THE LANCET Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission. We post it as supplied by the authors.

Supplement to: Llanos-Cuentas A, Casapia M, Chuquiyauri R, et al. Antimalarial activity of single-dose DSM265, a novel plasmodium dihydroorotate dehydrogenase inhibitor, in patients with uncomplicated *Plasmodium falciparum* or *Plasmodium vivax* malaria infection: a proof-of-concept, open-label, phase 2a study. *Lancet Infect Dis* 208; published online June 13. http://dx.doi.org/10.1016/S1473-3099(18)30309-8.

Appendix

Contents

	Screen / Day 1 (Pre-Dose)	Day 1 (Dosing)	Day 2	Day 3	Day 5- 7[n]	Day 10 or 11	Day 14	Day 17 or 18	Day 21	Day 24 or 25	Day 28[a]	Day 35
Informed Consent	X											
Inclusion/Exclusion criteria	X											
Dosing		X										
Medical history and demographics	X											
Height & weight	X										X wt	Xwt
Physical exam	X										X	X
Pregnancy test [b]	X										X	X
Vital signs [c]	X	2, 4, 8, 12, 16, 20h PD	24, 30, 36, 42h PD	48, 54, 60, 66, 72h PD	X	X	X	X	X	X	X	X
12 lead ECG [d]	X		36h PD	48h PD							X	X
Holter ECG [e]		←	—	→								
PK blood sample [f]	X	0.5, 1, 2, 4, 6, 8, 12, 16h PD	24 h PD	48, 72h PD	X	X	X		X		X	X
Haematology, Clinical chemistry & urinalysis	X		X	X	X	X	X		X		X	X
Parasite genotyping [h]	X										X	
Pharmacogenetics - genetic polymorphism	X											

qPCR[i] (for parasitaemia)	X (Screen and -0.5h)	4, 8, 12, 16, 20h PD	24, 30, 36, 42h PD	48, 54, 60, 66, 72h PD	X	X	X	X	X	X	X	
qPCR[i] (for gametocytemia)	X (-0.5h)	16h PD	24h PD	48, 72h PD	X	X	X		X		X	
Blood films[j]	X (Screen and -0.5h)	4, 8, 12, 16, 20h PD	24, 30, 36, 42h PD	48, 54, 60, 66, 72h PD	X X X X X X X If parasites not cleared within 72h (evidenced by two consecutive negative microscopy readings), continued every 6h until cleared or withdrawal when parasitaemia levels rise							
Temperature [k]	X	2, 4, 8, 12, 16, 20h PD	24, 30, 36, 42h PD	48, 54, 60, 66, 72h PD								
Adverse events	Continuous ←											→
Concomitant medications[l]	As required from screening until completion											→

Definitive malaria treatment [m]											X	

Table S1: Clinical assessments

Abbreviations: ECG=electrocardiogram; PD=post-dose; PK=pharmacokinetic; qPCR=quantitative polymerase chain reaction.

[a] Day 28, or day at premature discontinuation (Note: all Day 28 trial evaluations were to be done before definitive antimalarial treatment was received).

[b] Pregnancy test females of childbearing potential – serum test at screening. Urine test at Days 28 and 35 or at withdrawal.

[c] Vital signs: (supine blood pressure and pulse rate) were taken after the patient had been supine for at least 5 minutes. Blood pressure and pulse rate: at screening and at pre-dose and then time-points as listed in table (±30 minutes). Measurements were to be taken on Day 1 (pre-dose, 2, 4, 8, 12, 16, 20 and 24 hours) and on Days 2 and 3 (every 6 hours) during hospitalization period (until two negative parasite levels obtained). Vital signs were measured as scheduled and as clinically indicated.

[d] 12-lead ECG (including QTcF assessment): As scheduled and as clinically indicated. Baseline (pre-dose) ECG was recorded in single; Day 2, Day 3 and Day 28 (or at withdrawal) were provided in triplicate. Final ECG must have been completed before starting alternative antimalarial therapy.

[e] Holter recording from at least 15 minutes before dosing (Day 1) to at least 24 hours post dosing (Day 2).

[f] PK blood samples: Blood samples (1 mL) were collected as detailed (within ±5 minutes of the nominated time-point for the 30 minute and 1 hour samples, and within ±10 minutes for the remaining samples up to 24 hours then within ±30 minutes until 72 hours); and on Days 5, 7, 10 or 11, 14, 21 and 28. Post-definitive antimalarial therapy; at time of failure (if applicable) or in the case of any drug-related SAE.

[g] Haematology, biochemistry, urinalysis (by dipstick / microscopy if required) as scheduled and when clinically indicated. A sample was collected before rescue therapy was administered.

[h] *In vitro* blood samples for parasite genotyping pre-dose and at the time of recrudescence. Samples were taken **at the time of first reappearance of the parasites**, but only for patients for whom parasitaemia first re-appeared after Day 7.

[i] Filter card samples for qPCR (parasitaemia) were obtained at screening and on Day 1, at 0.5 hours pre-dose, at 4 hours post-dose and then every 4 hours (±30 minutes) until 24 hours and then examined every 6 hours (±30 minutes) for a minimum of 72 hours, or until two consecutive microscopically negative readings had been obtained within an interval of 6 to 12 hours. Following microscopically monitored clearance, qPCR samples were obtained from patients once they had been released from the clinic, as per their out-patient visits on Days 5, 7, 10 or 11, 14, 17 or 18, 21, 24 or 25 and 28.
Filter card samples for qPCR (gametocytemia) were obtained on Day 1 at 0.5 hours pre-dose, at 16 hours post-dose and then at 24h, 48h, 72h, until qPCR (parasitaemia) samples were no longer required. Following microscopically monitored clearance, qPCR

(gametocytemia) samples were obtained from patients once they had been released from the clinic, as per their out-patient visits on Days 5, 7, 10 or 11, 14, 17 or 18, 21, 24 or 25 and 28.

[j] Thick and thin blood films: were obtained on screening to confirm inclusion/exclusion criteria. Thick and thin blood films were examined at screening and 0.5 hours pre-dose and 4 hours post-dose, then every 4 hours (±30 minutes) following first dose administration until 24 hours and then every 6 hours (±30 minutes) for a minimum of 72 hours. If parasites had not cleared within 72 hours (as evidenced by two consecutive negative readings) blood films continued to be taken every 6 hours until clearance. Thick and thin blood films were then examined as outlined in Attachment 6 of the protocol and on Day 28/point of premature discontinuation. A thin blood smear was also reserved in the event of recrudescence, to confirm *P. falciparum* or *P. vivax* species.

[k] Temperature: at screening and at pre-dose and then time-points as listed in table (±30 minutes). From 24 hours post-dose, measurements were to be taken every 6 hours every day (+ 0, 6, 12, 18 and 24 hours etc.) throughout the hospitalization period (until two consecutive negative parasite levels were obtained).

[l] Concomitant Medications were to be assessed at each study visit.

[m] Definitive malaria treatment beginning Day 28 or earlier as required.

[n] Depending on parasitaemia results at Day 3, as evidenced by thin/thick slide and/or qPCR. All patients attended a Day 7 visit, regardless of whether they attended at Day 5 or not. Day 5 was an extra visit, only for patients who still had evidence of parasitaemia at the time of discharge from the clinic.

Table S2: Criteria for the administration of rescue treatment

1. Clinical decline and lack of clinical improvement or decrease in platelets >20% for patients with a platelet count at inclusion of between 50,000/mm^3 and 74,999/mm^3 (thrombocytopenia grade 2) or a drop in platelet count to <50,000/mm^3 for patients with a platelet count of >75,000/mm^3 at inclusion, at 24 hours for *P. vivax* malaria and 12 hours for *P. falciparum* malaria patients with a ≥25% increase in parasitaemia in comparison to baseline with or without fever.
2. Development of any clinical complications (WHO definition of complicated/severe malaria).
3. Parasitaemia ≥100,000/µL at any time with or without fever.
4. Parasitaemia ≥baseline (from the slides taken 30 minutes before start of treatment) with or without fever, 48 hours after first dose for *P. vivax* and 36 hours after first dose for *P. falciparum*.
5. Any parasitaemia with axillary fever ≥37.5°C or parasitaemia ≥25% of the baseline parasitaemia with or without fever, 72 hours after first dose for *P. vivax* and 60 hours after first dose for *P. falciparum*.
6. Failure to clear all parasites by microscopy associated with axillary temperature ≥37.5°C or oral/rectal/tympanic temperature ≥38°C between 72 hours and 7 days for *P. vivax* and 60 hours and 5 days for *P. falciparum.*
7. Failure to clear all *P. vivax* parasites by microscopy with or without fever on Day 7 and *P. falciparum* parasites by microscopy with or without fever on Day 5.

Drug substance

DSM265 was provided as an amorphous spray-dried dispersion with HPMCAS-MF (25% loading), produced by Bend Research, Bend, USA, using Good Manufacturing Practice standards. DSM265 was administered as a single dose, suspended in a daily prepared vehicle consisting of 0.1% Methocel A4M, 0.1% Polysorbate 80, 0.005% simethicone emulsion containing 30% simethicone), 0.5% sucralose, and 0.05% ethyl vanillin pre-dissolved in sterile water. The total volume of DSM265 vehicle suspension was 240 mL.

