

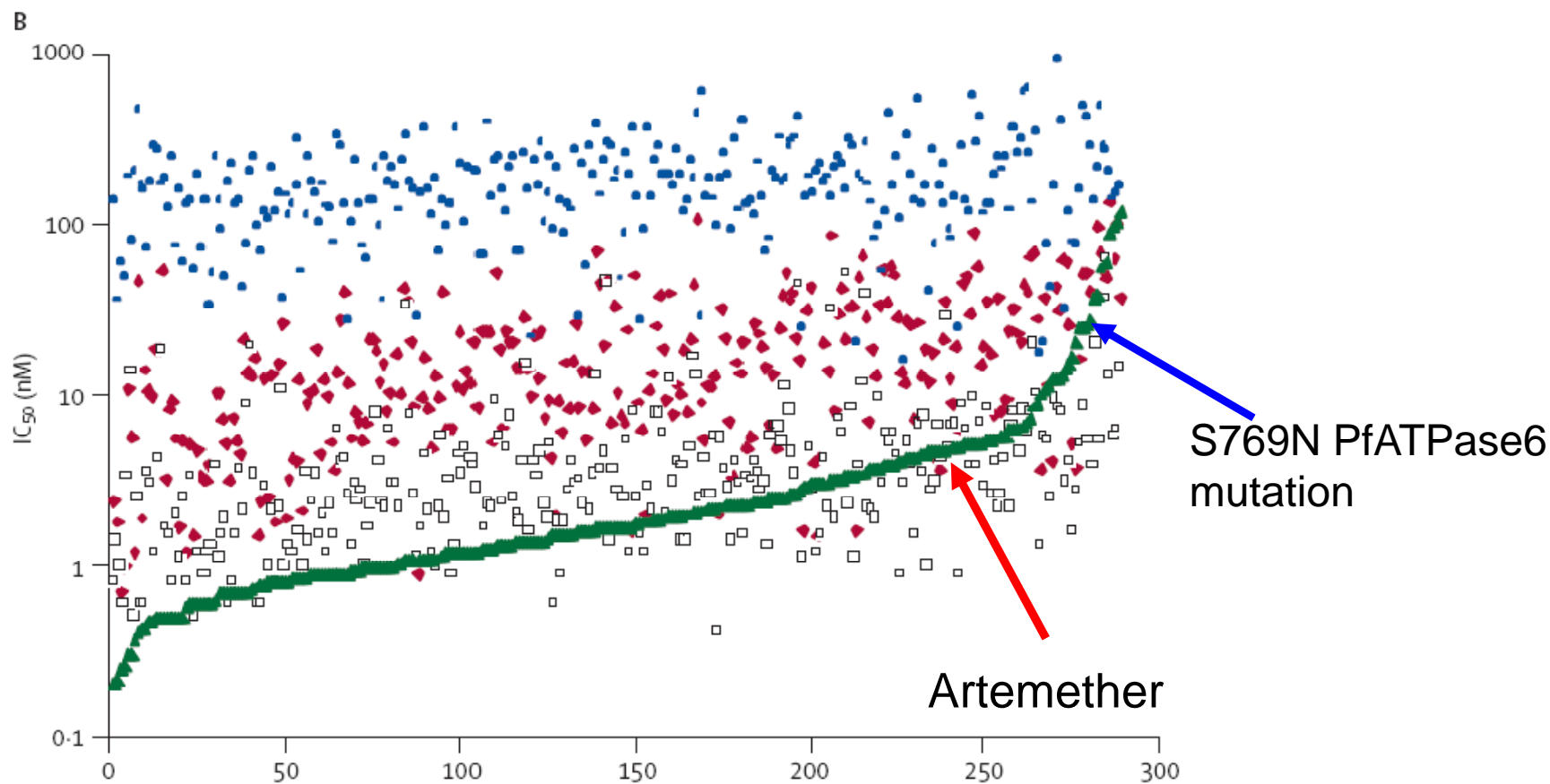
# Is there Artemisinin Resistance in Western Cambodia?



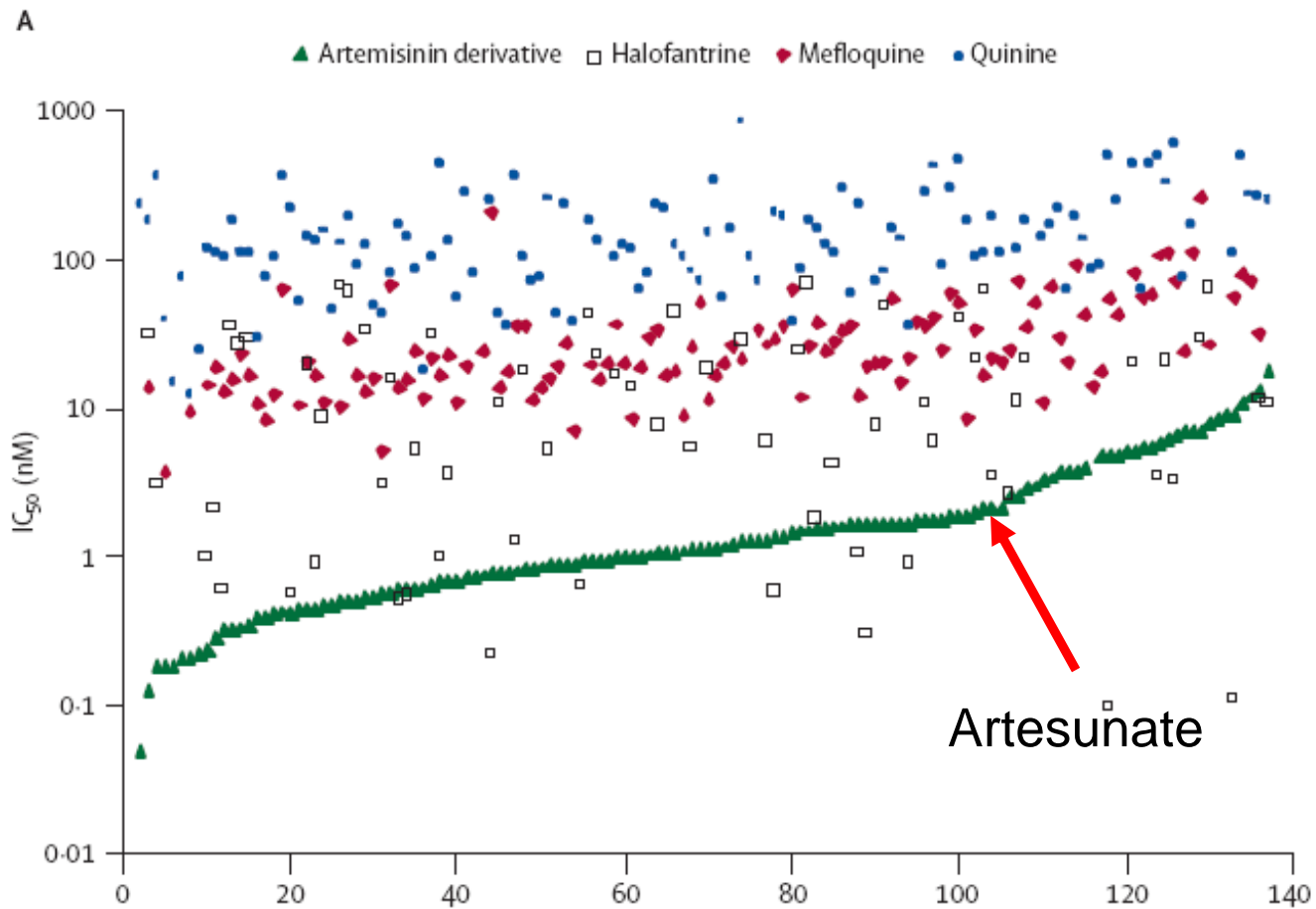
Preliminary results, February 2008



Arjen Dondorp on behalf of the “Task  
Force on Antimalarial Drug Resistance in Cambodia”



French Guiana 2002-2003; hypoxanthine uptake at 48 hours; no clinical data.  
*Jambou et al. Lancet 2005; 366: 1960-63.*



Cambodia 2001; hypoxanthine uptake at 48 hours.  
*Jambou et al. Lancet 2005; 366: 1960-63.*

# Antimalarial drug use in Western Cambodia

- 1955-9: DDT ± weekly mass drug administration
- 1960-61: Distribution of 77 tons of sea salt containing 0.05% pyrimethamine to a population of 20,000
- 1961-62: Distribution of 75 tons of salt containing 0.6% chloroquine
- Now: ACT (MAS3) first line treatment for uncomplicated falciparum malaria. However: substantial artesunate monotherapy use in the private sector



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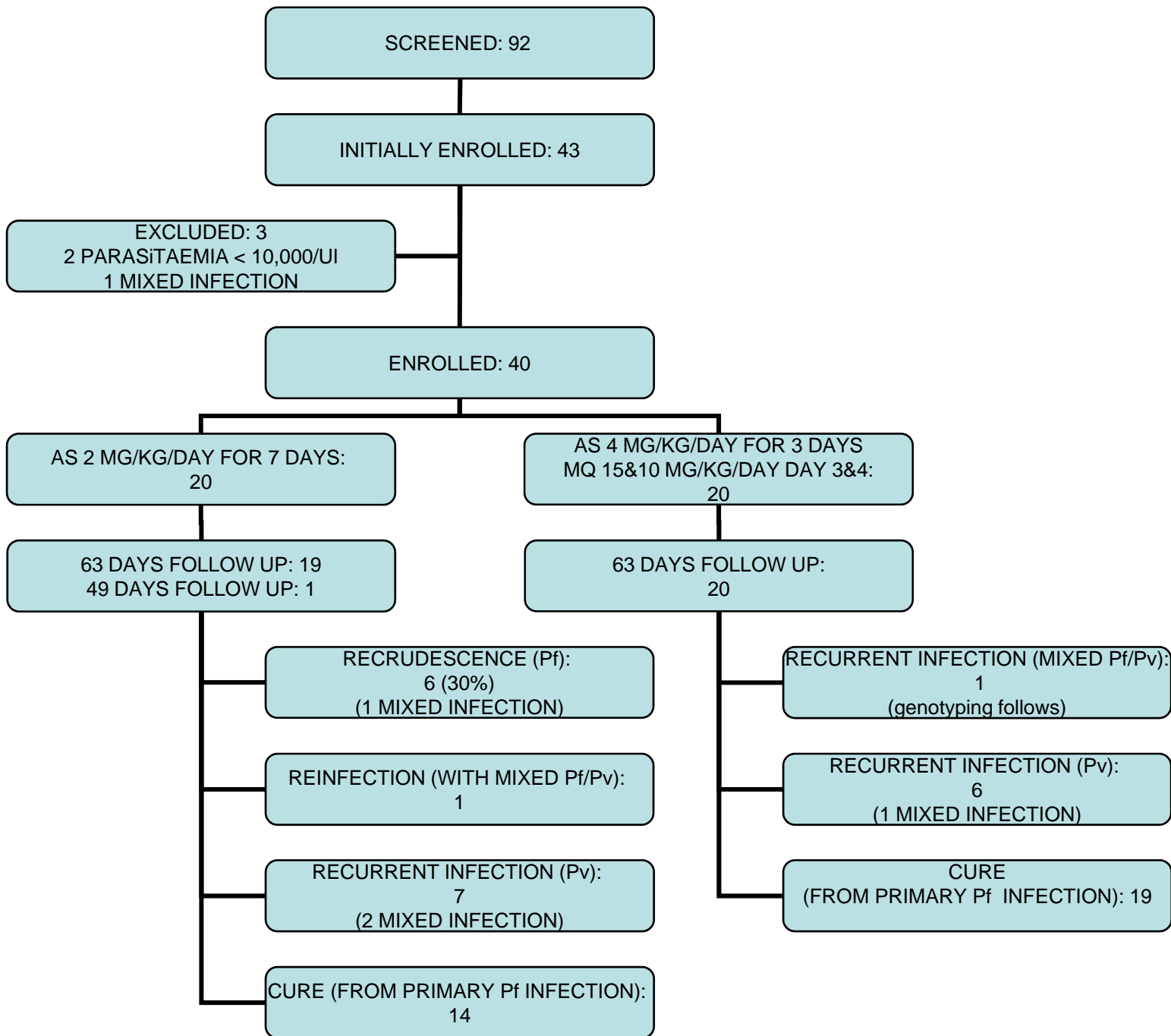
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# ACT efficacy in Western Cambodia

