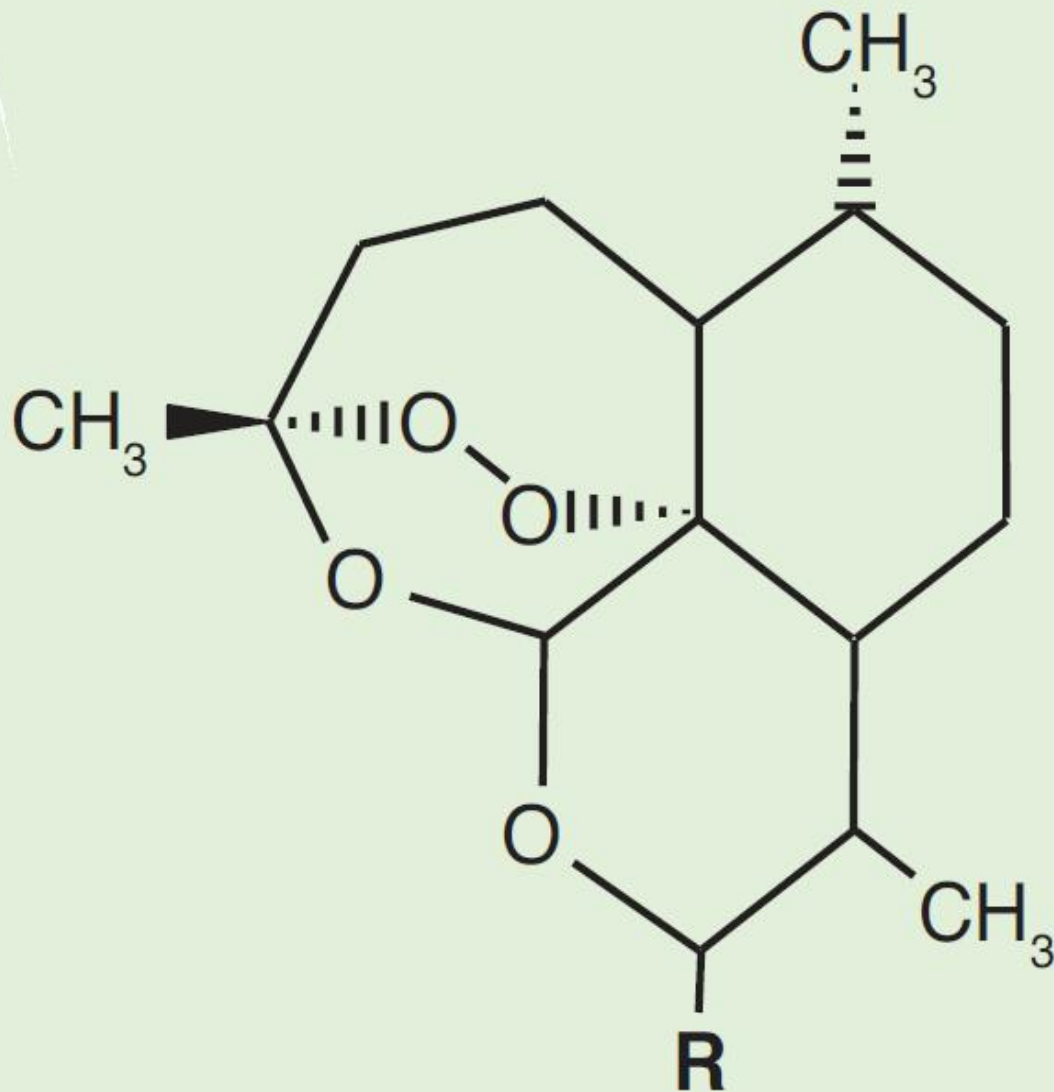


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Artemisinin and its derivatives. 1. artemisinin; 2. artemether;  
3. artemotil (arteether); 4. artesunate.