Definitive antimalarial therapy

The following definitive anti-malarial therapy for *P. vivax* and *P. falciparum* were given, in accordance with Peruvian national guidelines:

For *P. vivax* (adults, uncomplicated malaria):
Chloroquine (CQ) 10 mg/kg/day for 3 days + primaquine (PQ) 0.5 mg/kg/day for 7 days; CQ 250 mg (150 mg base), PQ 15 mg each tablet.
Schedule:

Day 1: CQ 600 mg (4 tablets) + PQ 30 mg (2 tablets)
Day 2: CQ 600 mg (4 tablets) + PQ 30 mg (2 tablets)
Day 3: CQ 300 mg (2 tablets) + PQ 30 mg (2 tablets)
Day 4 - Day 7: 30 mg x day

For *P. falciparum* (adults, uncomplicated malaria):
Artesunate (AS) 4 mg/kg/day for 3 days + mefloquine (MQ) 12.5 mg/kg/day for 2 days.
Artesunate: 250 mg each tablet and mefloquine 250 mg each tablet
Day 1: AS 250 mg (1 tablet)
Day 2: AS 250 mg (1 tablet) + MQ 750 mg (3 Tablets)
Day 3: AS 250 mg (1 tablet) + MQ 750 mg (3 Tablets)
Day 3: Primaquine 30 mg/day (only one day)

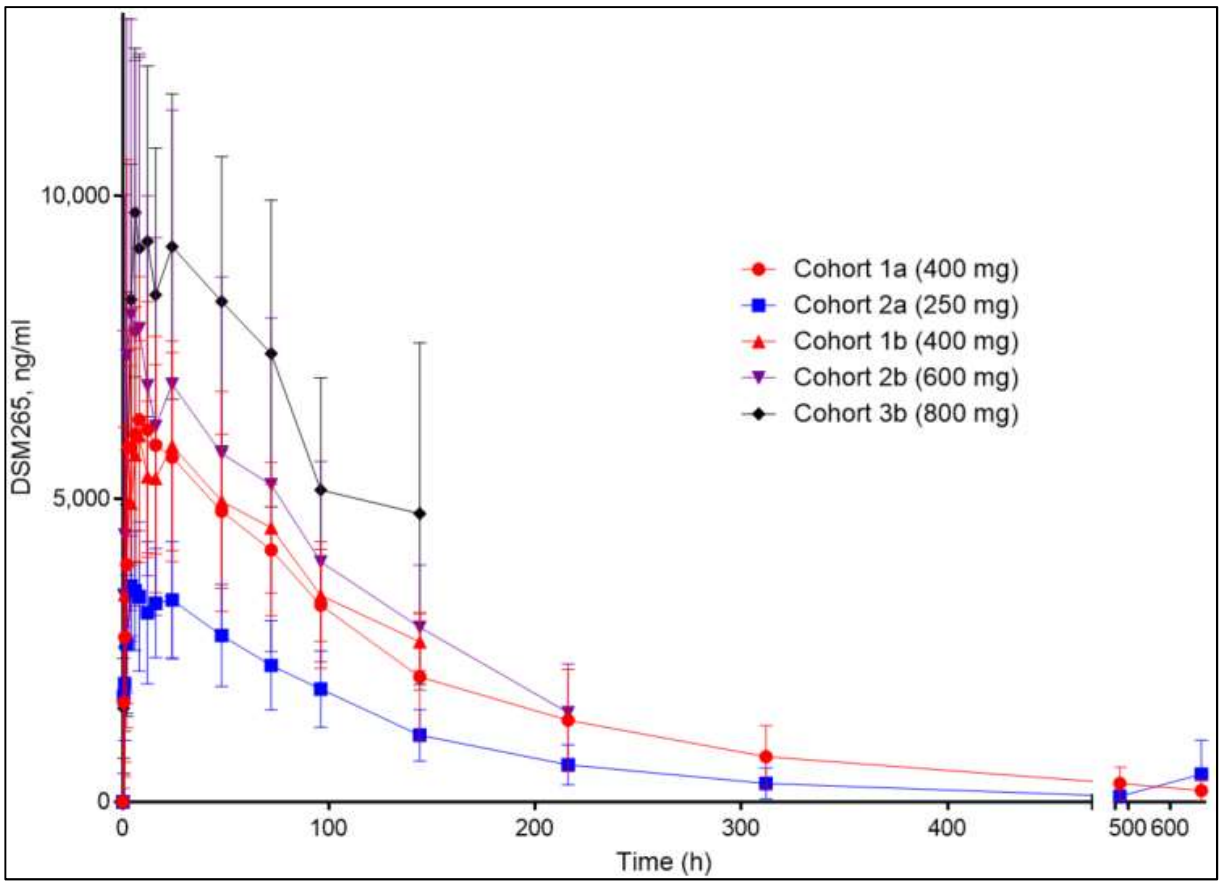
Table S3: Pharmacokinetic parameters for DSM265. [a]Median (and range) for t_{max}. [b]$t_{1/2}$, estimated terminal phase half-life. AUC_{0-t}, area under-the-concentration-time curve from zero to time t of the last measured concentration above the limit of quantification; CV%, coefficient of variation; N, Number of patients.

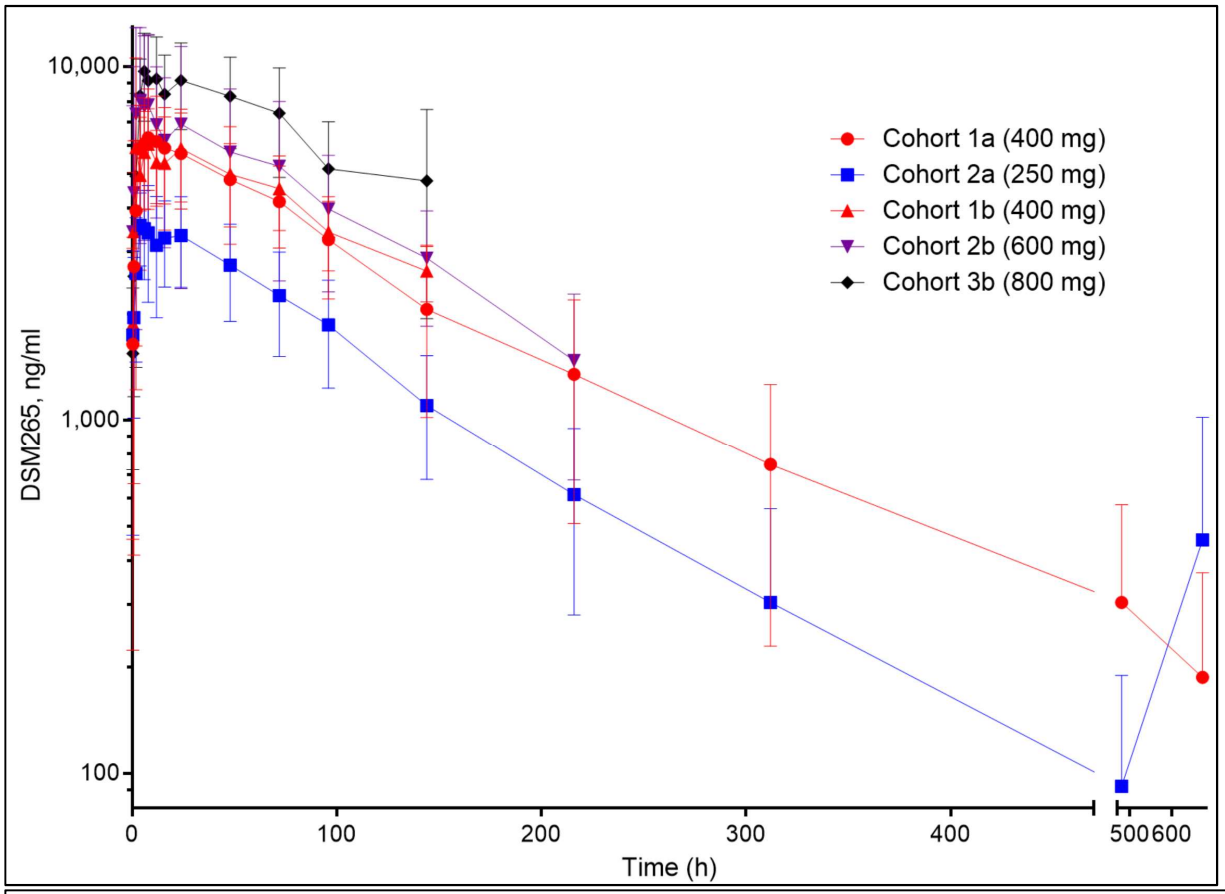
Cohort	Dose (mg)	Parameter	t_{max}[a] (h)	C_{max} (ng/mL)	AUC_{0-168} (ng.h/mL)	$t_{1/2}$[b] (h)
1a	**400**	N	13	13	13	13
		Mean	8.1	7,030	638,000	96.7
		Range or CV%	2.0-24	30	24	37
1b	**400**	N	5	5	5	5
		Mean	6	7,880	657,000	115
		Range or CV%	2.0-24	36	28	36
2a	**250**	N	11	11	10	9
		Mean	6.0	4,250	340,000	86.9
		Range or CV%	0.50-24	26	33.	43
2b	**600**	N	9	9	8	7
		Mean	6.0	8,440	744,000	91.3
		Range or CV%	2.0-48	45	43	25
3b	**800**	N	6	6	6	5
		Mean	10.1	10,100	1,080,000	110
		Range or CV%	4.0-24	26	31	70