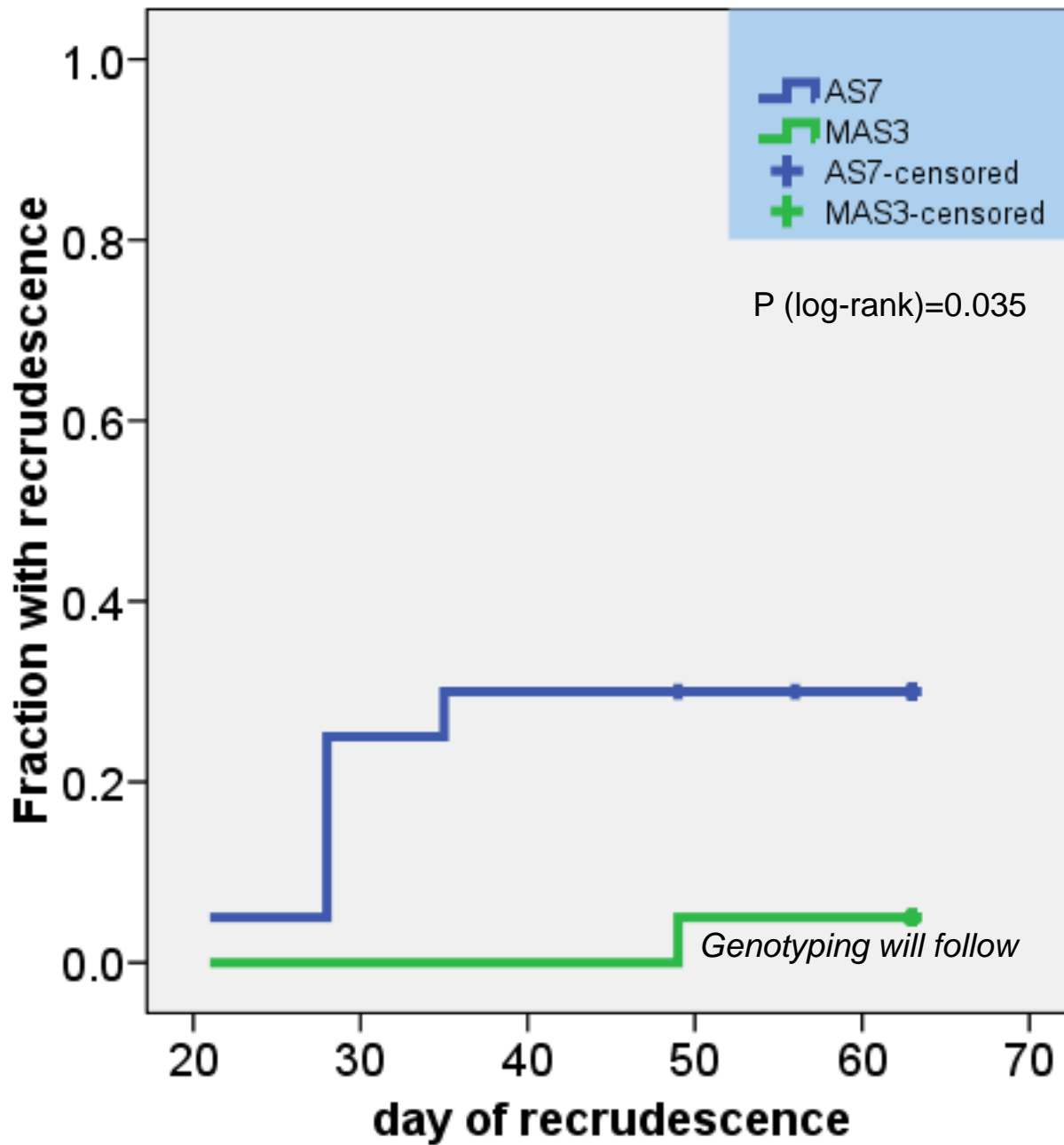
- **Coartem** 28 day cure rate
  - **Batambang** 2002 (no food): **71.1%** (but low day 7 blood levels)
  - **Batambang** 2003 (with food): **86.5%**
- **Artesunate-mefloquine** 28 day cure rate
  - **Batambang** 2001: **96%**
  - **Pailin** 2002: **86%**
  - **Batambang** 2003: **92.4%**
  - **Pailin** 2004: **90%**
  - **Trat** province Thailand (just across the border) with a 2 days regimen: **79%**
- Unconfirmed reports of prolonged parasite clearance times (as PD measure for the artemisinins)

# Study site



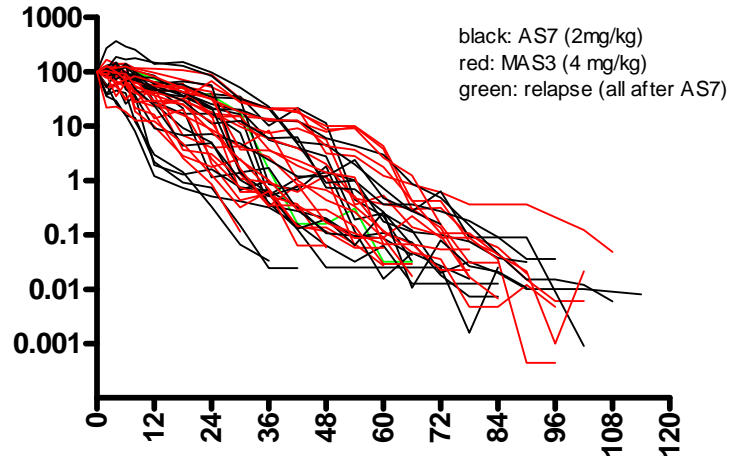




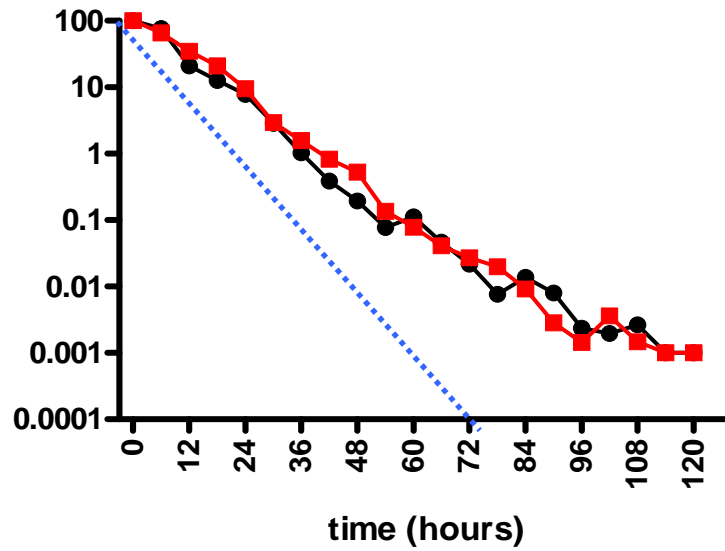


### parasite clearance curves (n=40)

parasitaemia as % from admission  
(individual data)



parasitaemia as % from admission  
(geometric mean)

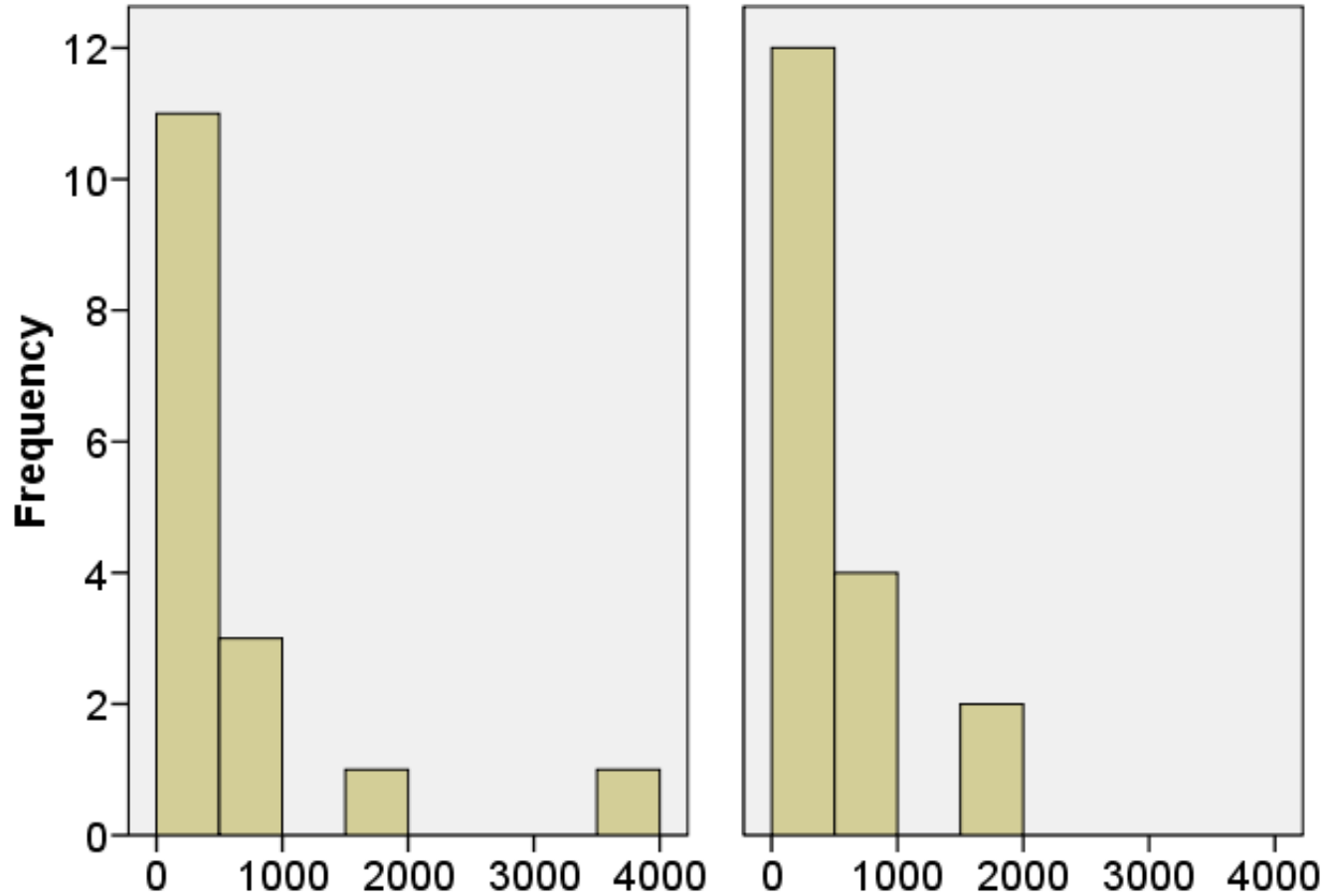


AS 2 mg/kg/d

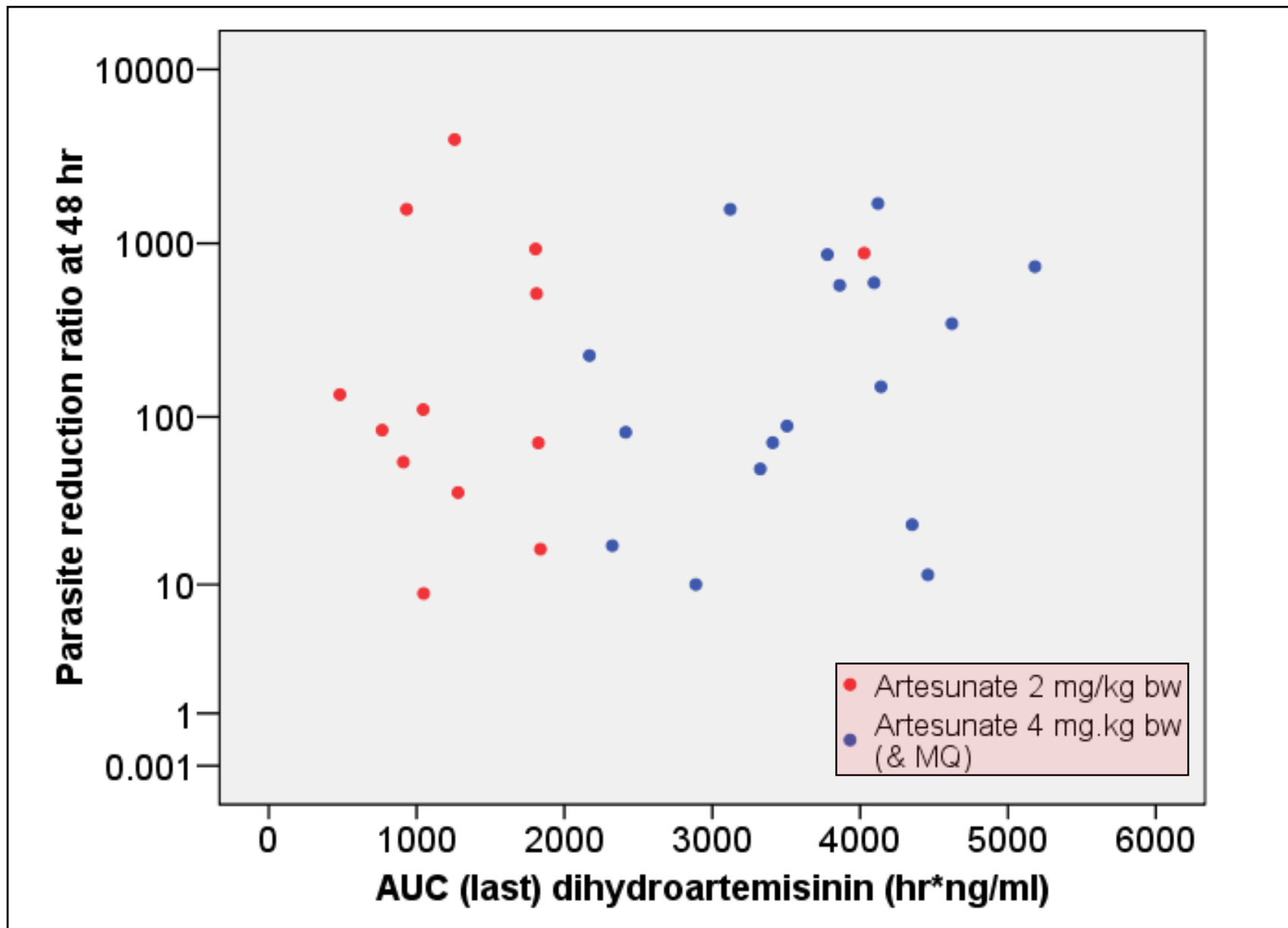
Median (range): 114 (9-3956)