1. **R: =O**

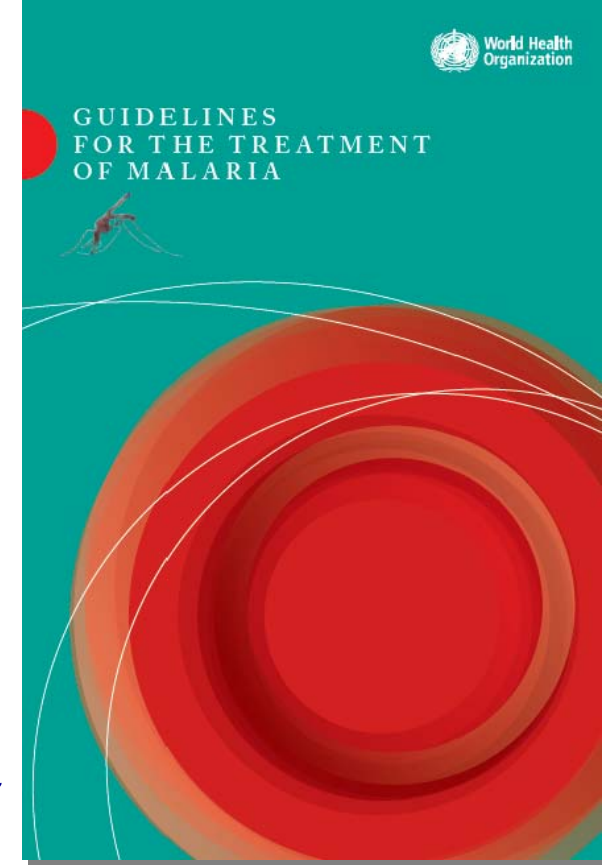
2. **R: βO-CH<sub>3</sub>**

3. **R: βO-C<sub>2</sub>H<sub>5</sub>**

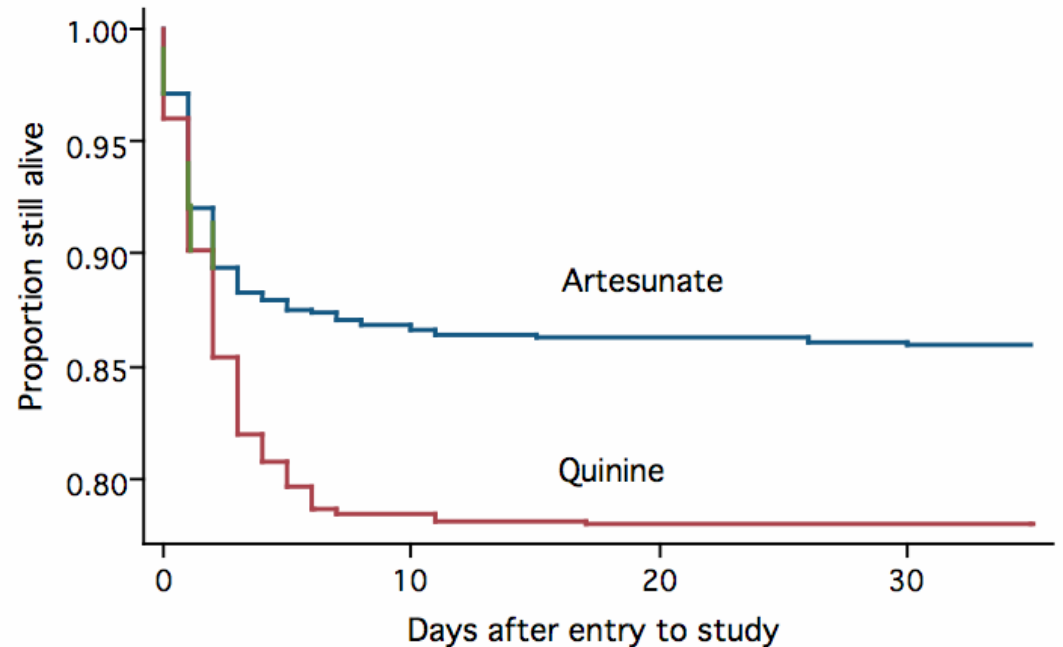
4. **R: αCO(CH<sub>2</sub>)<sub>2</sub>COO**

# Artemisinin Combination Therapies (ACT)

1<sup>st</sup> line treatments of Uncomplicated F.Malaria



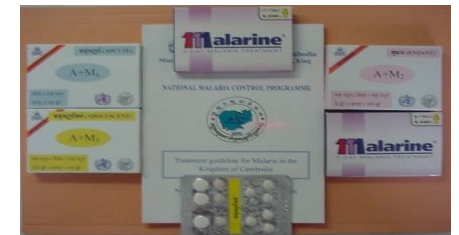
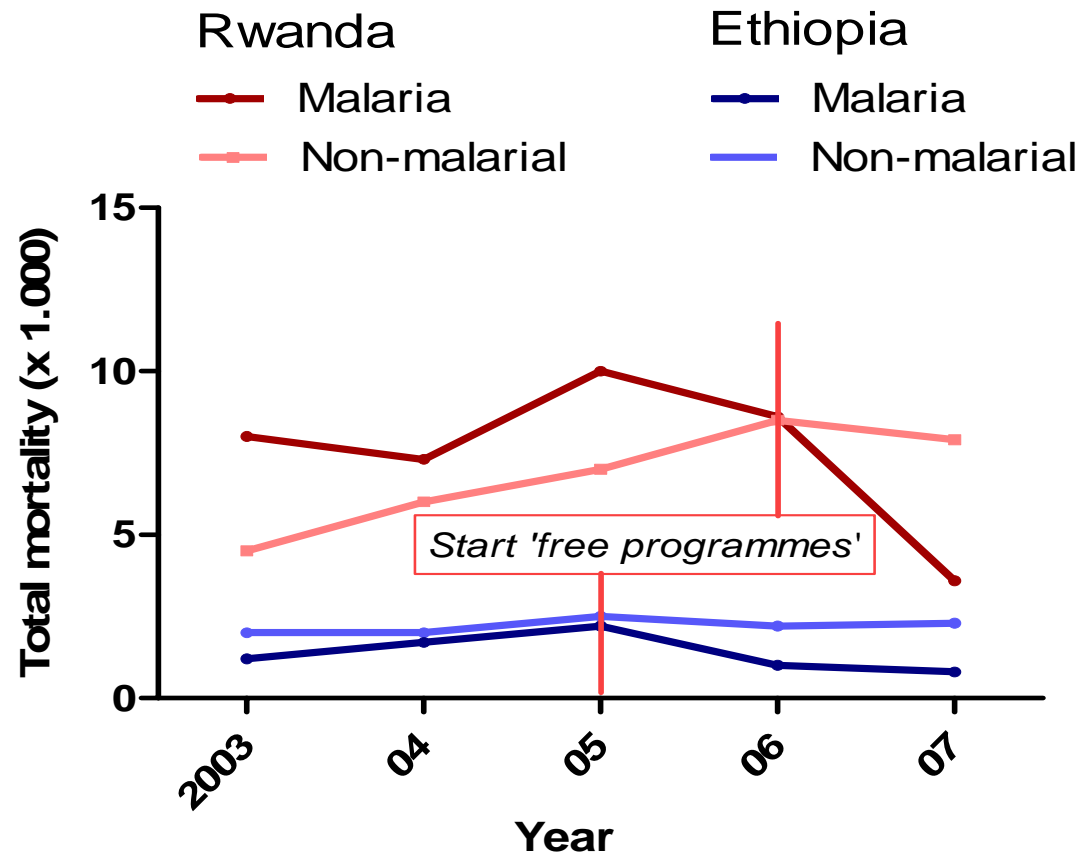
## Quinine vs Artesunate in Severe Malaria (SEAQUAMAT)