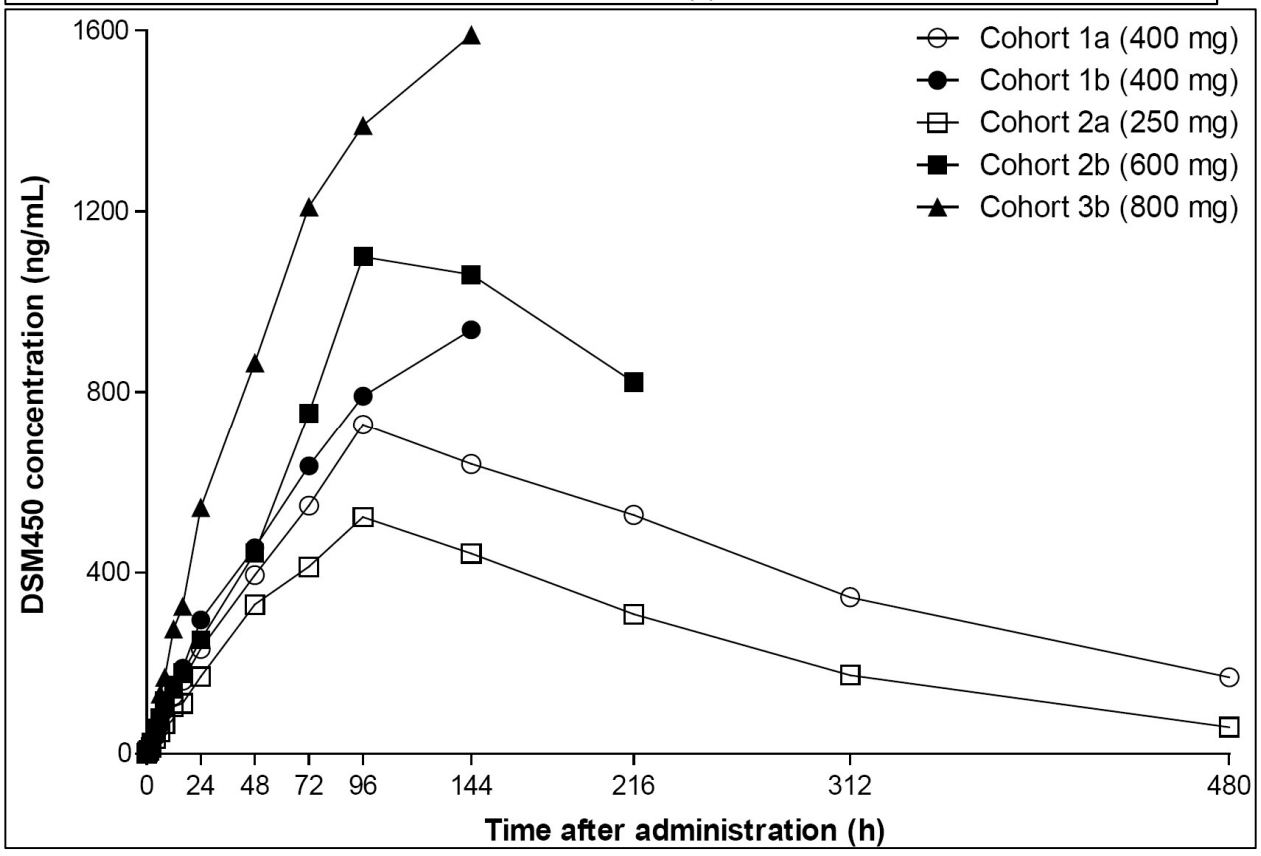
Table S4: Pharmacokinetic parameters for DSM450. *Data are the median (and range) for tmax. $t_{1/2}^{\#}$ is estimated terminal phase half-life.

Cohort	Dose (mg)	N	t_{max}^{*} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	AUC_{0-168h} (ng.h/mL)	$t_{1/2}^{\#}$ (h)
1a	400	N	13	13	13	13	13	13
		mean	113	738	189,000	214,000	78,200	134
		CV%	72.0, 233)	39.7	46.9	42.4	40.5	35.9
1b	400	N	3	3	3	9	10	9
		mean	114	995	131,000	131,000	50,300	126
		CV%	(114, 157)	59.8	51.8	36.5	62.2	29.6
2a	250	N	10	10	10	3	6	3
		mean	112	442	108,000	254,000	102,000	101
		CV%	38.5, 230)	61.6	70.8	80.2	80.6	29.6
2b	600	N	8	8	8	3	4	3
		mean	114	897	148,000	389,000	147,000	108
		CV%	71.8, 261)	72.7	116.1	27.1	64.1	43.6
3b	800	N	4	4	4	13	13	13
		mean	136	1,350	362,000	214,000	78,200	134
		CV%	72.0, 160)	43.0	26.4	42.4	40.5	35.9

Figure S 1: Mean concentration versus time profiles of DSM265 and DSM450 (linear A, C and semi logarithmic B, D, scales) in patients with confirmed *P. falciparum* (Cohorts 1a and ; A and B) or *P. vivax* (Cohorts 1b, 2b and 3b; C and D) mono infection after oral administration.




10,000
1,000
100
DSM265, ng/ml
0
100
200
300
400
500
600
Time (h)
Cohort 1a (400 mg)
Cohort 2a (250 mg)
Cohort 1b (400 mg)
Cohort 2b (600 mg)
Cohort 3b (800 mg)


1600
1200
800
400
0
DSM450 concentration (ng/mL)
0
24
48
72
96
144
216
312
480
Time after administration (h)
Cohort 1a (400 mg)
Cohort 1b (400 mg)
Cohort 2a (250 mg)
Cohort 2b (600 mg)
Cohort 3b (800 mg)

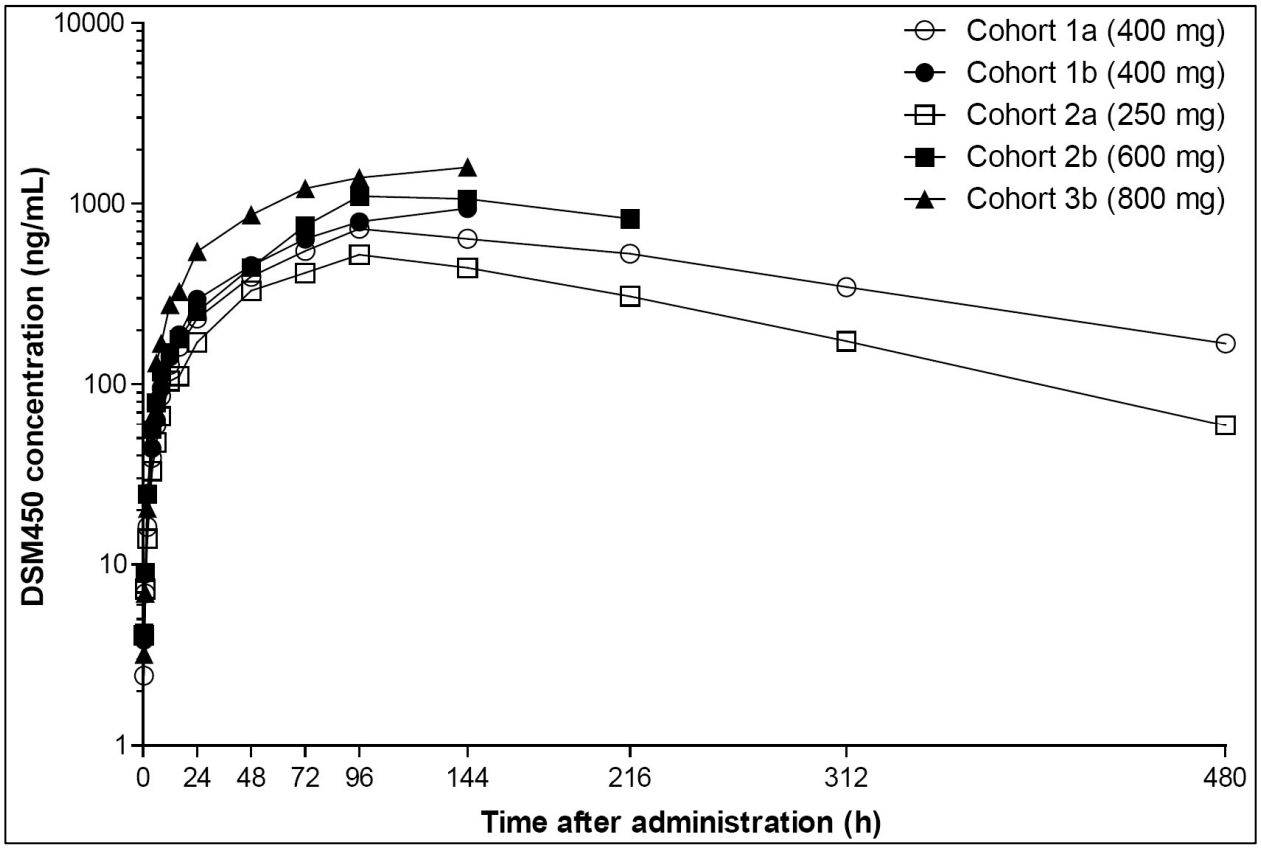

Cohort 1a (400 mg)
Cohort 1b (400 mg)
Cohort 2a (250 mg)
Cohort 2b (600 mg)
Cohort 3b (800 mg)
DSM450 concentration (ng/mL)
10000
1000
100
10
1
0
24
48
72
96
144
216
312
480
Time after administration (h)

Table S5: Safety assessment details.