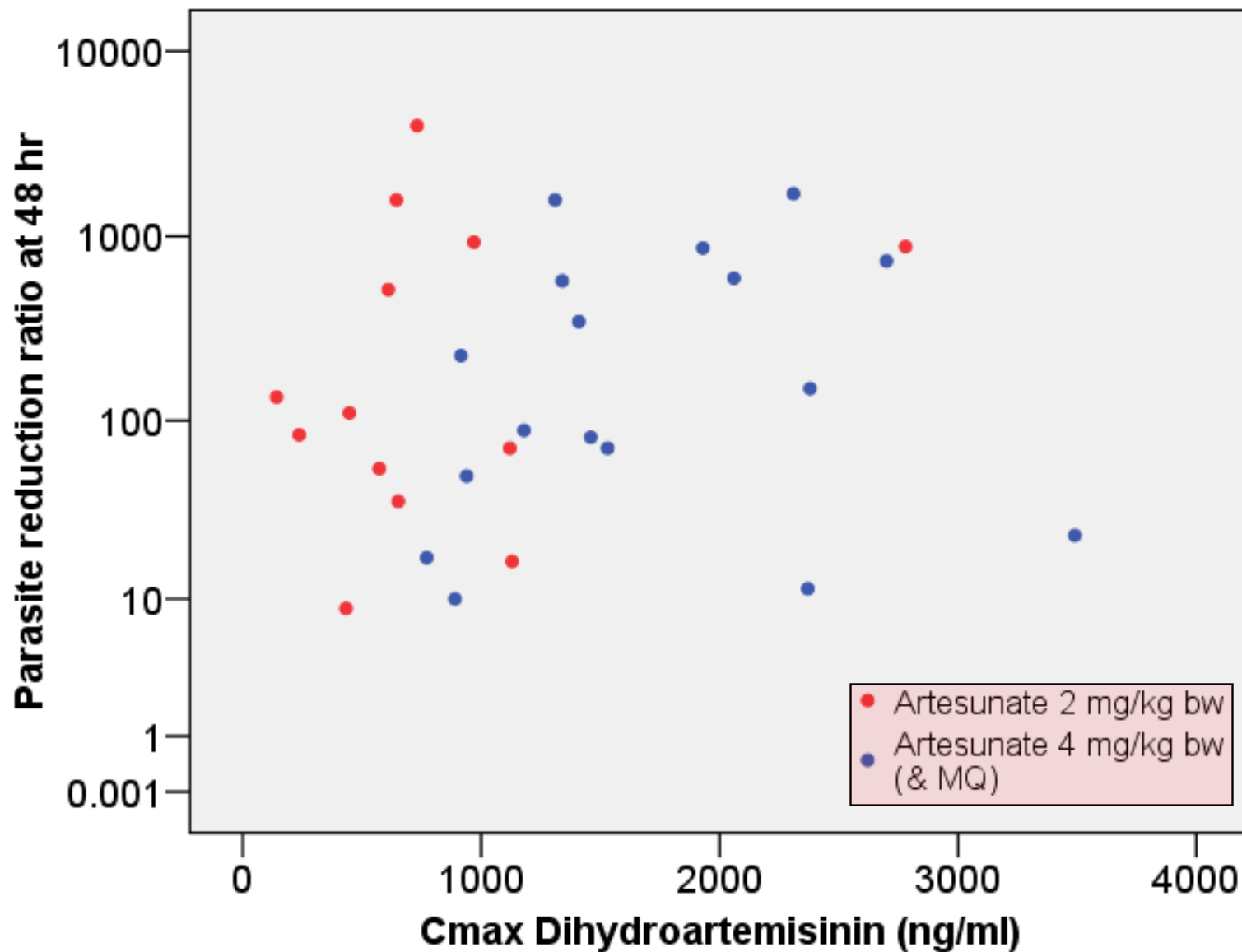
AS 4 mg/kg/d & MQ

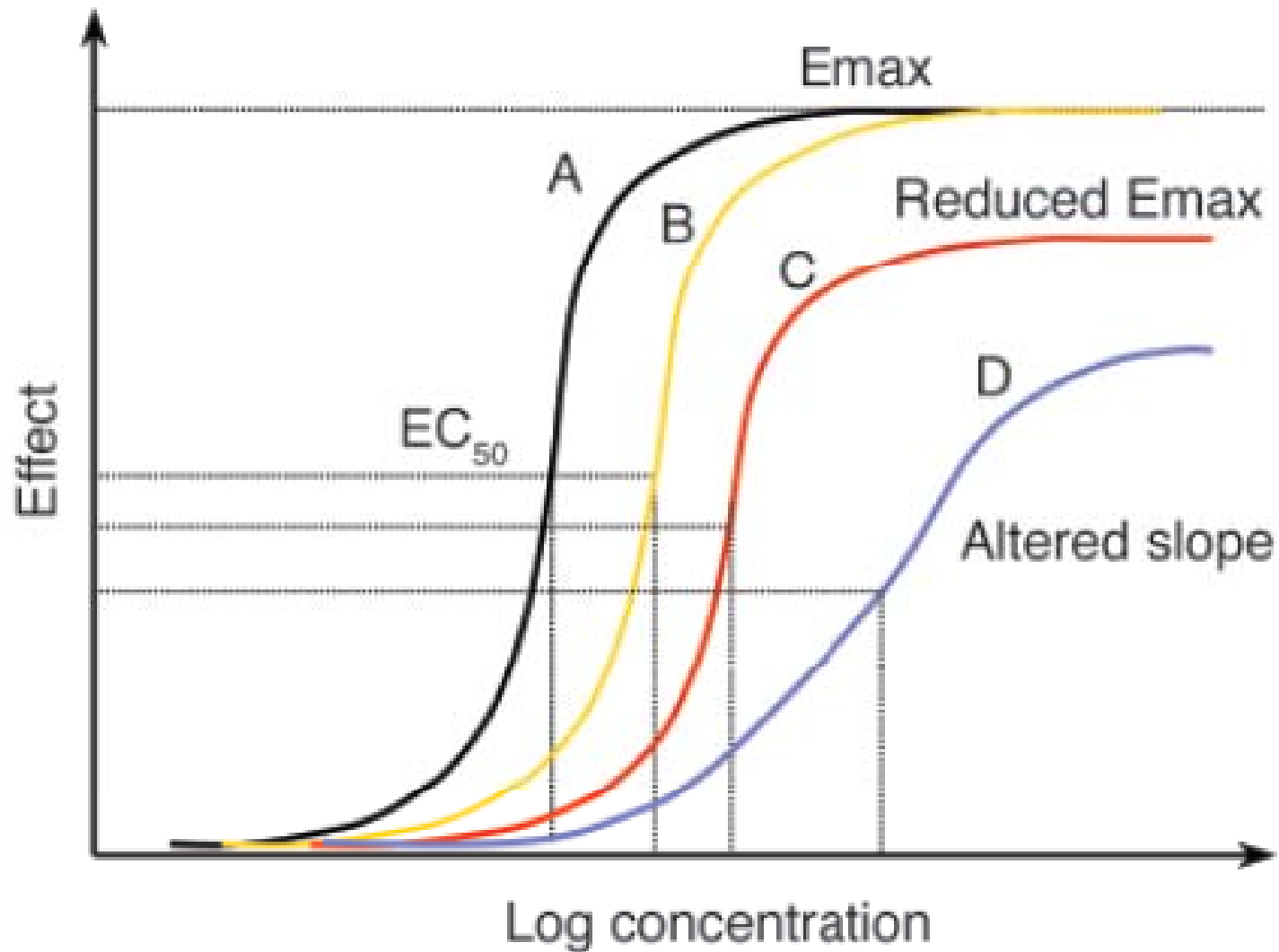
Median (range): 119 (10-1696)



Parasite reduction ratio at 48 hours

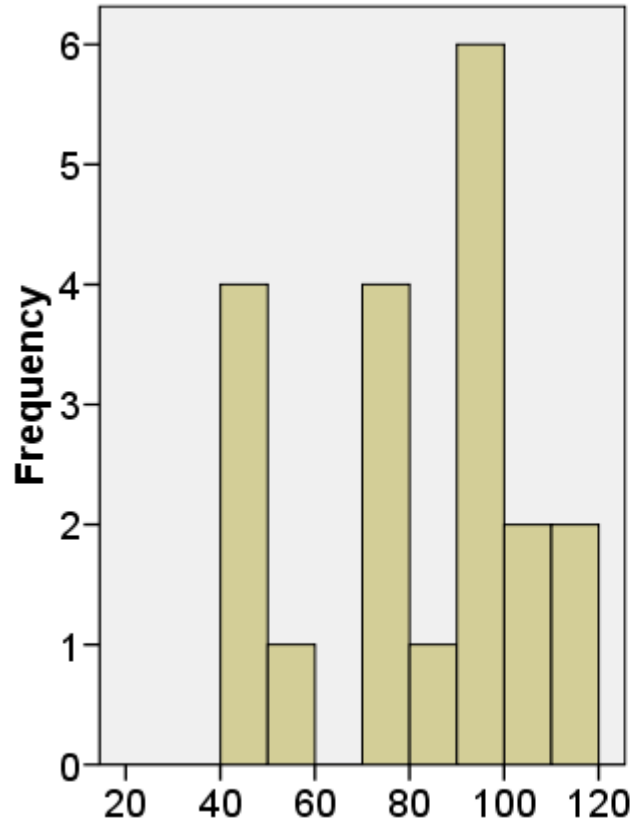






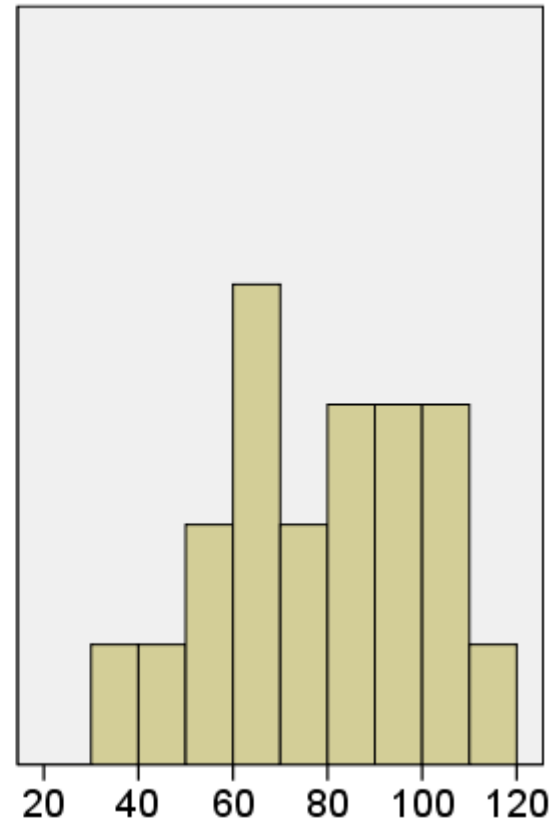
### AS 2 mg/kg/d

Median (range): 87 (42-120)