# Artemisinin Combination Therapies (ACT)

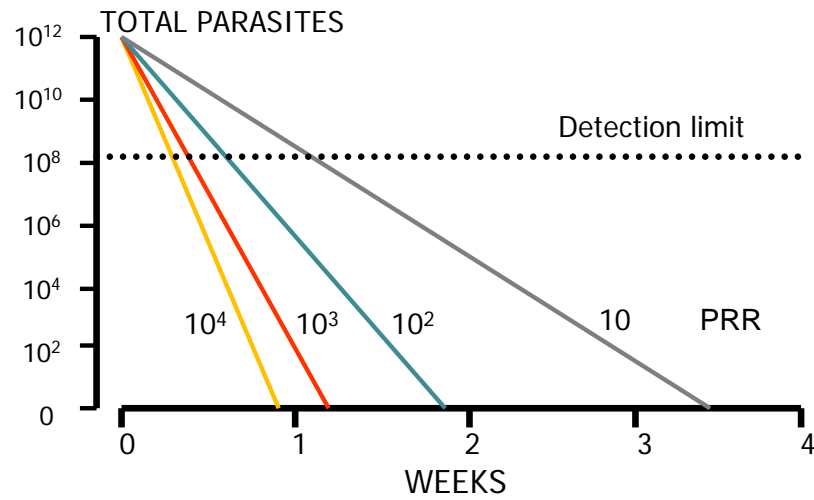
Accelerate therapeutic response, Increase cure rates  
Reduce Transmissibility, Prevent resistance