System Organ Class Preferred term	*P. falciparum*		*P. vivax*		
	Cohort 1a N=13 n (%)	**Cohort 2a N=11 n (%)**	**Cohort 1b N=5 n (%)**	**Cohort 2b N=9 n (%)**	**Cohort 3b N=7 n (%)**
Overall	10 (76.9)	7 (63.6)	4 (80.0)	7 (77.7)	6 (85.7)
Blood and lymphatic system disorders	0	0	1 (20.0)	0	0
Thrombocytopenia	0	0	1 (20.0)	0	0
Cardiac disorders	2 (15.4)	0	0	2 (22.2)	0
Sinus bradycardia	2 (15.4)	0	0	0	0
Eye disorders	1 (7.7)	0	0	0	0
Vision blurred	1 (7.7)	0	0	0	0
Gastrointestinal disorders	3 (23.1)	4 (36.4)	1 (20.0)	2 (22.2)	2 (28.6)
Abdominal pain	0	1 (9.1)	1 (20.0)	0	0
Diarrhoea	3 (23.1)	1 (9.1)	0	0	2 (28.6)
Nausea	2 (15.4)	3 (27.3)	0	0	0
Stomatitis	0	0	0	1 (11.1)	0
Vomiting	0	1 (9.1)	0	1 (11.1)	0
General disorders and administration site conditions	4 (30.8)	4 (36.4)	4 (80.0)	5 (55.6)	4 (57.1)
Asthenia	1 (7.7)	0	0	0	0
Chills	1 (7.7)	0	0	0	0
Fatigue	0	2 (18.2)	0	0	0
Pyrexia	4 (30.8)	3 (27.3)	4 (80.0)	5 (55.6)	4 (57.1)

PK assessments

The concentrations of DSM265 and DSM450 in blood were measured using a validated liquid chromatography-tandem mass spectrometry method at Swiss BioQuant AG, Reinach, Switzerland. The lower limit of quantification (LLOQ) was 10.0 ng/mL for DSM265 and 2.0 ng/mL for DSM450. Non-compartmental PK analysis was performed using Phoenix WinNonlin (version 6.3, Pharsight Corporation, Mountain View, CA, USA). All patients who received DSM265 were included in the analysis (all-treated set).

Asexual clearance parameters

Methodology

The microscopy counts of asexual parasites were uploaded in the WWARN calculator to calculate the parasite clearance rate constant, the clearance half-life, the lag phase and the time needed to clear 50, 90, 95 and 99% of the baseline parasitaemia. The baseline parasitaemia was measured half an hour before administration of DSM265 for all patients, except for patients F1001 (5 hours before DSM265 administration), V1001 (4 hours prior to drug administration) and V1002 (3.4 hr prior to drug administration). In all cases, the value of this pre-dose parasitaemia was considered to be the baseline parasitaemia at time of drug administration since WWARN does not accept negative times.

The maximum PRR48 was calculated from the clearance rate constant k as followed:

$$MaximumPRR48 = \frac{k * 48}{log(10)}$$

It was thus assumed the clearance rate was at its maximum over the 48 hours.

For some patients, the parameters were not calculated due to the WWARN calculator criteria:

- Patients' baseline parasitaemia was below the threshold of 1000 parasites per µL blood (F1002, F1003, F1004, F1006, F1908, F2006, F2010, F2910, V1005).
- Patients had less than 3 parasite measurements (V1003).
- Patient's parasitaemia remained at high levels (F2003).

The true PRR48 as defined by the reduction ratio of parasitaemia at time point 48 hr after drug administration to the parasitaemia at the time of drug administration in log unit was calculated with the ratio of the pre-dose parasitaemia (P0) measured half an hour prior to DSM265 administration for most patients and the parasitaemia measured at time point 48 hr or extrapolated between the two measured values around 48 hours assuming a log linear decline (P48).

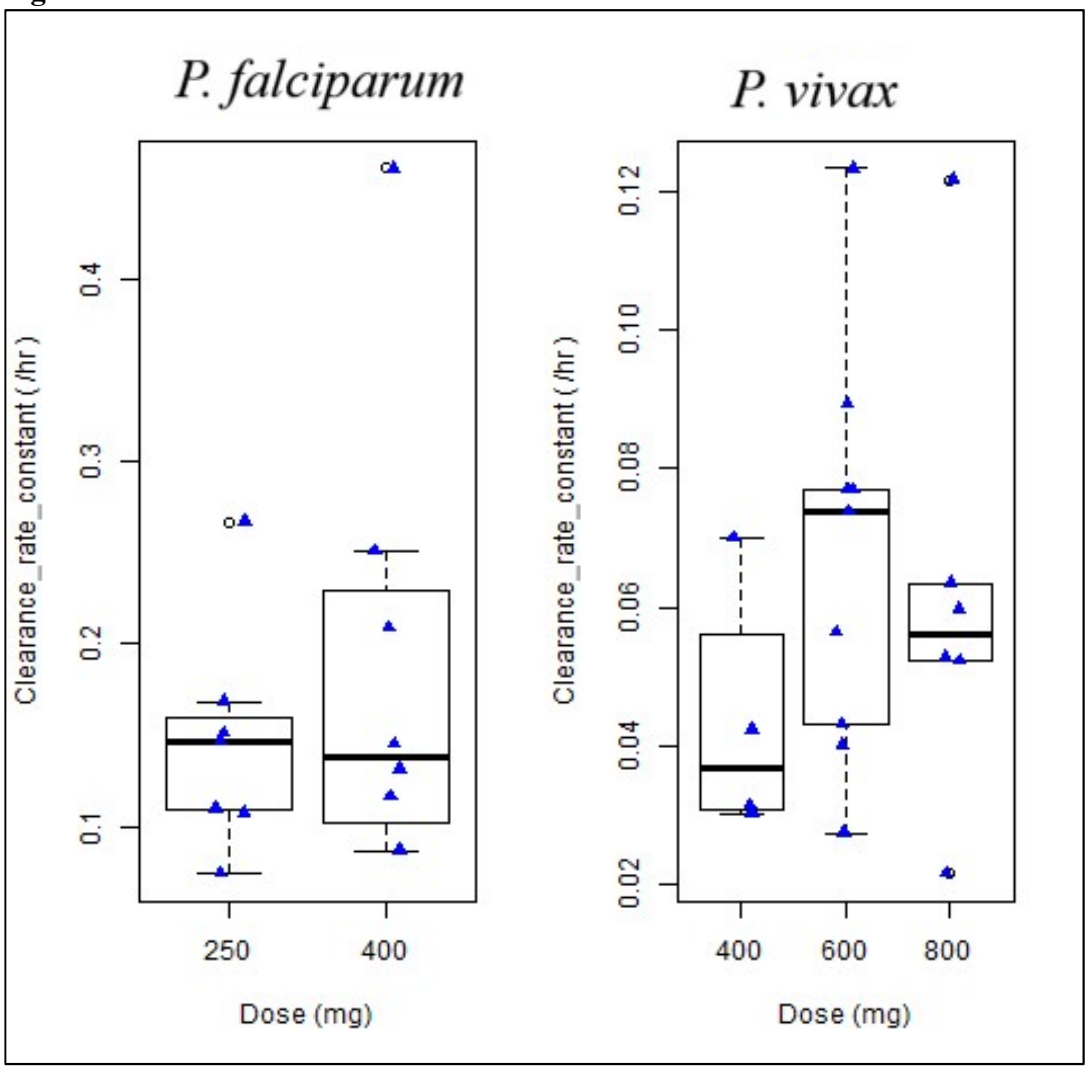
$$PRR48 = log10\left(\frac{P0}{P48}\right)$$

Parasite clearance rate constant

Table S6: Summary statistics of clearance_rate_constant (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	**Cohort 2a**	**Cohort 1b**	**Cohort 2b**	**Cohort 3b**
N	8	7	4	9	6
Mean	0.186	0.147	0.0435	0.0675	0.0619
SD	0.125	0.062	0.0185	0.0293	0.0328
Median	0.139	0.146	0.0369	0.0737	0.0562
Min;Max	0.0868; 0.46	0.0744; 0.267	0.0303; 0.0701	0.0274; 0.123	0.0216; 0.122