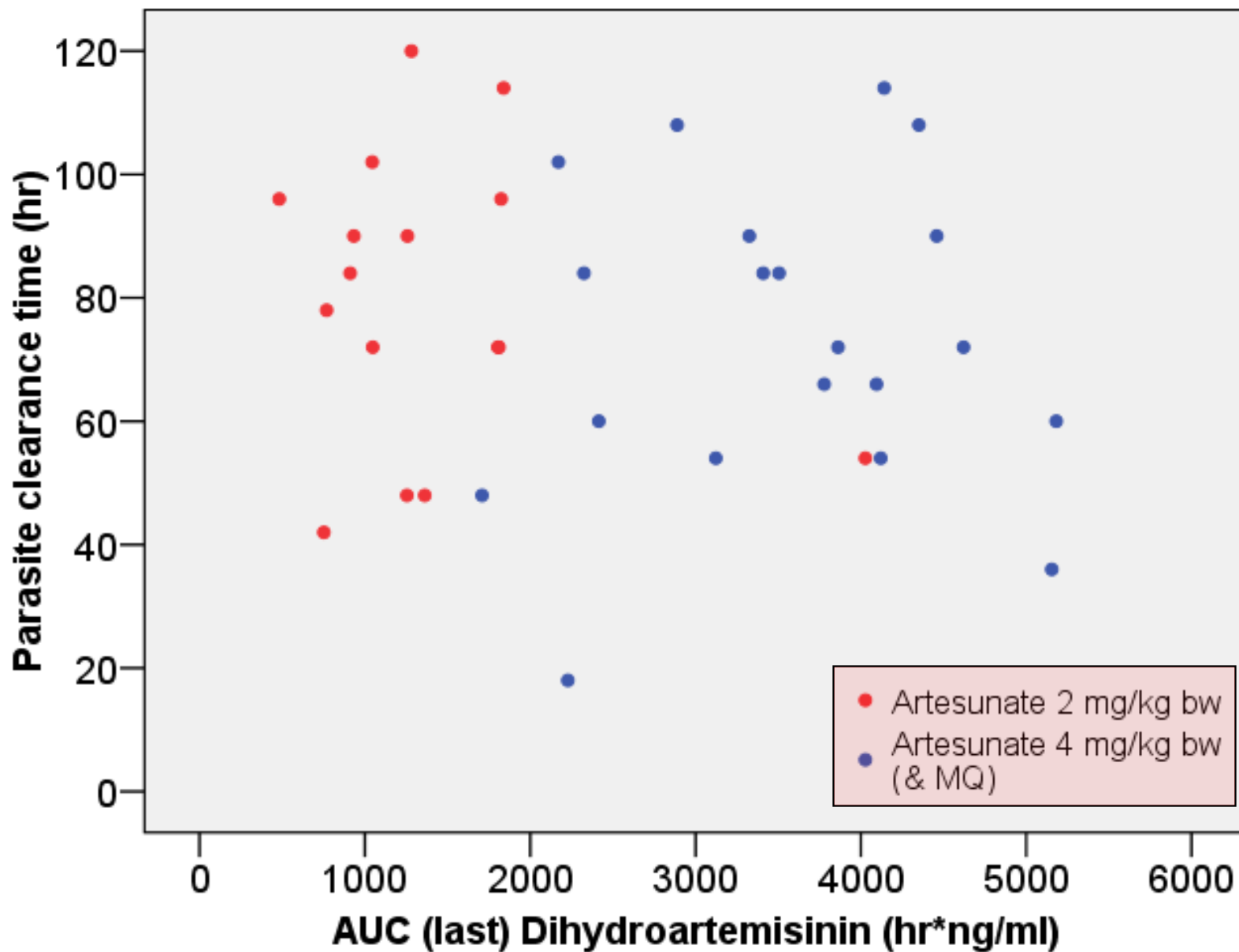


### AS 4 mg/kg/d & MQ

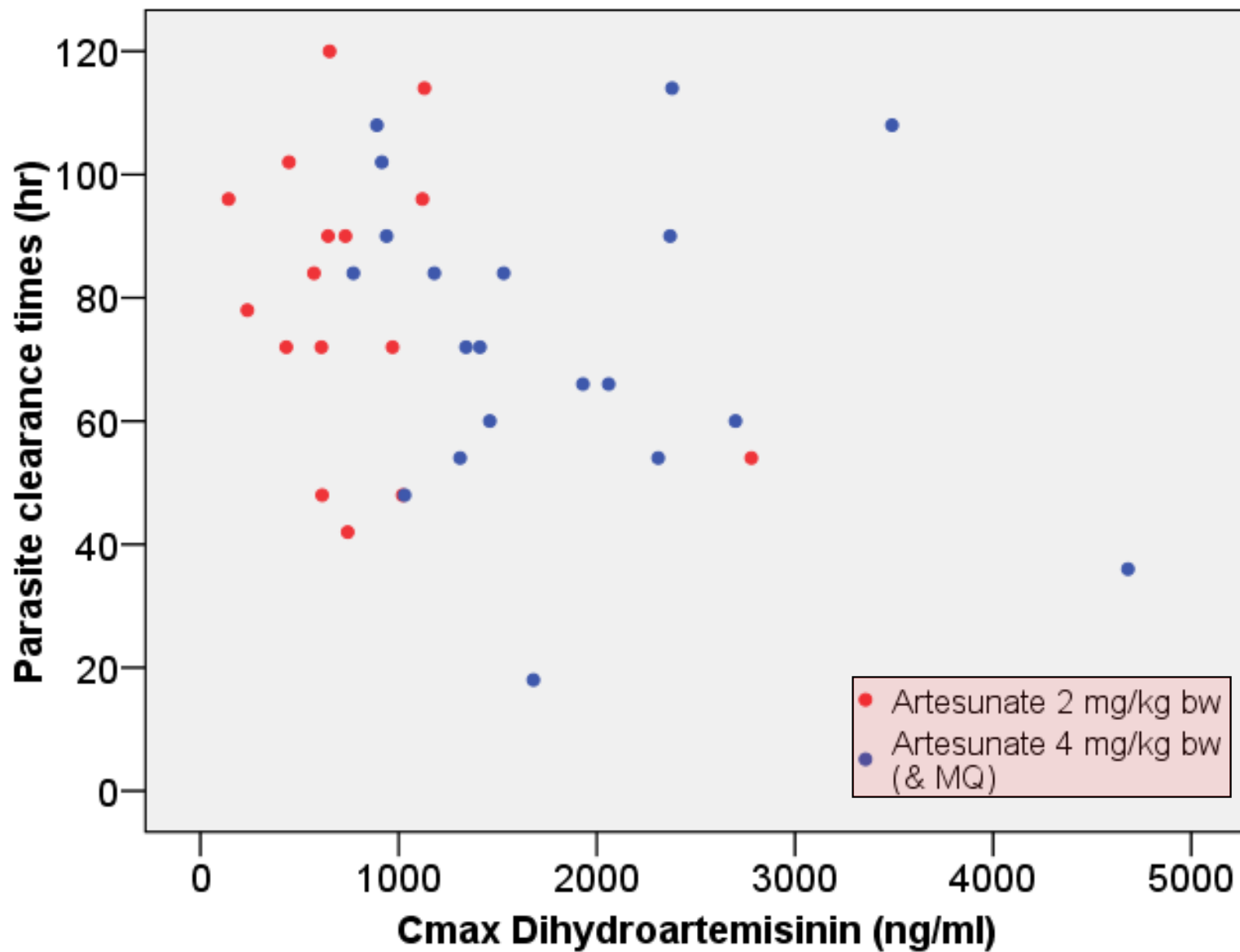
Median (range): 78 (36-114)

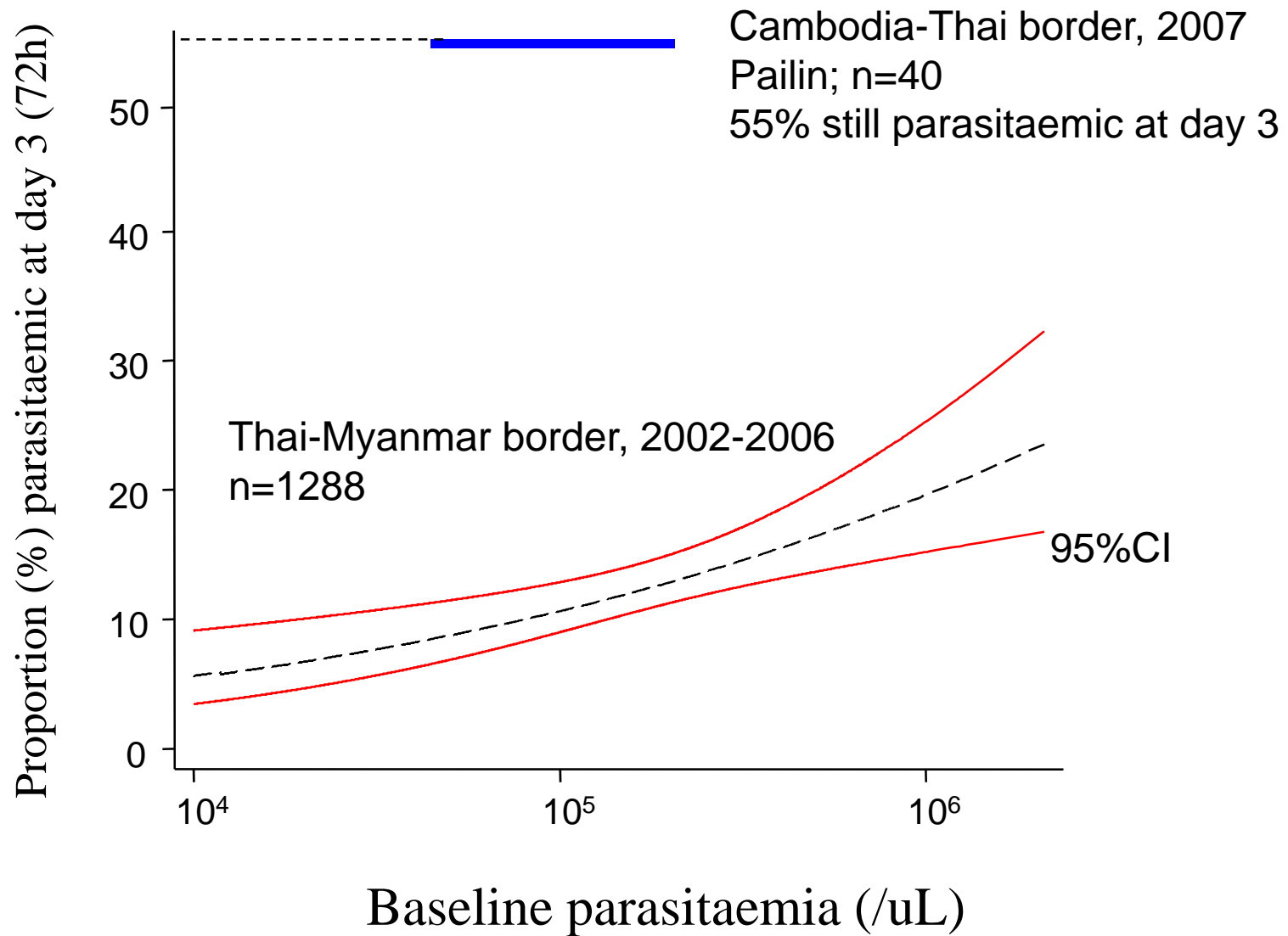


Parasite clearance time (hours)



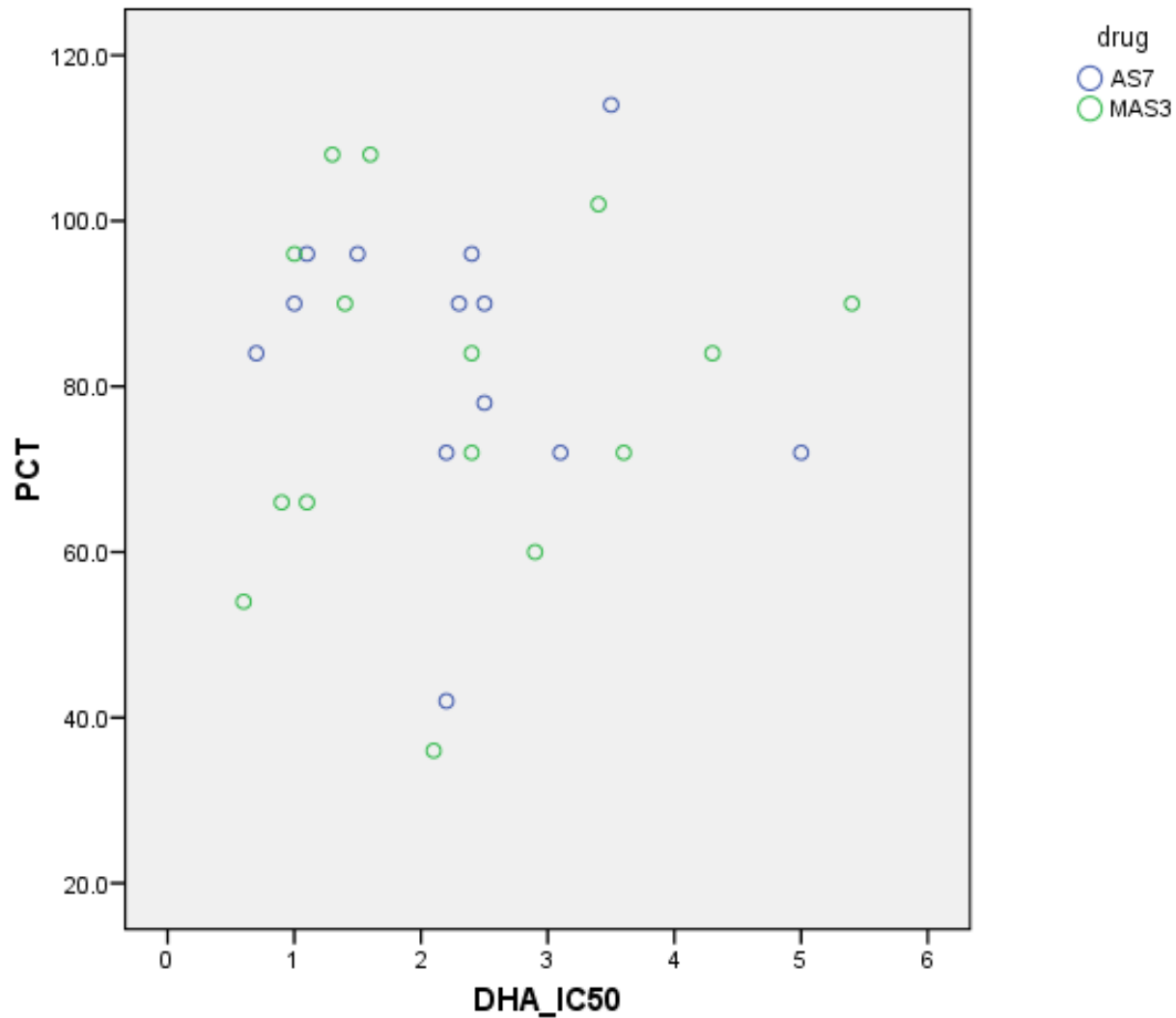











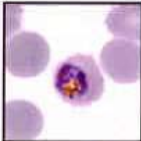
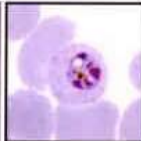
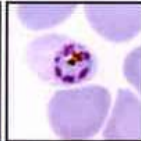
Gametocytaemia during follow up: 8/40 (20%)  
 (high compared to historical SMRU data (3%))

Arm	Patient No	Adm. P count	Gametocyte detection (Hour)	PCT(Hour)
AS7 (n=20)	P002	25/1000	72 until 78	90
	P013	2/1000	36 (once)	42
	P022	9/1000	78 (once)	96
	P035	4/1000	0 until 120	48
MAS3 (n=20)	P015	7/1000	30 until 336	66
	P027	24/1000	72 until 336	102
	P029	9/1000	24 until 144	60
	P034	3/1000	8 (once)	36



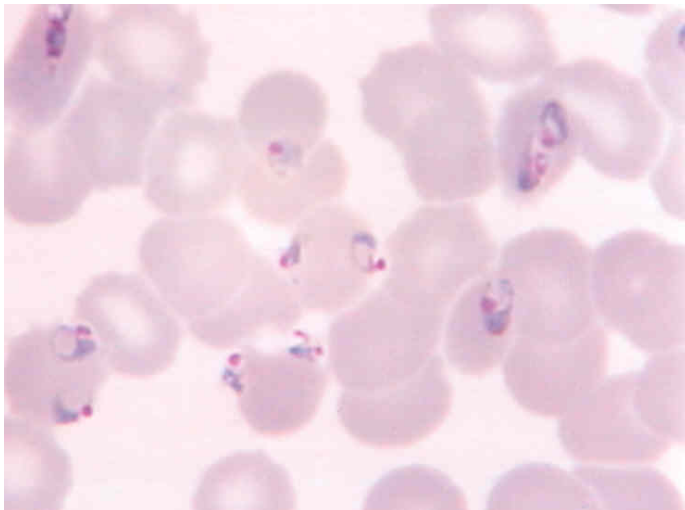
No correlation PCT and  $IC_{50}$   
(hypoxanthine uptake after 24 h)