## Effects on mortality of free impregnated bednets and ACT

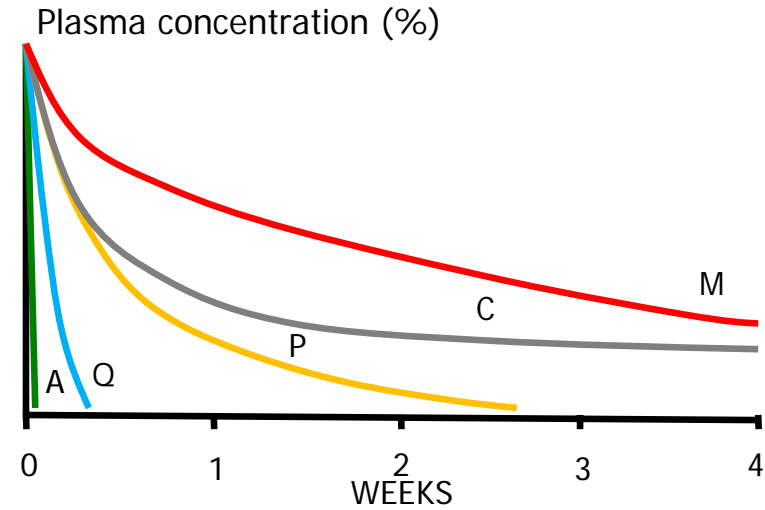


# The ACT rationale

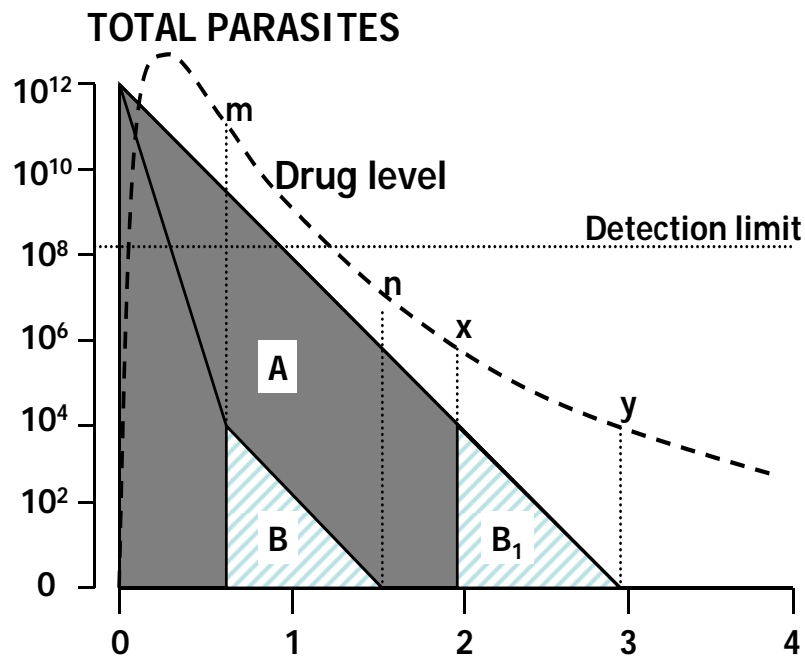
N.J. White



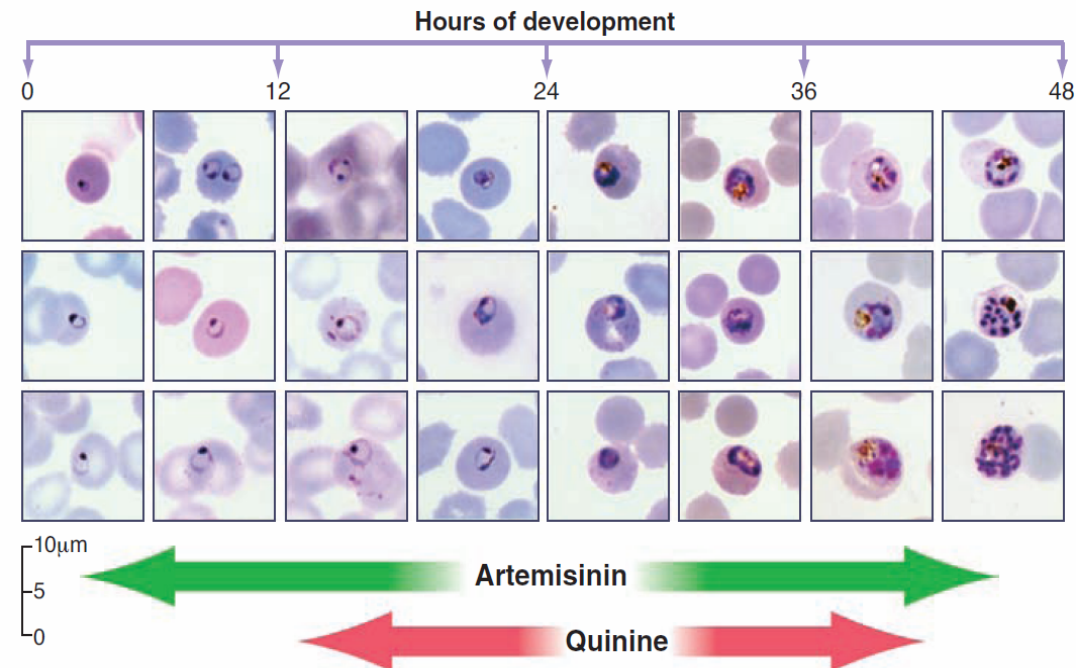
Rapid parasite clearance



The selective window



Mutual protection



Broad stage specificity



## ACT efficacy in Western Cambodia

- **Artemether-lumefantrine** 28 days cure rate
  - **Batambang** 2002 (no food): **71.1%** (but low day 7 plasma concentrations)
  - **Batambang** 2003 (with food): **86.5%**
- **Artesunate-mefloquine** 28 days cure rate
  - **Batambang** 2001: **96%**
  - **Pailin** 2002: **86%**
  - **Batambang** 2003: **92.4%**
  - **Pailin** 2004: **90%**