Figure S2: Clearance rate constants

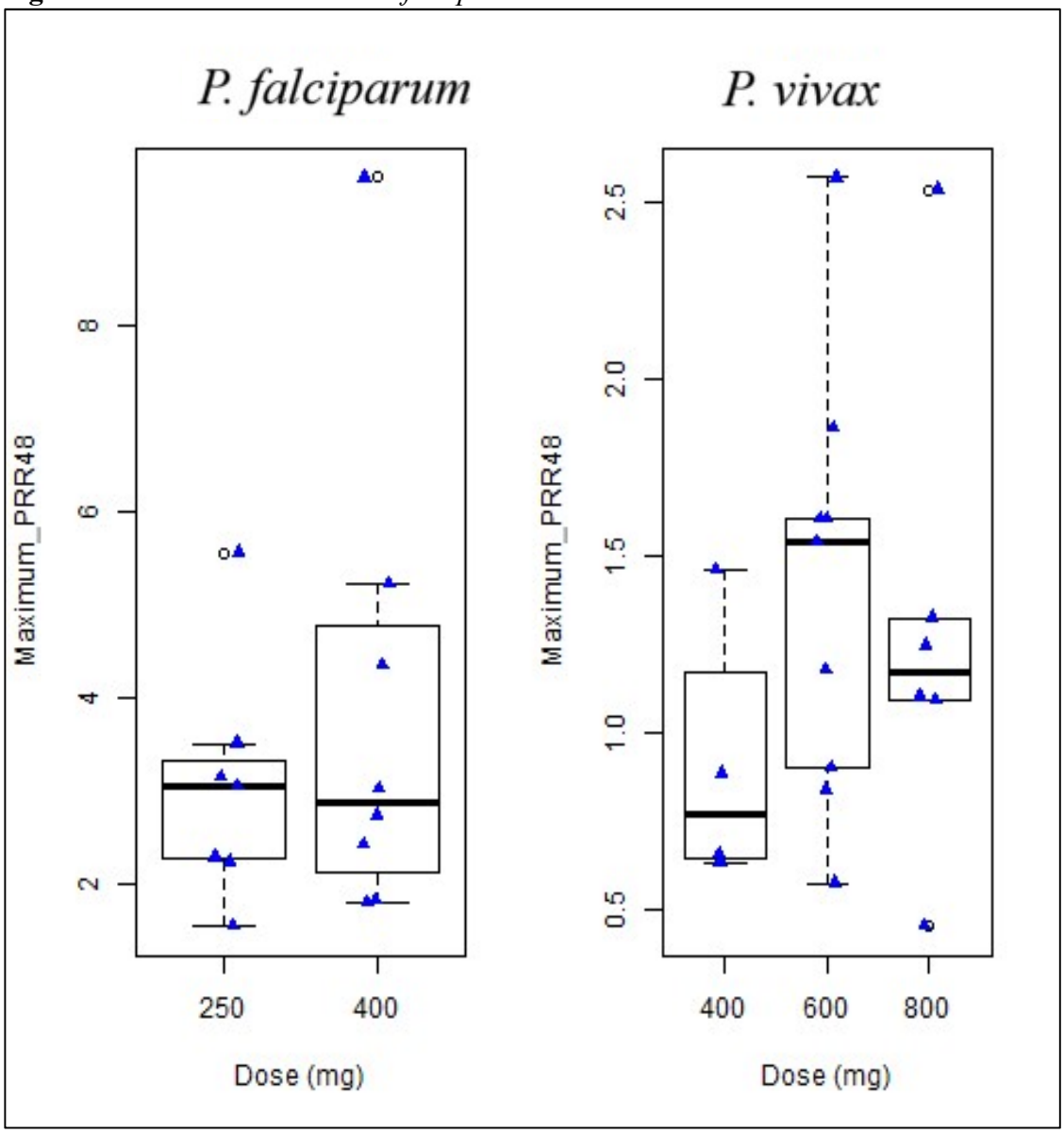


Maximum Parasite Reduction Ratio over 48 hours (PRR48)

Table S7: Summary statistics of maximum_PRR48 (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	**Cohort 2a**	**Cohort 1b**	**Cohort 2b**	**Cohort 3b**
N	8	7	4	9	6
Mean	3.9	3.0	0.91	1.4	1.3
SD	2.6	1.3	0.38	0.61	0.68
Median	2.9	3.1	0.77	1.5	1.2
Min;Max	1.8 ; 9.6	1.6 ; 5.6	0.63 ; 1.5	0.57 ; 2.6	0.45 ; 2.5

Figure S3: Maximum PRR48 *P. falciparum*

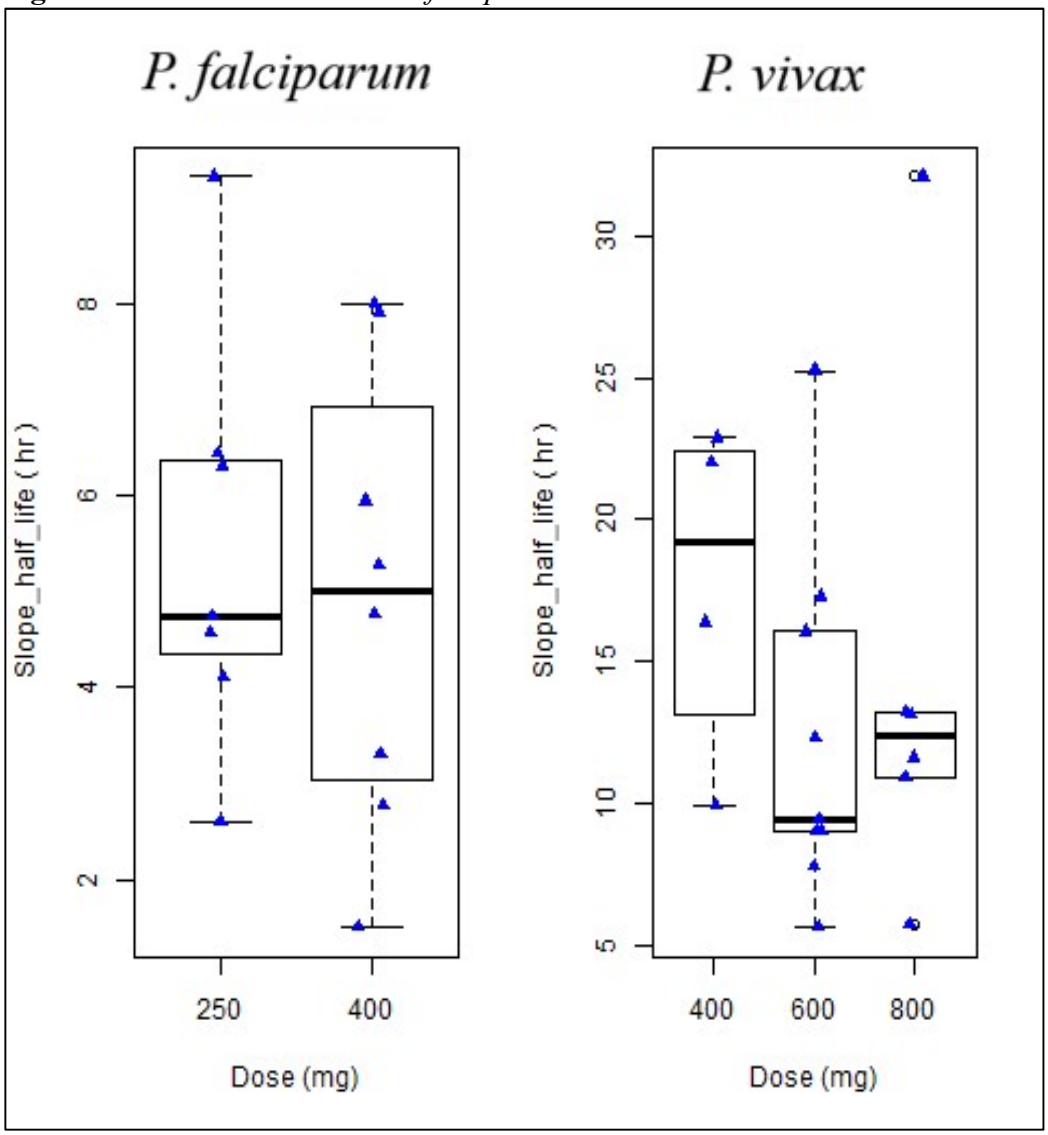


Asexual Parasite clearance half life

Table S8: Summary statistics of slope_half_life (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	**Cohort 2a**	**Cohort 1b**	**Cohort 2b**	**Cohort 3b**
N	8	7	4	9	6
Mean	4.9	5.4	18	12	14
SD	2	2	6	6	9
Median	5.0	4.7	19	9.4	12
Min;Max	1.5 ; 8.0	2.6 ; 9.3	9.9 ; 23	5.6 ; 25	5.7 ; 32