*Frederique Arieu, preliminary data*

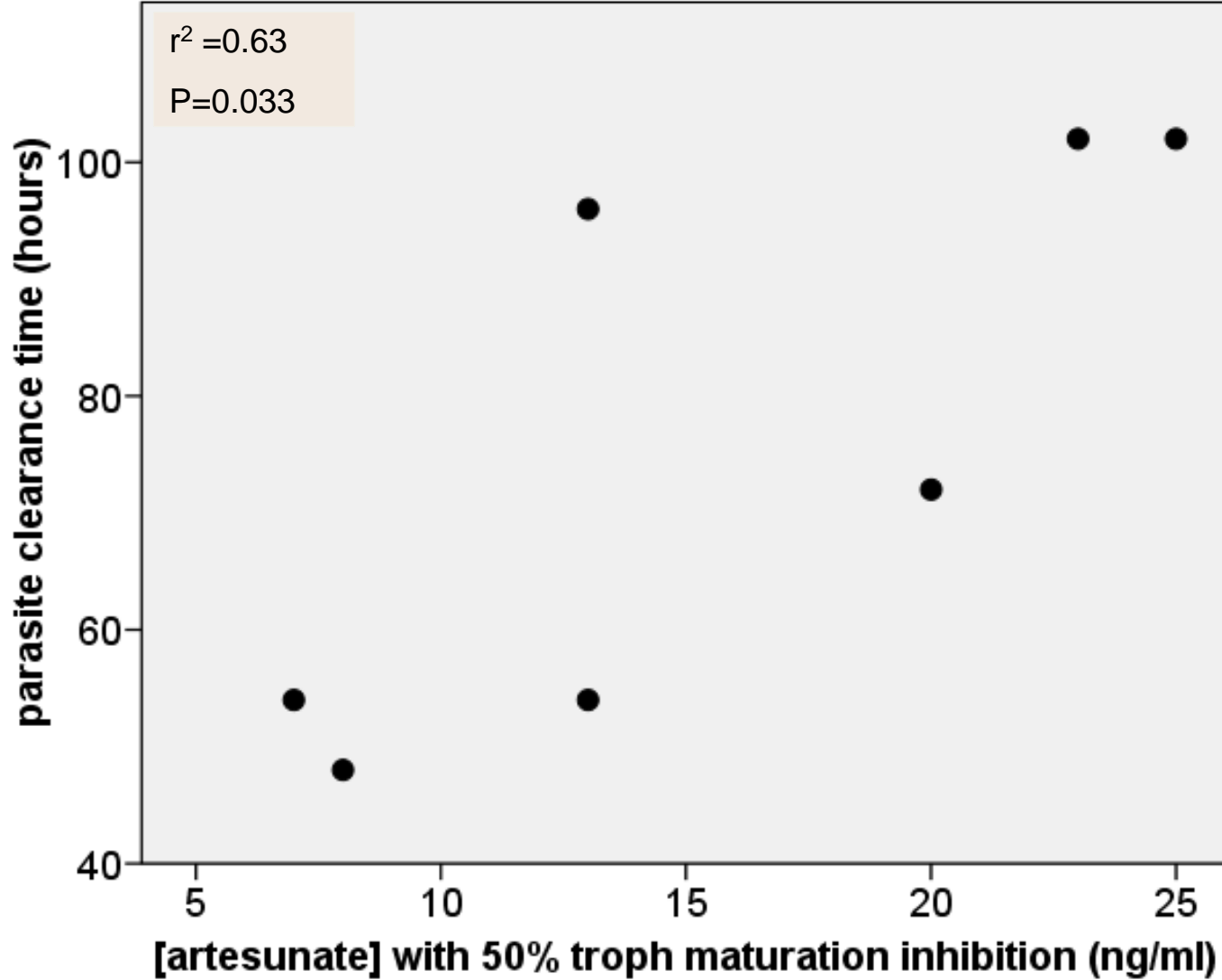
0-6 H	6-16 H	16-26 H	26-30 H	30-34 H	34-38 H	38-44 H	44-48 H
							
<b>TINY RINGS</b>	<b>SMALL RINGS</b>	<b>LARGE RINGS</b>	<b>EARLY TROPH.</b>	<b>MID TROPH.</b>	<b>LATE TROPH.</b>	<b>SCHIZONTS</b>	<b>SCHIZONTS</b>
width of cytoplasm < 1/2 nucleus	width of cytoplasm ≥ 1/2 nucleus	width of cytoplasm ≥ nucleus	light brown pigment appears as faint pale area or visible dots	brown pigment, dark cytoplasm nucleus and cytoplasm enlarge	dark brown pigment, irregular shaped nucleus ≤ 2	dark brown pigment, ≤ 5 nuclei	dark brown pigment, > 5 nuclei

*P.falciparum* staging ( *in vitro* culture )

Wellcome unit, Bangkok 2000



- ‘Trophozoite inhibition assay’
  - Parasitemia/ 1000 red cells and stage assessed
  - Parasite development assessed in 200 asexual parasites
  - % trophozoites compared to control well without drug.



*Kesinee Chotivanich, preliminary data*

# Conclusions

- Prolonged parasite clearance times confirmed, with unacceptably high failure rate in AS7 arm
- Not explained by inadequate drug levels; variability in C<sub>max</sub> and AUC is considerable
- No dose (or concentration)-response relationship within current range of [AS]
- Increased dose/ split dose?
- No/ just 1 failure in MAS3 arm suggests continued susceptibility to mefloquine
- *In vitro* test under evaluation
- Molecular marker?



# Acknowledgements

- Staff Pailin Referral Hospital
- Mallika Imwong, Kesinee Chotivanich, Joel Tarning, Frederique Arieu, Shunmay Yeung, Nick Day, Nick White
- Staff of Mahidol - Oxford Tropical Medicine Research Unit
- National Malaria Control Programme, Cambodia (Dr. Duong Socheat)
- Institut Pasteur, Pnomh Penh
- AFRIMS, Bangkok
- FHI, Bangkok
- Li Ka Shing Foundation
- WHO
- Wellcome Trust







# Pailin, SW Cambodia



# Pailin clinical trial

- Inclusion:
  - Uncomplicated *P. falciparum* infection
  - Parasitaemia > 10,000/ uL
  - No mixed infection on light microscopy
    - But 11/40 (28%) mixed Pf/Pv by PCR
  - No pregnancy
  - No previous antimalarial treatment (48H)

# Randomisation

- Artesunate 2 mg/kg bw per day for 7 days  
(AS7)

OR

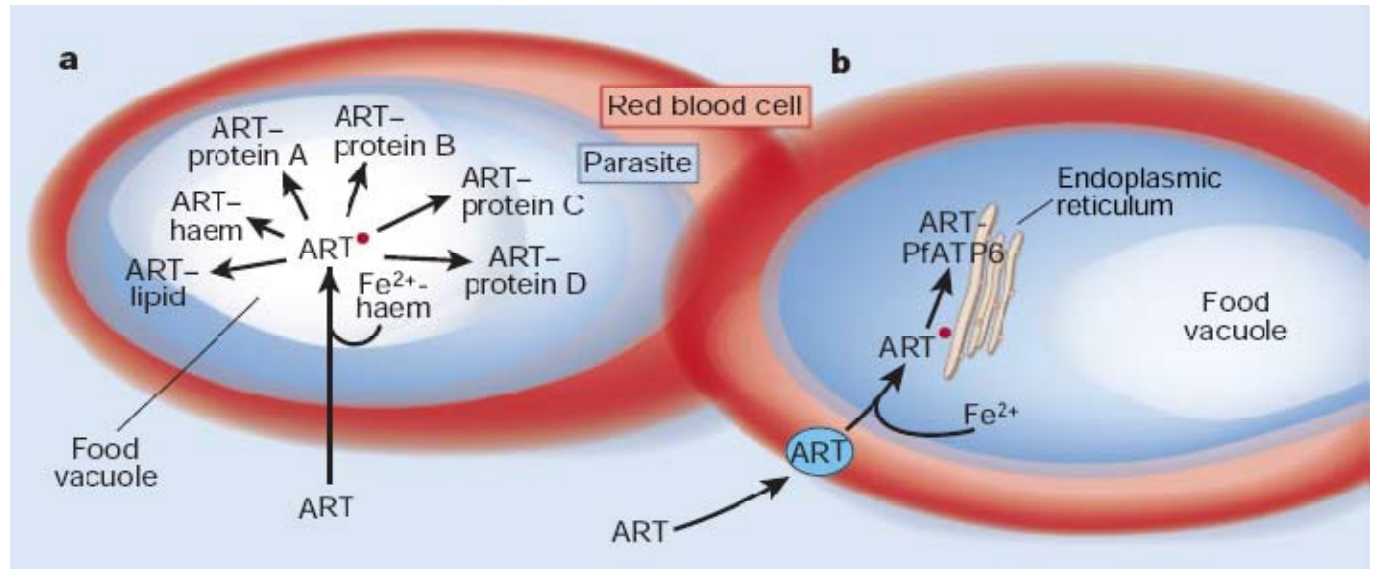
- Artesunate 4 mg/kg bw per day for 3 days  
& mefloquine 15 and 10 mg/kg bw per day  
on day 3 and 4  
(MAS3)