# Study sites



**Wang Pha, Thailand**  
**Pailin, Cambodia**





# Artesunate efficacy in Pailin (W-Cambodia) vs. Wang Pha (NW-Thailand)



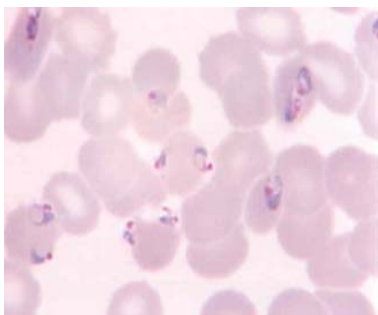
## Inclusion

- Uncomplicated *P. falciparum* infection
- Parasitaemia > 10,000/ uL
- No mixed infection on light microscopy
- No pregnancy
- No previous antimalarial treatment (48H)



## Randomisation

- Artesunate 2 mg/kg per day for 7 days (AS7) vs
- Artesunate 4 mg/kg per day for 3 days & mefloquine 15 and 10 mg/kg per day on day 3 and 4 (MAS3)

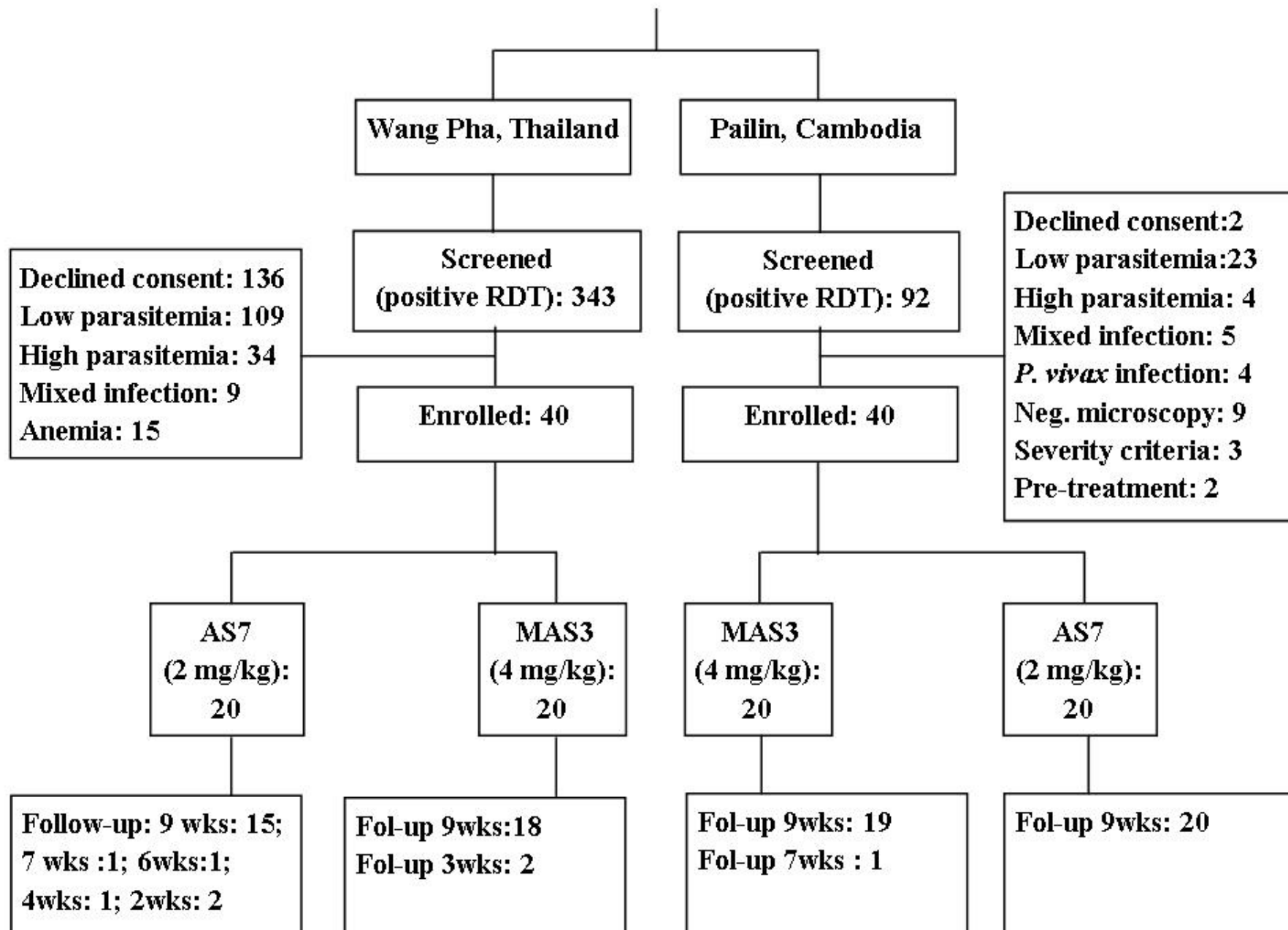


## Outcome measures

- Clinical and parasitological cure
- Parasite clearance times
- Artesunate Pharmacokinetics
- *In-vitro* sensitivity tests
- Molecular markers