Figure S4: Maximum PRR48 *P. falciparum*

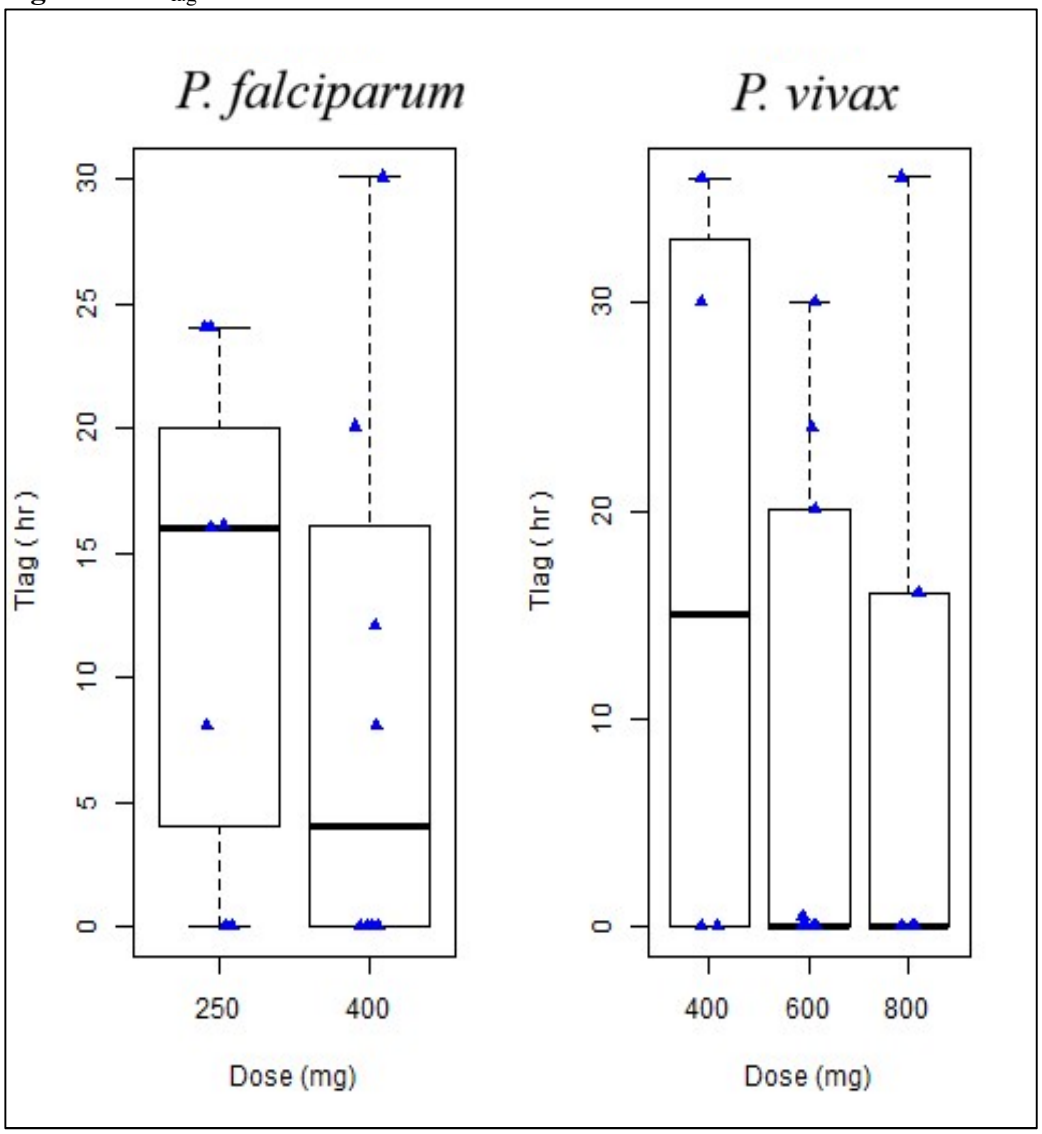


Asexual Lag phase

Table S9: Summary statistics of T_{lag} (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	**Cohort 2a**	**Cohort 1b**	**Cohort 2b**	**Cohort 3b**
N	8	7	4	9	6
Mean	8.78	12.6	16.5	8.29	8.67
SD	11.3	10.2	19.2	12.6	14.9
Median	4.01	16	15	0	0
Min;Max	0 ; 30.1	0 ; 24	0 ; 36	0 ; 30	0 ; 36

Figure S5: T_{lag}



Time to 50, 90, 95 and 99% reduction in asexual parasite density

Table S10: Summary statistics of PC_{50} (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	**Cohort 2a**	**Cohort 1b**	**Cohort 2b**	**Cohort 3b**
N	8	7	4	9	6
Mean	16.1	19	31.8	22.5	22.3
SD	10.4	10.4	24.1	12.8	10.7
Median	14.6	24	34.1	16.1	22.6
Min;max	1.04 ; 29.3	2.04 ; 30.4	4.66 ; 54.2	9.72 ; 45.3	8.44 ; 36.3

Figure S6: PC50

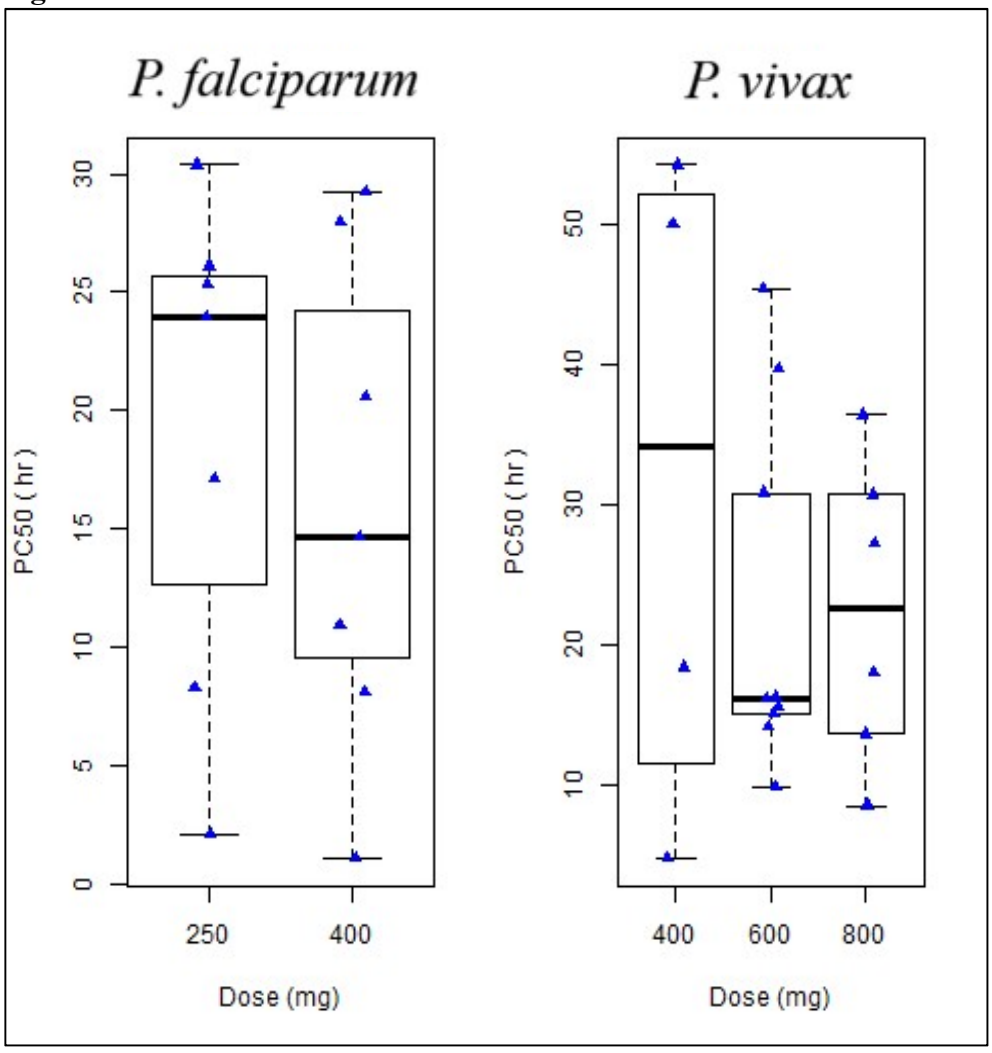


Table S11: Summary statistics of PC90 (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	**Cohort 2a**	**Cohort 1b**	**Cohort 2b**	**Cohort 3b**
N	8	7	4	9	6
Mean	25.1	31.7	73.1	51.3	55.9
SD	12.1	8.93	14.9	19.8	24.9
Median	26.8	31.3	72.2	53.5	49.1
Min;Max	4.54 ; 40.2	16.7 ; 45.3	55.8 ; 92.2	22.8 ; 74.1	38.9 ; 105