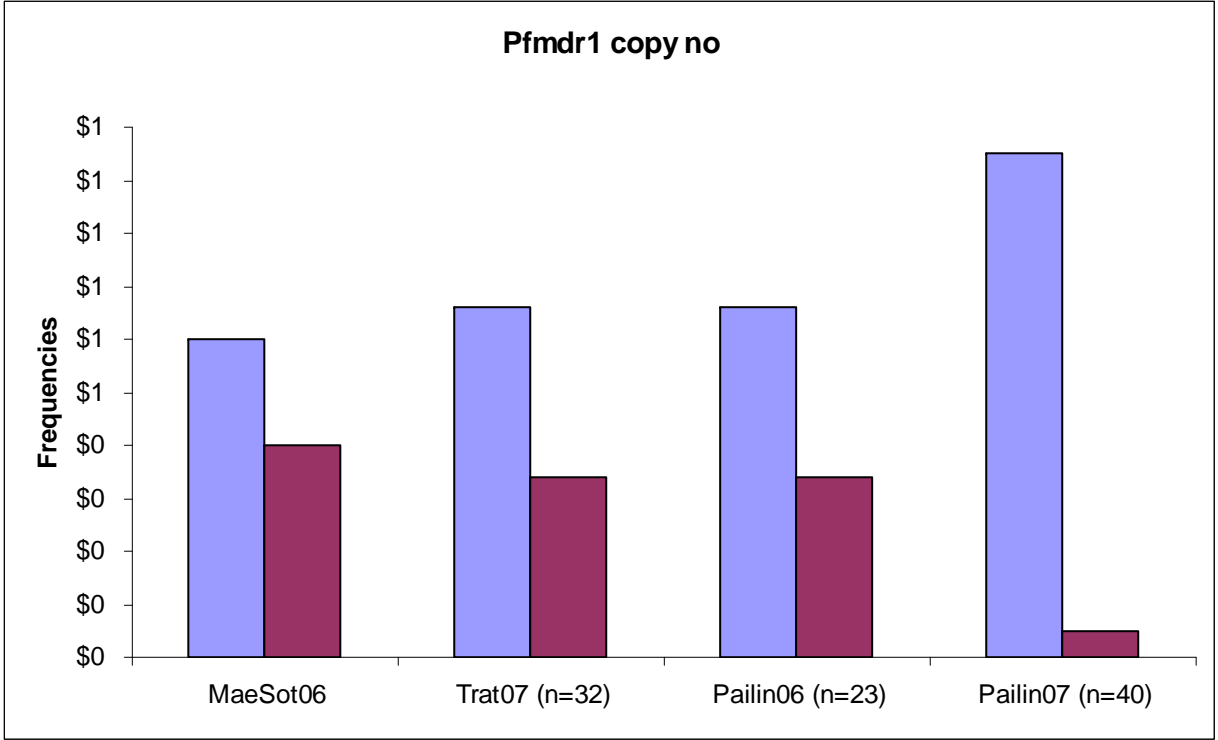
# Outcome measures

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

# Molecular marker for artemisinin resistance?

- *pfmdr1* mutation and copy no.
- *pfserca*
- *pf mitochondrial DNA*

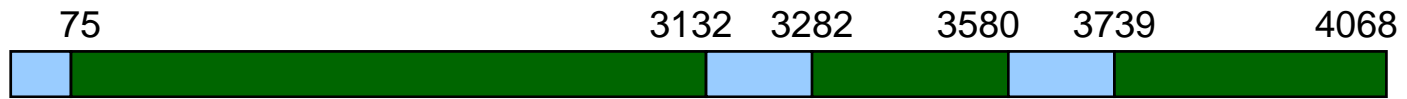








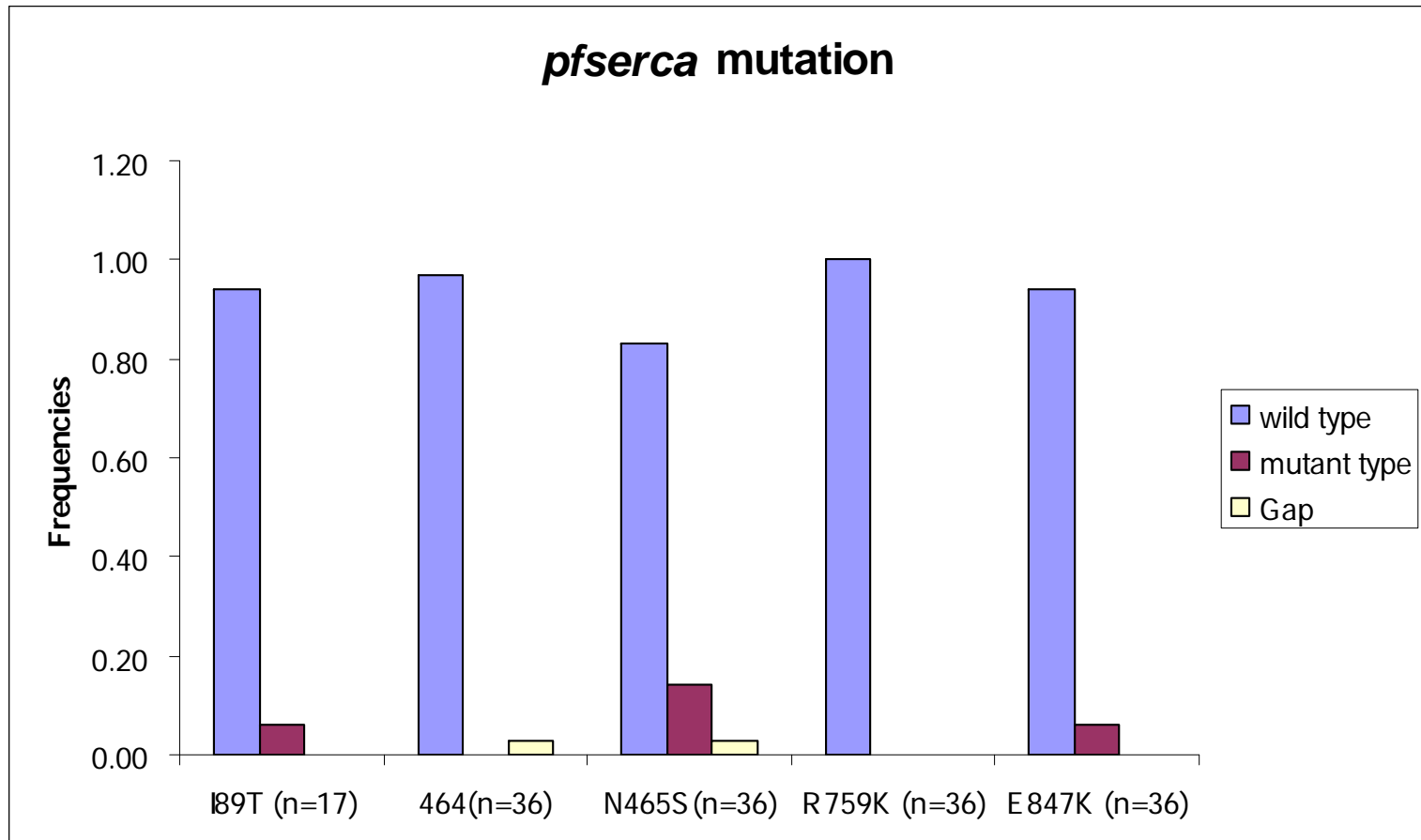
Single copy no

Multiple copy no

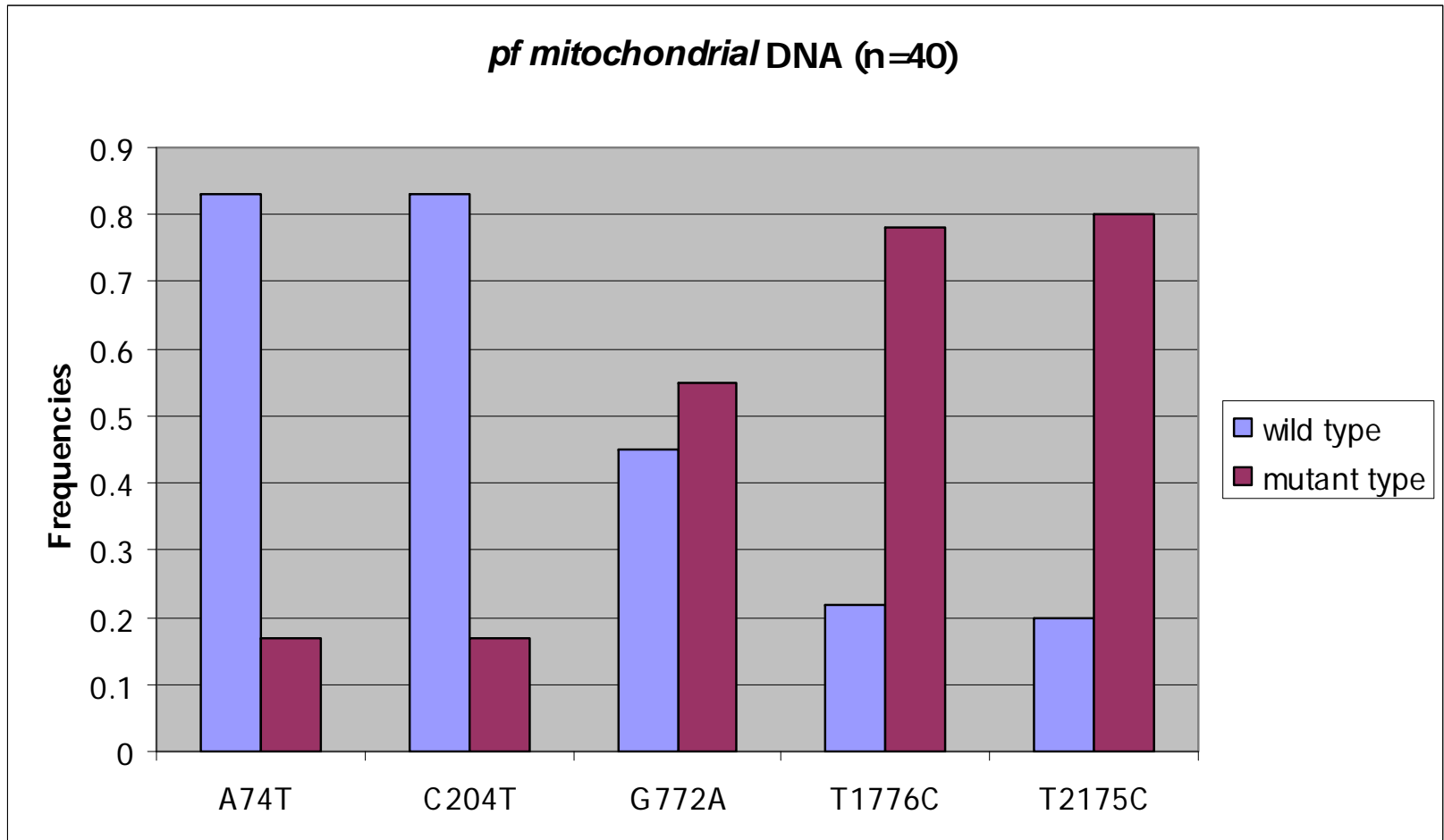
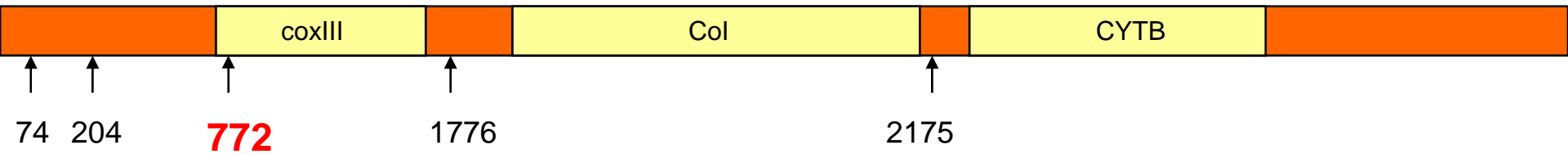
# *Pfserca* SNP: full length sequence



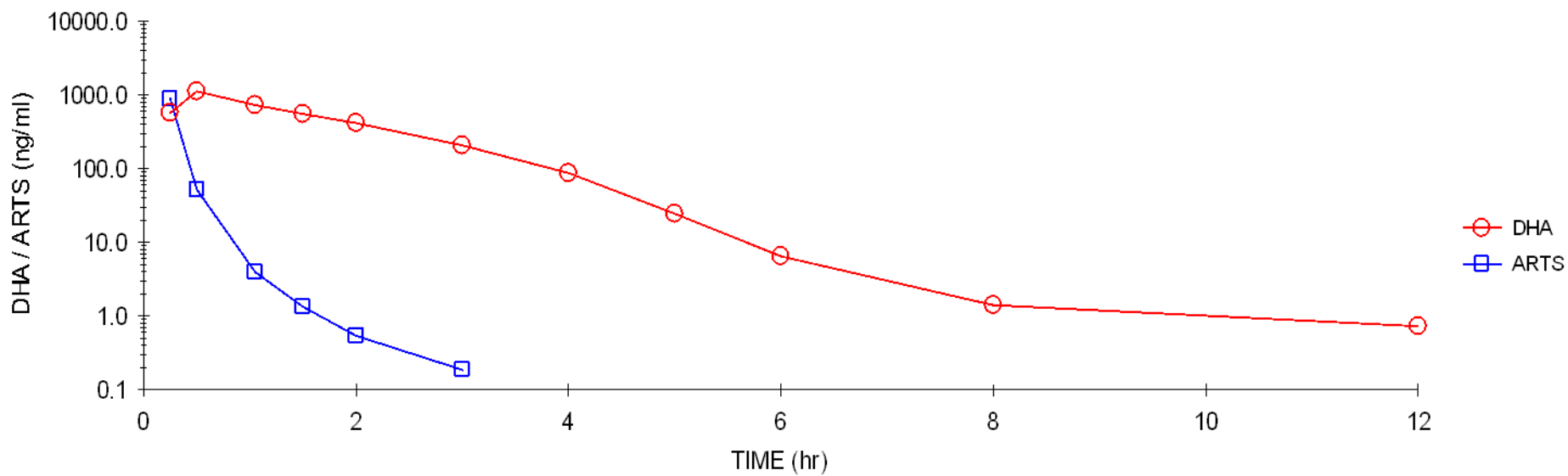
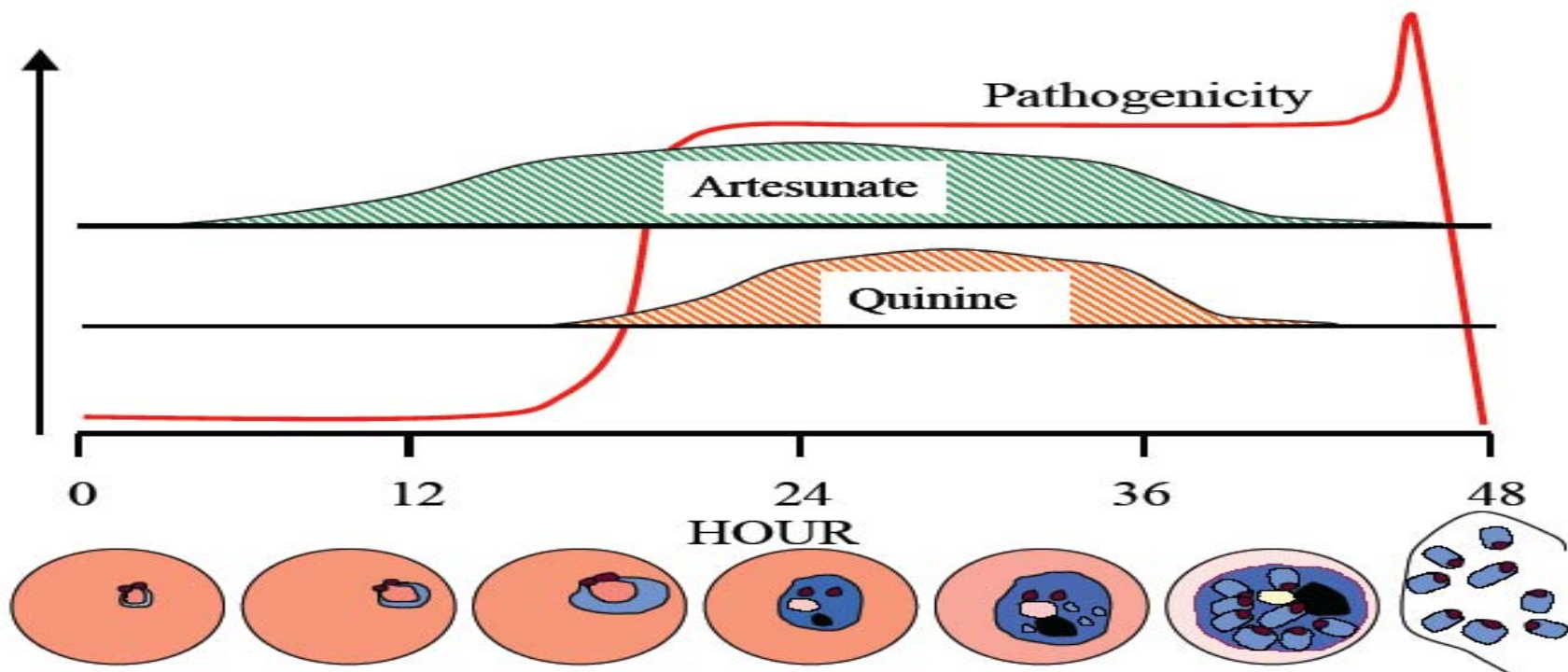
SNP  89  464  759  847  
465