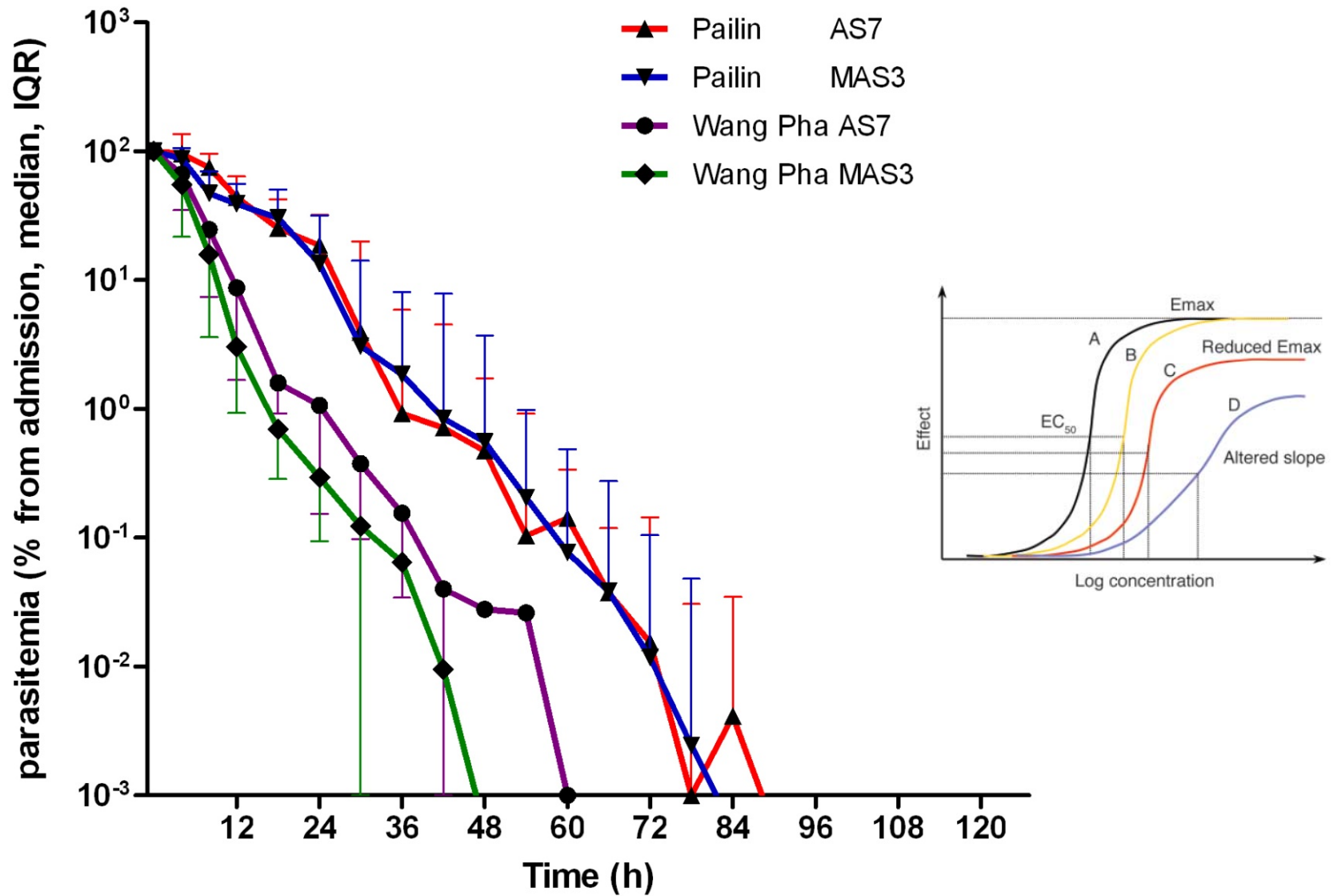


# Trial Profile

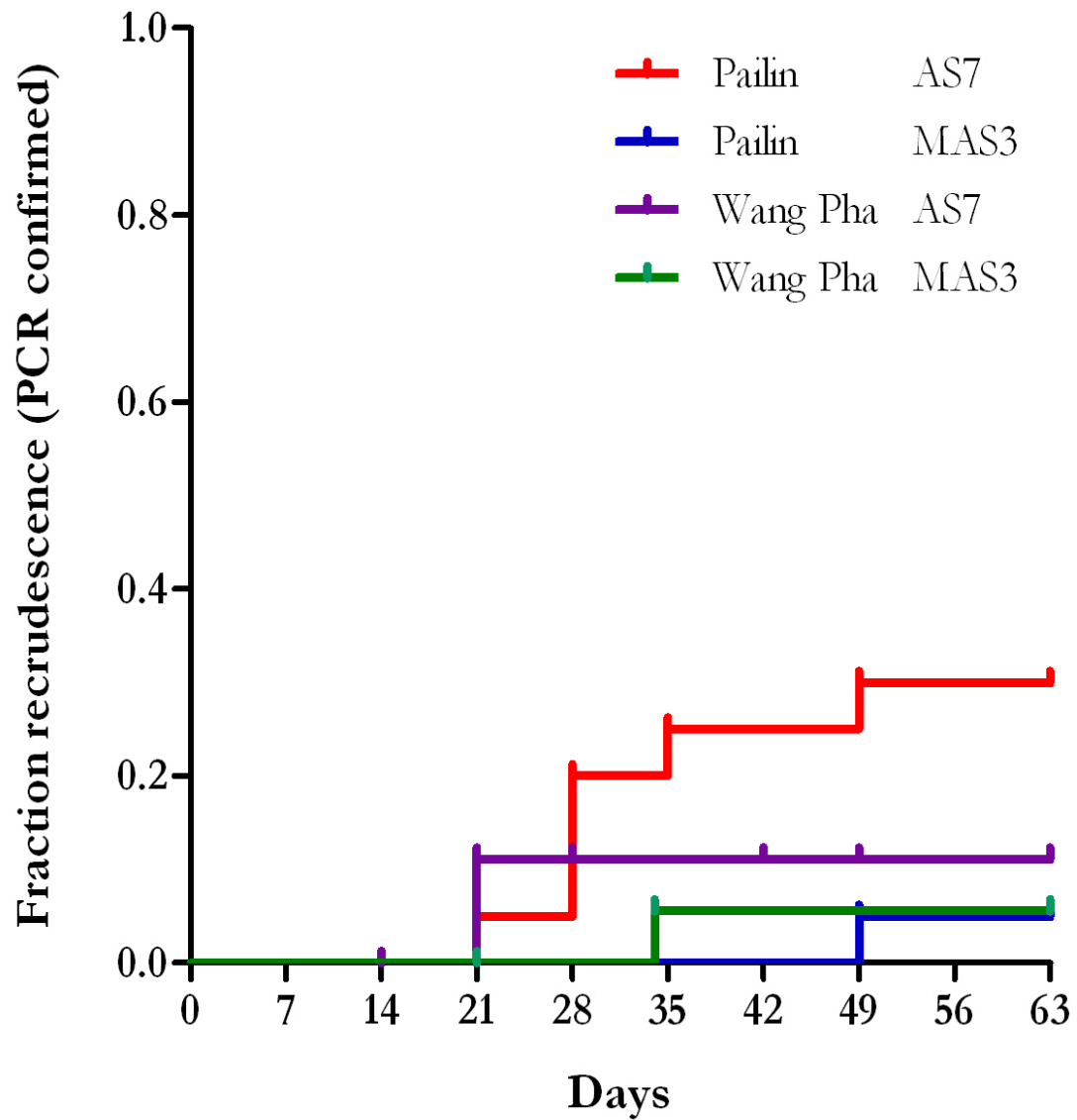


	Wang Pha		Pailin		p-value
	AS7 (n=20)	MAS3 (n=20)	AS7 (n=20)	MAS3 (n=20)	
PCT (IQR), h	54 (42, 72)	48 (30, 54)	84 (54, 96)	72 (60, 96)	<0.001
PCT (IQR), h, by baseline prst.					
	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	
10 <sup>4</sup> to 10 <sup>4.99</sup> /μL	54 (42, 72)	48 (30, 54)	72 (48, 96)	66 (60, 84)	0.009
	18	15	11	15	
10 <sup>5</sup> /μL and above	54 (54, 72)	60 (-)	90 (84, 96)	96 (90, 108)	0.003
	2	1	9	5	
No. recrudescences (%)	2 (10)	1 (5)	6 (30)	1 (5)	0.31
Gametocyte duration days	19 (7, 19)	13 (1, 19)	10 (7, 19)	18 (6, 23)	0.41

# Parasite Clearance Curves (p=0.0001 for $\Delta$ slopes between sites)

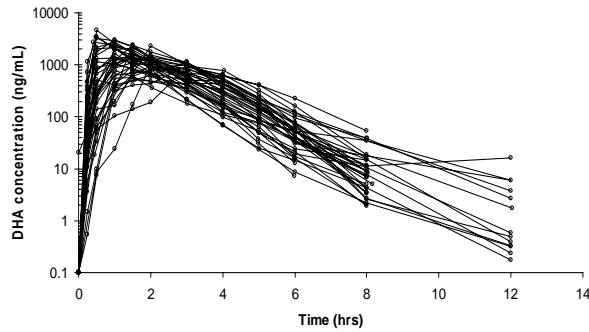


# Kaplan-Meier plot (p=0.068)



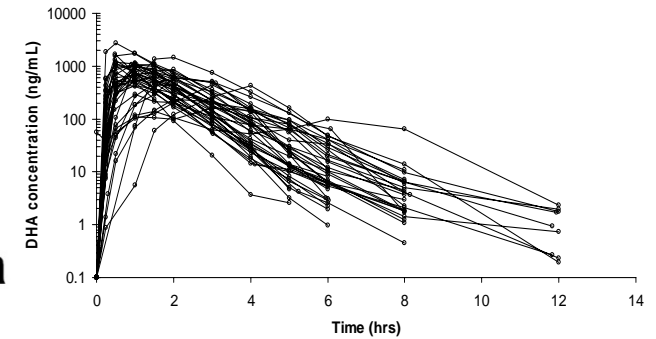
# Difference in PD (=PCT), but not in PK

MAS3 (4 mg artesunate/kg)