Figure S7: PC90

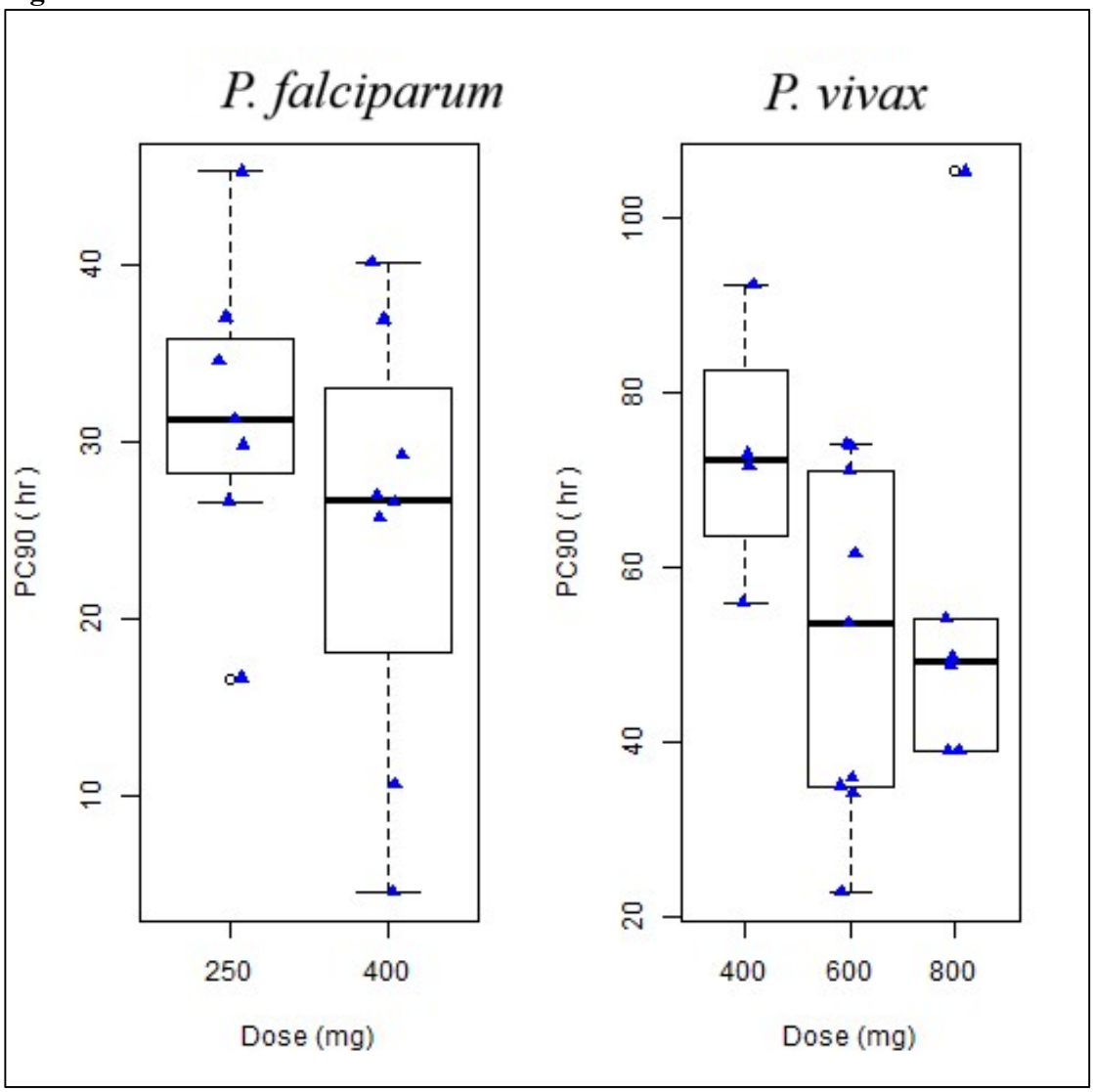


Table S 12: Summary statistics of PC95 (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	**Cohort 2a**	**Cohort 1b**	**Cohort 2b**	**Cohort 3b**
N	8	7	4	9	6
Mean	30	37.1	90.9	63.7	70.3
SD	12.9	9.09	13.7	24.8	33.3
Median	32.5	39.2	88.6	69.5	58.6
Min;Max	6.04 ; 45.4	23 ; 51.8	77.8 ; 109	28.4 ; 99.3	49.8 ; 137

../03-Output/PC95-microscopy.txt
2016-10-26 22:37:15

Figure S8: PC95

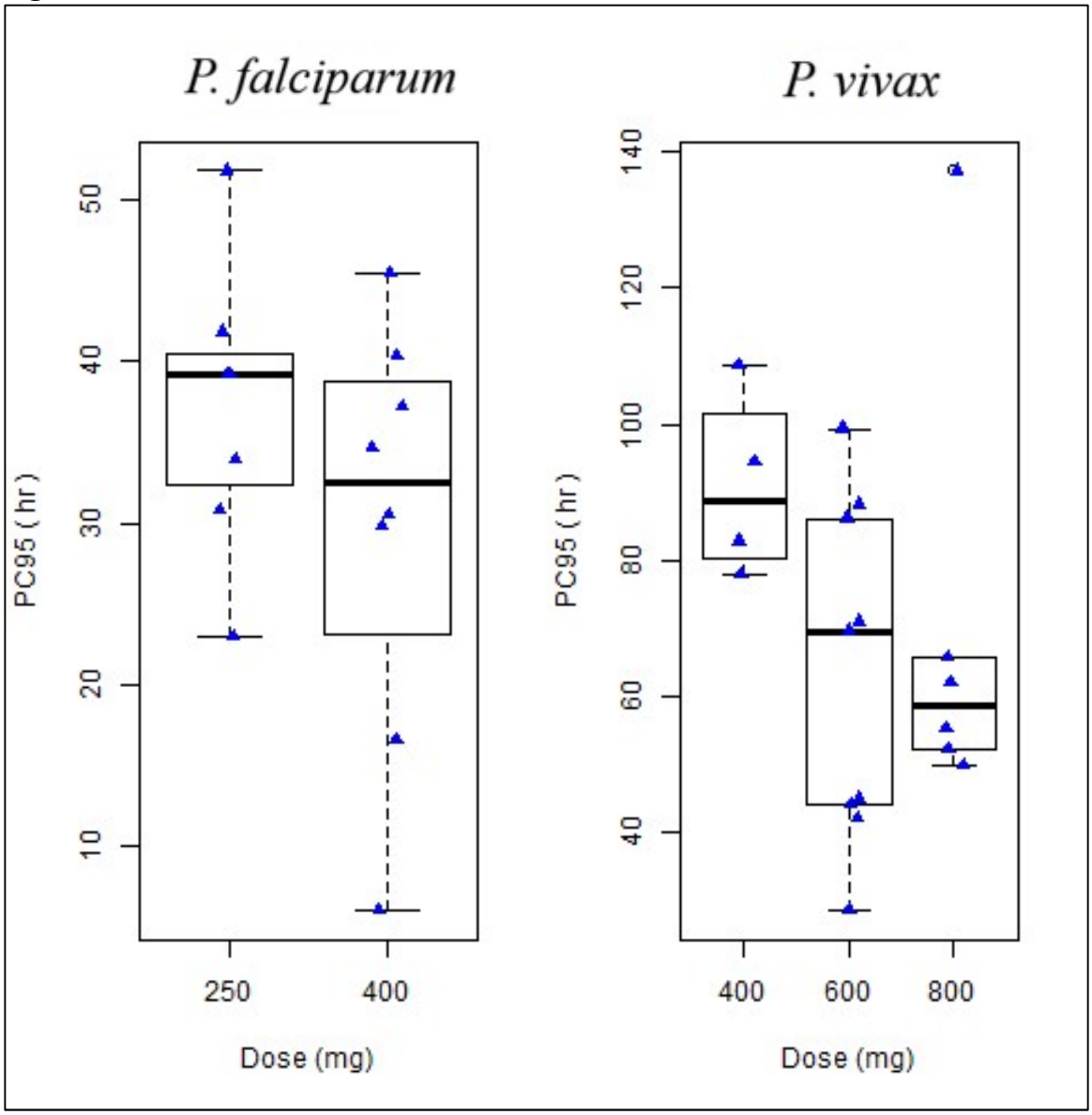
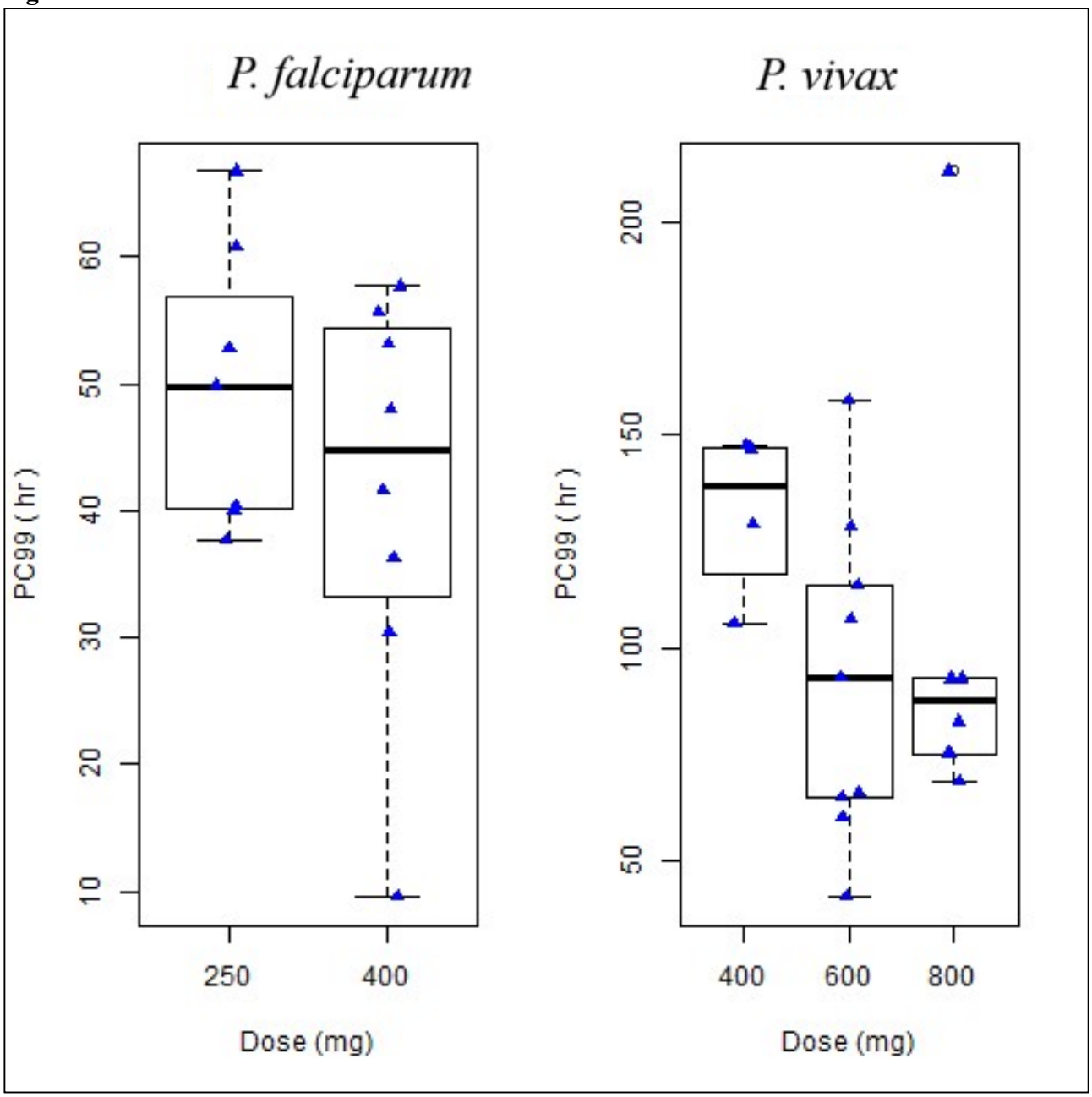