# *pf* mitochondrial DNA

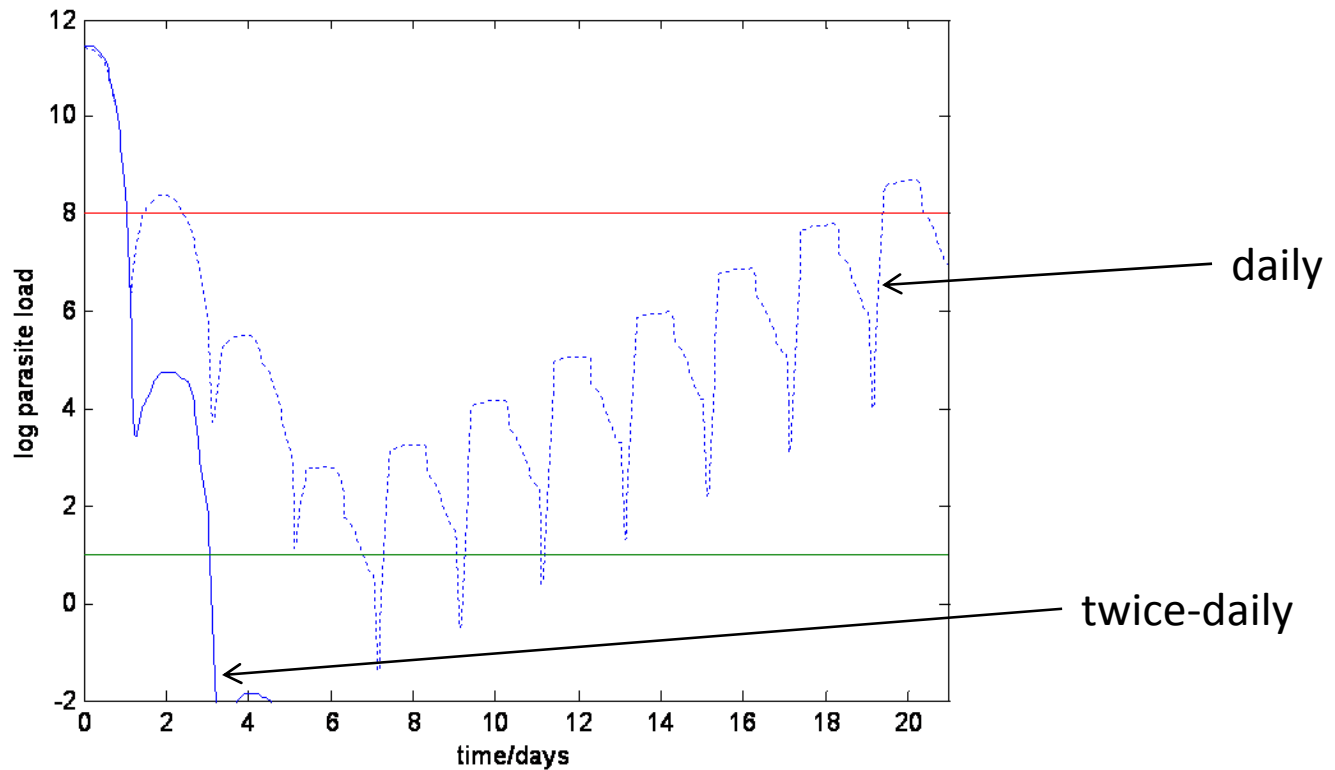




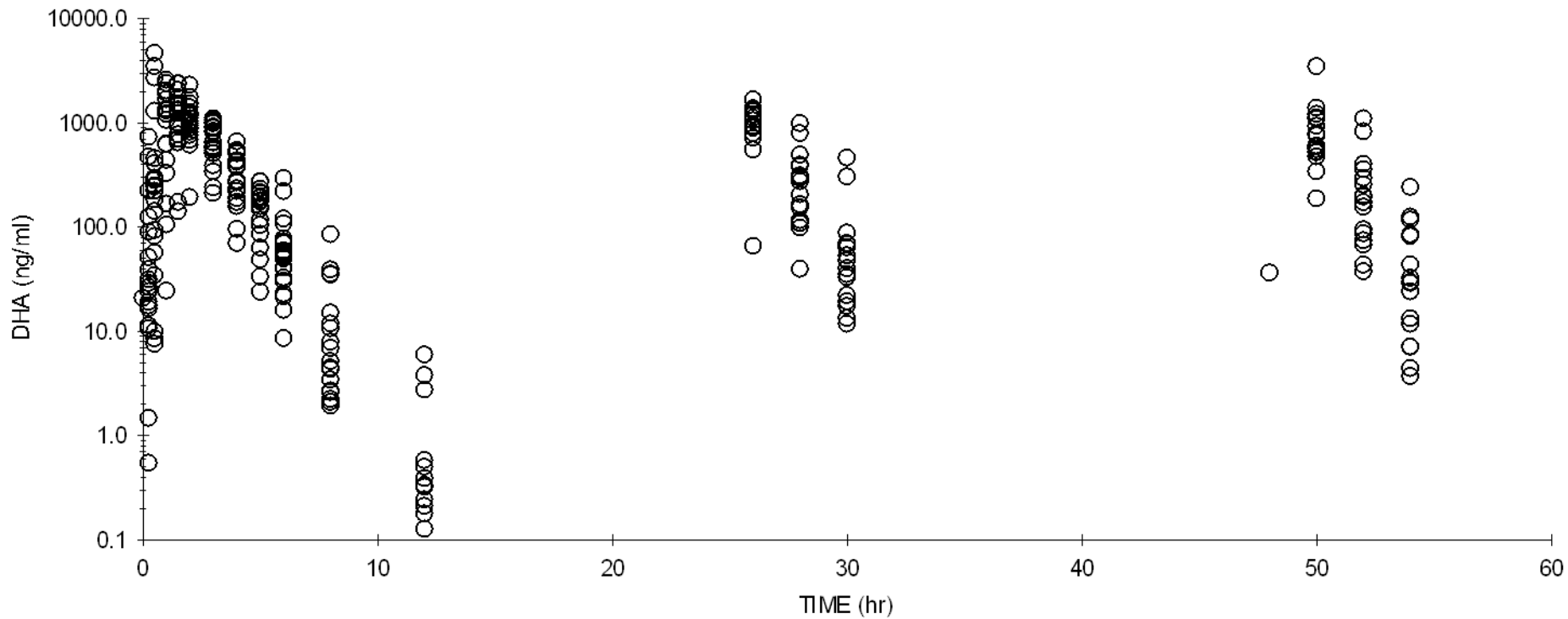


# Dosing regime

## Every 12 hours for a week

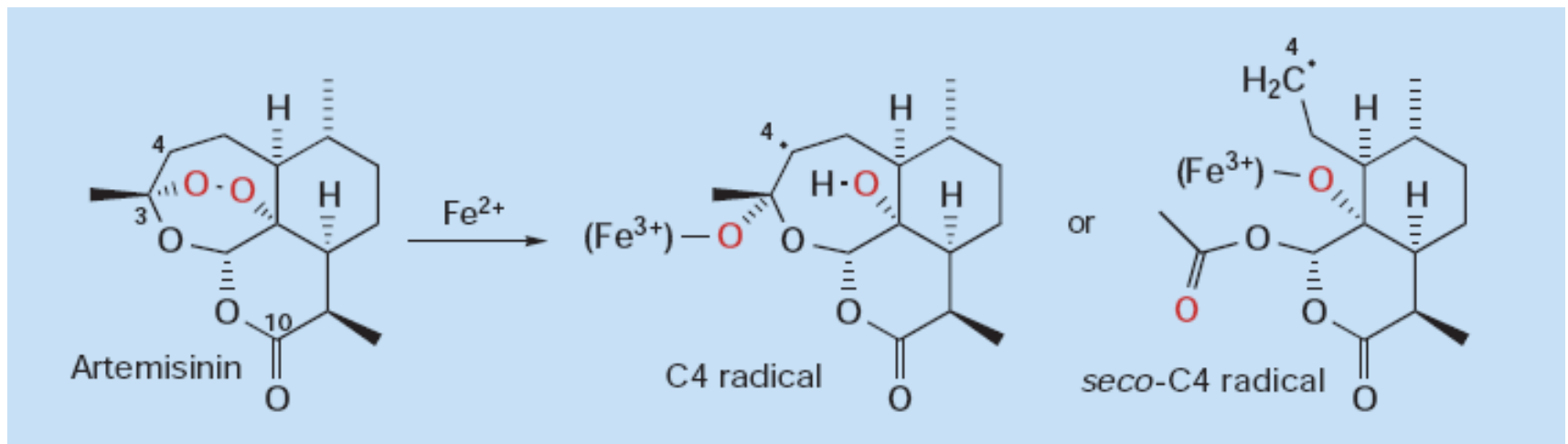
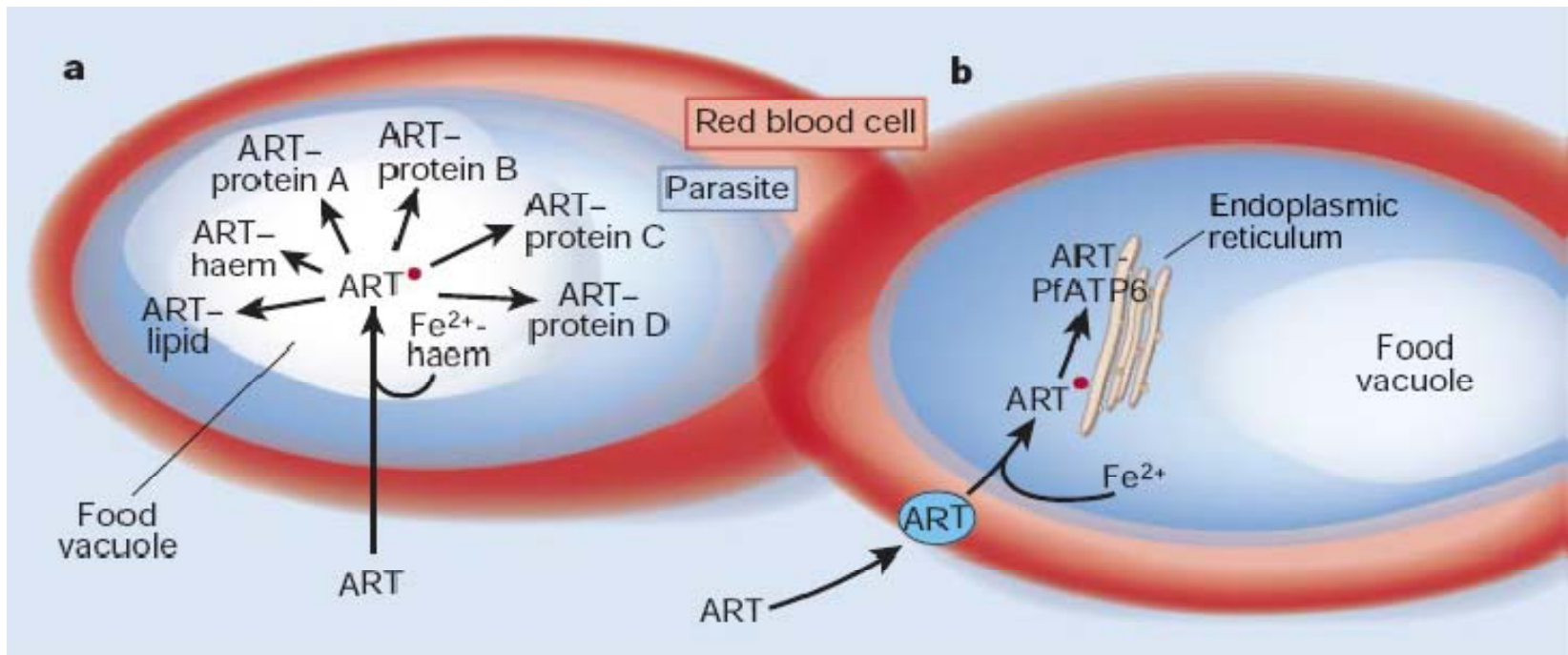