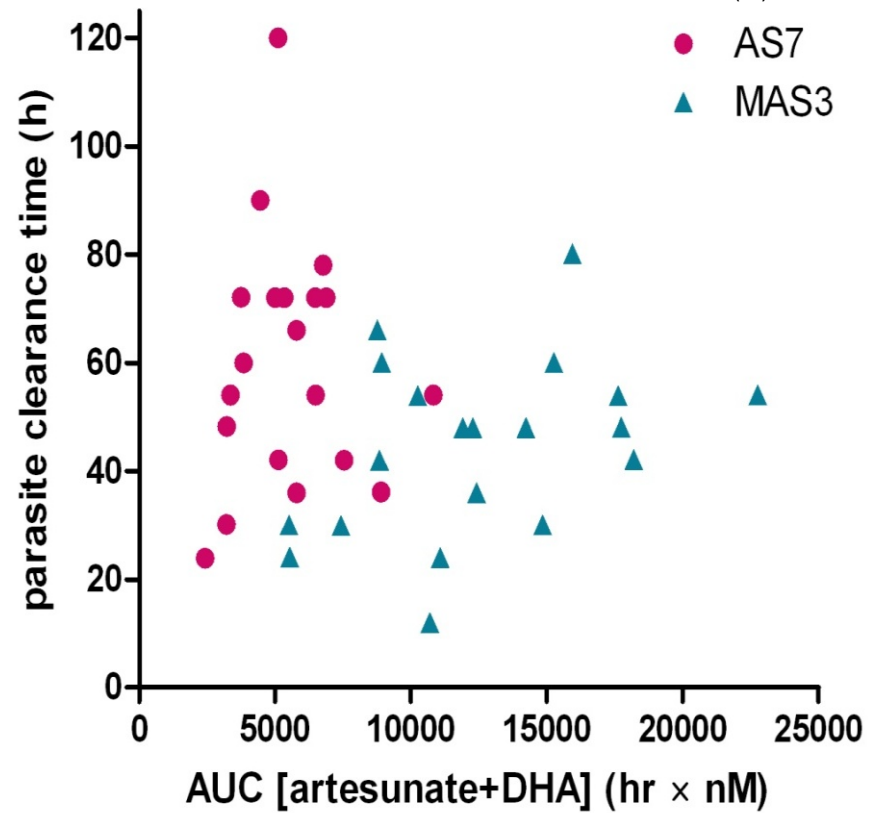
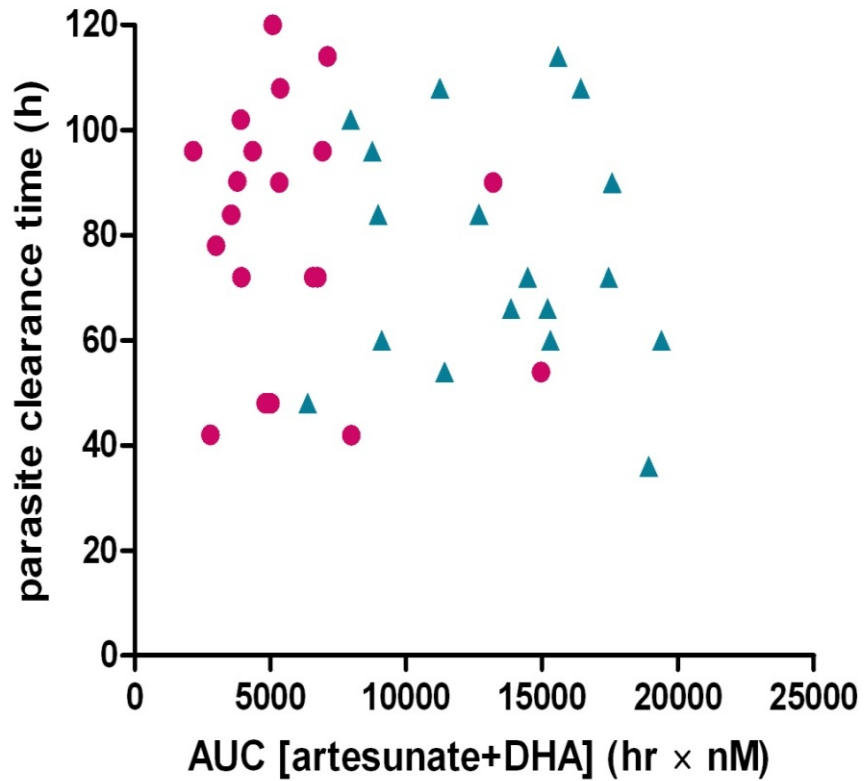


Pailin

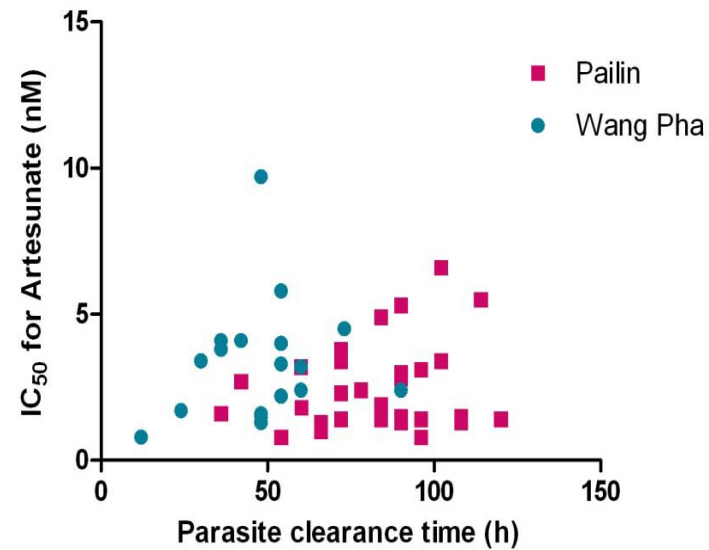
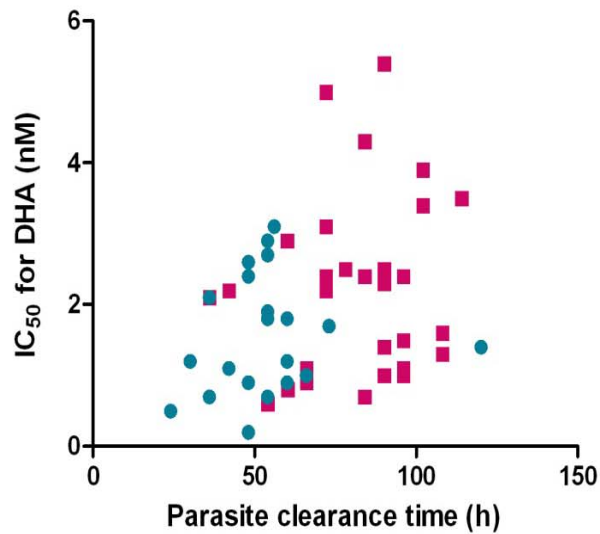
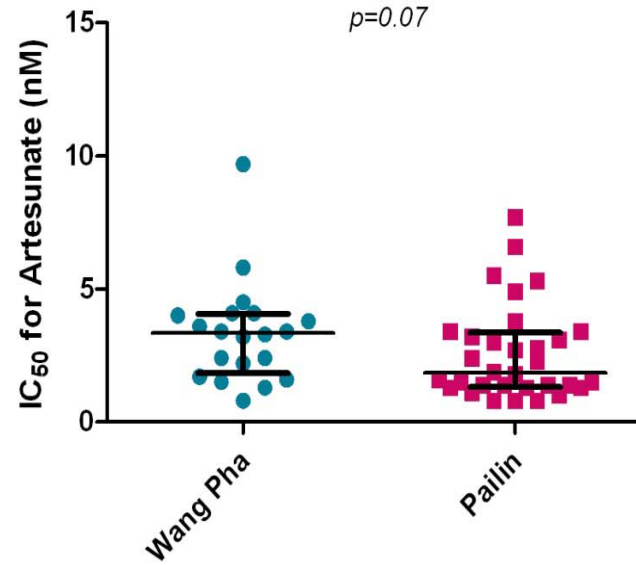
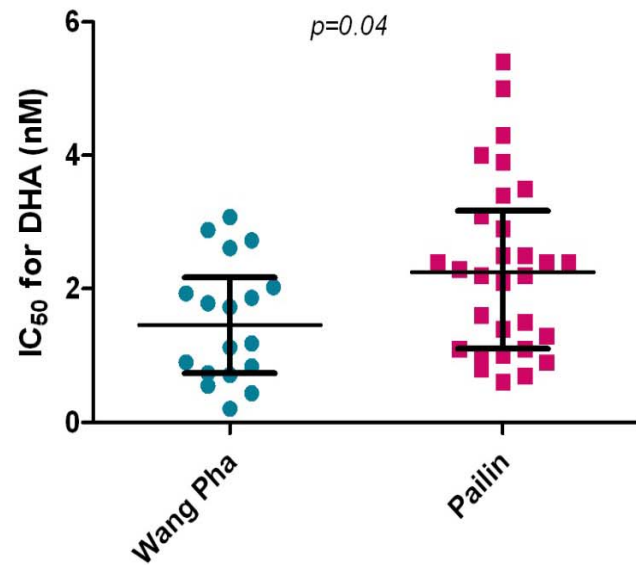
AS7 (2 mg artesunate/kg)