GROUP=MAS3

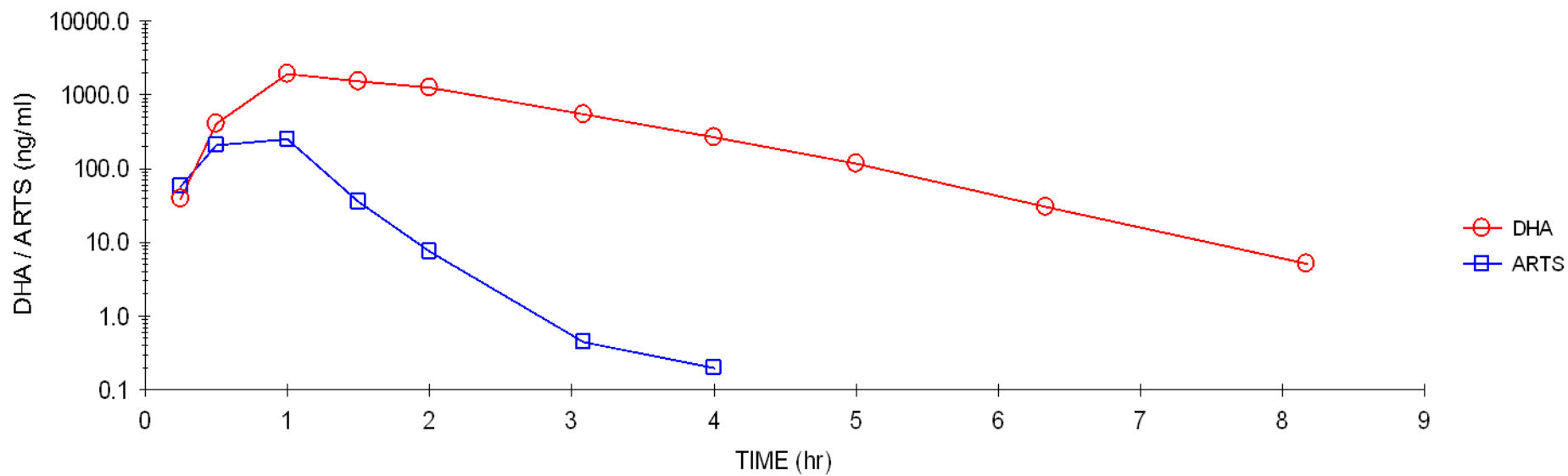


# Molecular mechanisms of resistance

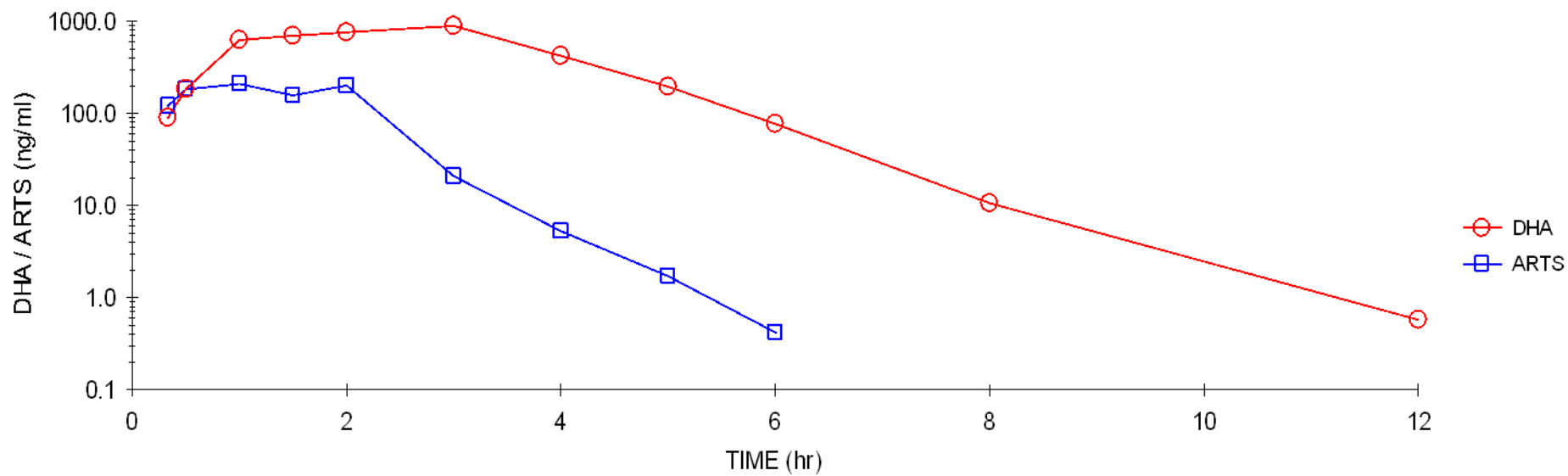
Drug	Low to medium level resistance	High-level resistance
Chloroquine	CRT; 76	CRT; 76 & others MDR; 86, + others
Mefloquine, halofantrine, lumefantrine	MDR; amplification of wild-type gene	
Pyrimethamine	DHFR; 108 then 51 and 59	DHFR; 108 + 51 + 59 + 164
Cycloguanil, chlorcycloguanil	DHFR; 16 + 108	DHFR; 108 + 51 + 59 + 164
Sulfonamides and sulfones	DHPS; 436, 437, 540, 581, 614	
Atovaquone	None reported	Cytochrome <i>b</i> ; 133 ± 280



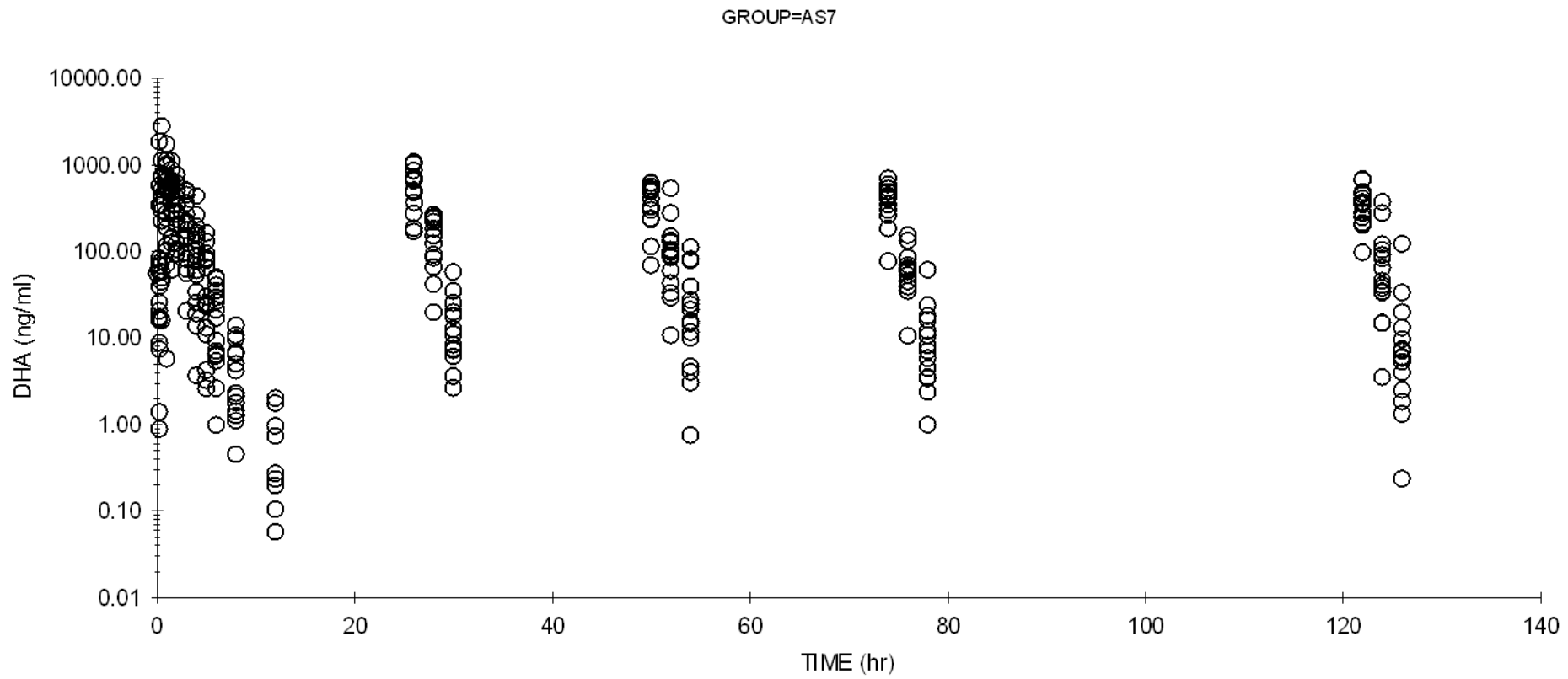
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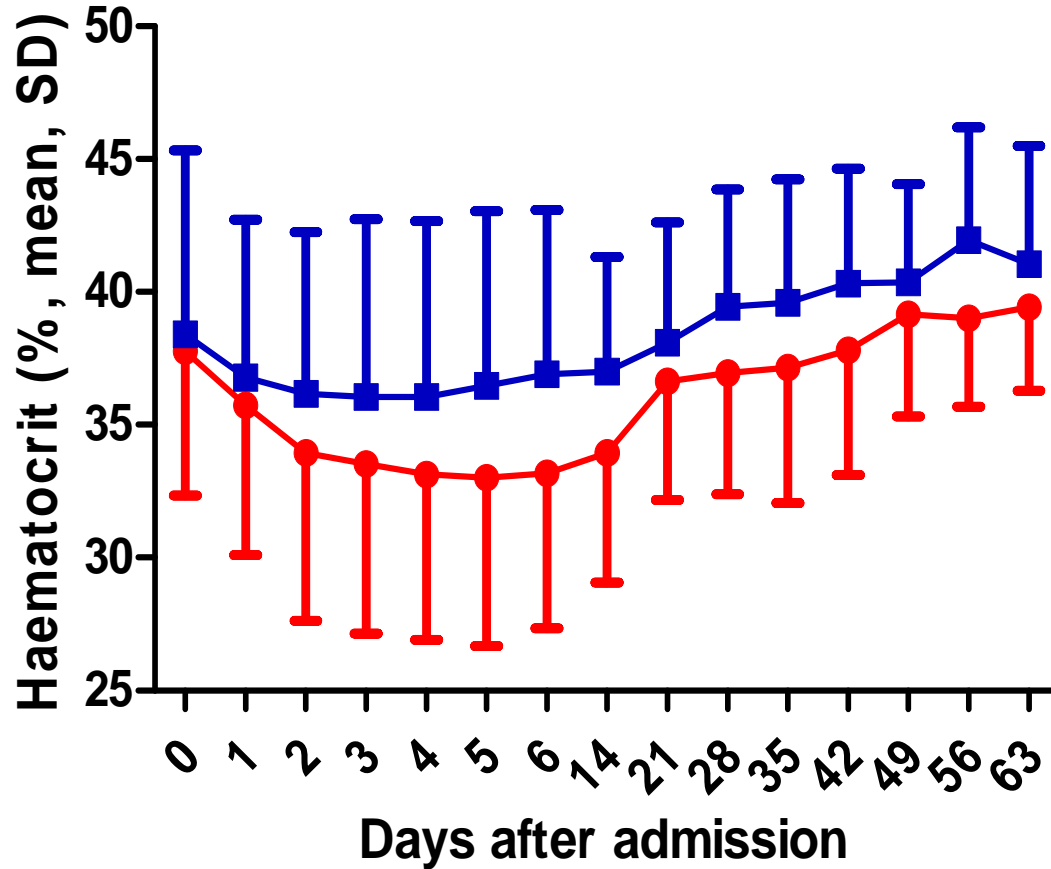


ID=7, GROUP=MAS3



# DHA





—●— artesunate 2 mg/kg bs (for 7 days)

—■— artesunate 4 mg/kg bw (for 3 days) & mefloquine on day 4 and 5



# Trophozoite inhibition IC<sub>50</sub> (preliminary data)

<i>P. falciparum</i>	IC <sub>50</sub> (ng/mL) Mean (95% CI)
Lab strain (TM267)	0.7 (0.5-0.9)
Isolates from	
<b>Pailin, Cambodia</b> (N=9)	13 (7-19)
<b>Mae Sot, Thailand</b> (N=3)	3.9 (0-16)

**AS7 (2 mg/kg)****MAS3 (4 mg/kg)**

DHA	Mean	% CV	min	max		Mean	% CV	min	max
Cmax (ng/mL)	802	75%	142	2780		1819	54%	771	4680
AUC (hr*ng/mL)	1400	58%	482	4027		3543	29%	1708	5182
C60min (ng/mL)	564	84%	6	1720		1103	71%	24	2570
C90min (ng/mL)	469	60%	61	1100		1227	52%	141	2380
C180min (ng/mL)	218	72	20	506		695	41%	211	1100
ARTS			<i>20-300 fold</i>						
Cmax (ng/mL)	269	103%	28	897		411	91%	34	1620
AUC (hr*ng/mL)	160	54%	53	340		317	48%	122	738
C60min (ng/mL)	65	93%	0.3	244		252	141%	6	1620
C90min (ng/mL)	27	80%	1.3	68		85	84%	3	260
C180min (ng/mL)	6	131%	0	22		14	128%	0	54

		AS7	MAS3
n		20	20
Sex	<i>F (%)</i>	5 (25%)	4 (20%)
	<i>M (%)</i>	15(75%)	16 (80%)
Age*	<i>(y)</i>	23 (9 to 50)	29 (9 to 56)
Height*	<i>(m)</i>	1.55 (1.23 to 1.67)	159 (123 to 173)
Weight*	<i>(kg)</i>	44.8 (20.5 to 60.0)	47.0 (21.0 to 62.0)
Temp	<i>(°C)</i>	38.5 (38.1 to 38.9)	38.5 (38.1 to 38.9)
Pulse rate	<i>(/min)</i>	96 (87 to 104)	96 (90 to 102)
Blood pressure	<i>Syst</i>	108 (103 to 114)	108 (103 to 113)
	<i>Diast</i>	65 (62 to 69)	60 (57 to 64)
Resp rate	<i>(/min)</i>	20 (18 to 21)	19 (18 to 20)
Liver* (from costal margin)	<i>(cm)</i>	0 (0 to 2)	0 (0 to 3)
Spleen* (from costal margin)	<i>(cm)</i>	0 (0 to 3)	0 (0 to 5)

Table. Baseline variables. Values are mean (95%CI) except \* median (range).

		<b>AS7</b>	<b>MAS3</b>
Parasitaemia*	(/ $\mu$ L)	64165 (39156 to 105147)	65298 (40813 to 104472)
Haematocrit	(%)	34 (31 to 37)	37 (33 to 40)
Haemoglobin	(g/dL)	11.1 (10.0 to 12.1)	12.0 (10.8 to 13.1)
WBC	( $10^9$ /L)	6.0 (5.2 to 6.8)	6.2 (5.2 to 7.1)
Neutrophils	(%)	63.1 (56.6 to 69.6)	67.3 (60.2 to 74.4)
Lymphocytes	(%)	24.8 (18.9 to 30.7)	21.2 (15.2 to 27.3)
Platelets	( $10^9$ /L)	125 (80 to 170)	123 (88 to 158)
Glucose	(mg/dL)	94.1 (89.5 to 98.7)	105.3 (92.8 to 117.9)
Sodium	(mmol/L)	137.7 (134.5 to 141.0)	138.0 (136.1 to 139.8)
Potassium	(mmol/L)	3.70 (3.58 to 3.82)	3.75 (3.58 to 3.92)
Chloride	(mmol/L)	98.6 (96.7 to 100.4)	98.1 (96.7 to 99.4)
Calcium	(mmol/L)	8.96 (8.80 to 9.10)	8.99 (8.82 to 9.13)
BUN	(mg/dL)	12.8 (11.6 to 14.0)	13.6 (11.7 to 15.4)
Creatinine	(mg/dL)	0.80 (0.70 to 0.90)	0.81 (0.66 to 0.95)
Total Bilirubin	(mg/dL)	1.35 (1.05 to 1.65)	1.72 (1.34 to 2.10)
ASAT	(U/L)	53.6 (38.7 to 68.4)	40.3 (36.1 to 44.3)
ALP	(U/L)	113.7 (90.0 to 137.2)	113.9 (89.2 to 138.5)
Albumin	(g/dL)	3.53 (3.33 to 3.72)	3.84 (3.63 to 4.04)

Baseline variables (2). Values are mean (95%CI), except \* geometric mean (95% CI)

# Summary of recurrent infections:

- Recrudescence *P. falciparum* infection:
  - N=6 (all in AS7 arm)
- Recurrent *P. falciparum* infection:
  - N=1 (MAS3 arm; genotyping follows)
- Recurrent *P. vivax* infection:
  - N=13 (7 in AS7 arm; 6 in MAS3 arm)
- Recurrent *mixed* infection:
  - N=2 (1 in each arm, 1 included in total Pf recurrence; 1 still needs genotyping)