Wang Pha



# *In-vitro* Drug Sensitivity



# Molecular markers of artemisinin resistance:

- ❖ No molecular marker has been identified yet.  
(Dr. Mallika Imwong's talk will follow)





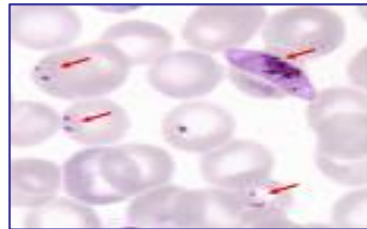
# Why has resistance emerged in W-Cambodia?



- repeated underdosing with artemisinin monotherapies.
- Counterfeit or substandard antimalarial drugs.
- underdosing more likely in pregnant women and children.



- ring stage resistance?



- Parasite factors.



# Has or will it spread??



- ❖ "If the same thing happens again, the spread ... from Asia to Africa, then that will have devastating consequences,"  
- **Prof. Nick Day**, BBC News.



- ❖ "We need to take a look at the past and what happened with chloroquine",- **Dr. Arjen Dondorp**, The Lancet.



- ❖ "It would be serious if these partially resistant parasites reached Africa where great gains in malaria control are currently being made using ACTs and ITNs,"  
- **Prof. Brian Greenwood**, BBC News.



- ❖ "The containment project's success is dependent on..time and money"- **Dr. Duong Socheat**, The Lancet.



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# Containment strategy (NMCPs & WHO)

- Early diagnosis and effective treatment
- Banning of artesunate monotherapy.
- ACT.
- Targeting migrant population.
- 100% coverage with LLINs.



## Conclusions:

- Artemisinin resistance in Western Cambodia; ring form resistance?
- Lack of a sensitive in vitro test
- Molecular basis for the resistance remains unknown
- Not explained by inadequate drug levels
- No dose – response relationship: high dose or split dose? Semisynthetic artemisinins or synthetic endoperoxides?



# Acknowledgements

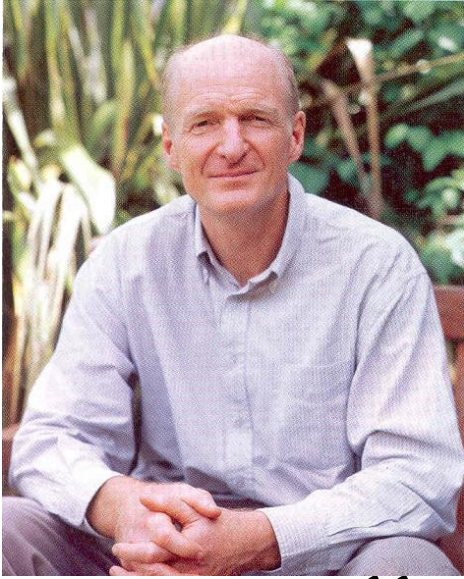
- Staff and patients Pailin Referral Hospital and Wang Pha clinic
- National Malaria Control Programme, Cambodia  
(Dr. Socheat, Dr. Poravuth Yi)
- Staff of Mahidol - Oxford Tropical Medicine Research Unit and SMRU  
( Arjen Dondorp, Nick White, Francois Nosten, Nick Day, Niklas Lindegardh, Mallika Imwong, Kesinee Chotivanich, Kamolrat Silamut, Rupam Tripura, Aung Phae Phyo, Khin Maung Lwin)
- Institut Pasteur, Pnomh Penh (Frédéric Ariey)
- AFRIMS, Bangkok
- FHI, Bangkok
- Li Ka Shing Foundation
- WHO
- Wellcome Trust
- Bill & Melinda Gates Foundation



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“Get rid of all malaria from Cambodia”  
“Eradicate it. Eliminate it.”  
- **Prof. Nicholas White**, The New York Times



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