Table 1: Summary statistics of PC99 (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	Cohort 2a	Cohort 1b	Cohort 2b	Cohort 3b
N	8	7	4	9	6
Mean	41.5	49.7	132	92.5	104
SD	16.1	11.2	19.6	37.8	53.7
Median	44.7	49.8	138	92.7	87.6
Min;Max	9.54 ; 57.6	37.6 ; 66.7	106 ; 147	41.4 ; 158	68.5 ; 212

Figure S9: PC99



Parasite Reduction Ratio over 48 hours (PRR48)

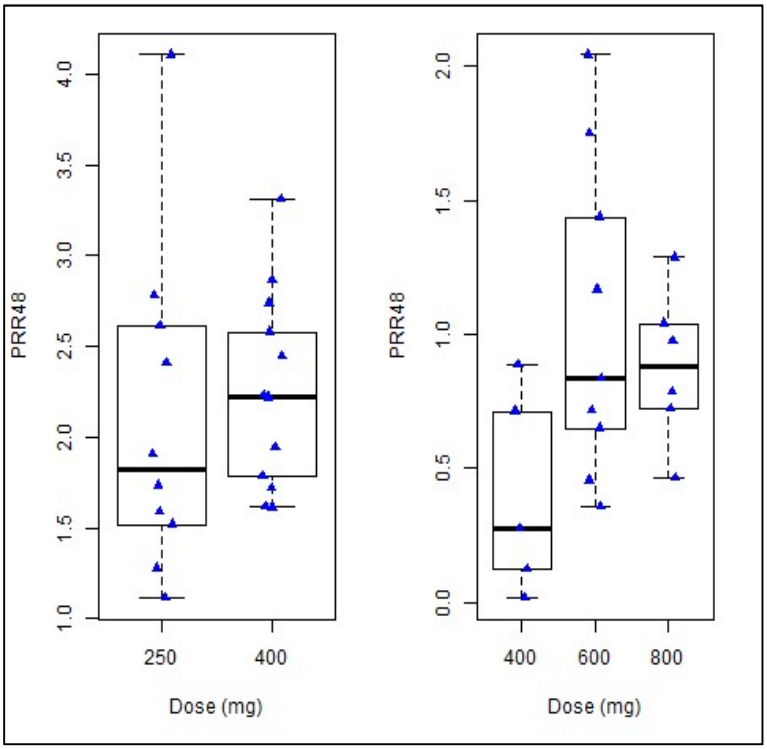
Table S13: Summary statistics of PRR48 (microscopy)

	P. falciparum		*P. vivax*		
	Cohort 1a	Cohort 2a	Cohort 1b	Cohort 2b	Cohort 3b
N	13	10	5	9	6
Mean	2.25	2.1	0.404	1.05	0.88
SD	0.525	0.9	0.378	0.591	0.285
Median	2.22	1.82	0.278	0.836	0.88
Min;Max	1.61 ; 3.31	1.11 ; 4.11	0.0163 ; 0.885	0.358 ; 2.04	0.466 ; 1.29

Table S14: Summary statistics of PRR48 (microscopy) for same patients as maximum PRR48

	P. falciparum		*P. vivax*		
	Cohort 1a	Cohort 2a	Cohort 1b	Cohort 2b	Cohort 3b
N	8	7	4	9	6
Mean	2.26	2.18	0.5	1.05	0.88
SD	0.606	0.997	0.357	0.591	0.285
Median	2.22	1.91	0.496	0.836	0.88
Min;Max	1.61 ; 3.31	1.11 ; 4.11	0.126 ; 0.885	0.358 ; 2.04	0.466 ; 1.29

Figure S10: PRR48



Conclusion on efficacy

Excellent efficacy was observed in *P. falciparum* patients. In contrast, efficacy in *P. vivax* patients did not meet study objectives. This is likely due to the clearance rate being twice as high in *P. falciparum* patients as in *P. vivax* patients. The clearance rate did not increase with dose or other exposure parameters (AUC_{168h}), either for *P. falciparum* or *P. vivax*. It is possible that the observed clearance rate corresponds to the maximum attainable rate at which parasites can be cleared with DSM265.
As a result of a lower clearance rate in *P. vivax* patients, the time needed to clear 99% of the initial parasitaemia after drug administration was about 4 days compared to 2 days in *P. falciparum*-infected patients.
A significant lag phase of up to 40 hours was observed in some *P. falciparum* and *P. vivax* infected patients. The presence and duration of lag phase did not seem to be related to DSM265 exposure nor the baseline parasitaemia. The maximum PRR48 calculated from the clearance rate constant showed the same trend as the clearance rate constant: it did not increase with dose or exposure parameters. The true PRR48, which takes the lag phase into account, was reduced by one log unit compared to the maximum PRR48 for *P. falciparum* patients and by 0.5 log unit for *P. vivax* patients. This was mainly due to the lag phase. The clearance constant rate was not calculated for some patients who fell outside the criteria of the WWARN calculator (e.g. too low a baseline parasitaemia). However, the statistics on the true PRR48 excluding or including these patients were similar (see **Table S13**).

Comparison qPCR and microscopy

The count of parasites by qPCR did not correlate well with the count of asexual parasites by microscopy for *P. falciparum* (see **Figure S11**) or *P. vivax* (see

Figure S12). One explanation is that qPCR detects all blood stages including the sexual stages that were not scored by microscopy.

Figure S11: Count of total parasites by qPCR vs asexual parasites by microscopy for *P. falciparum* patients

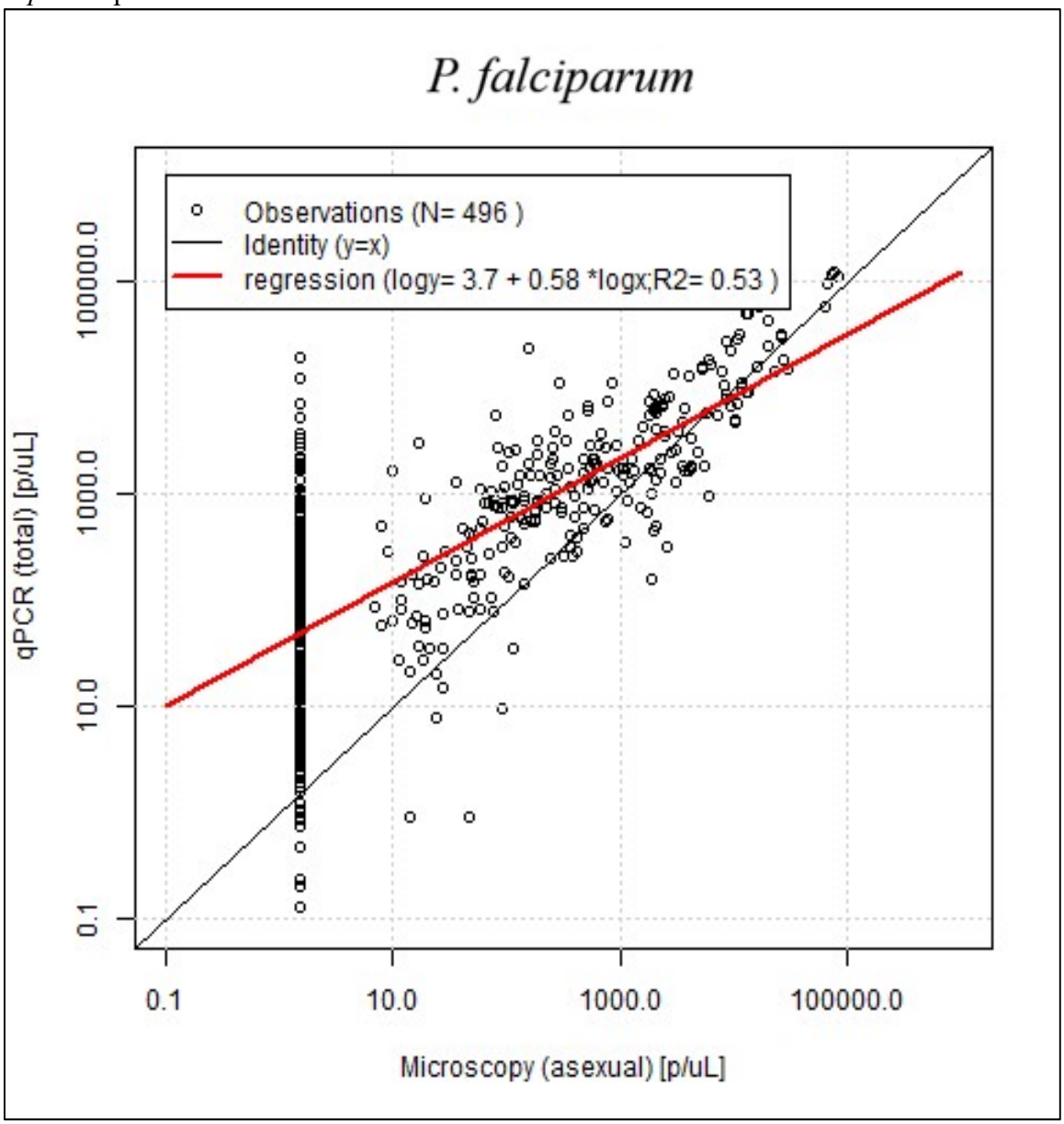
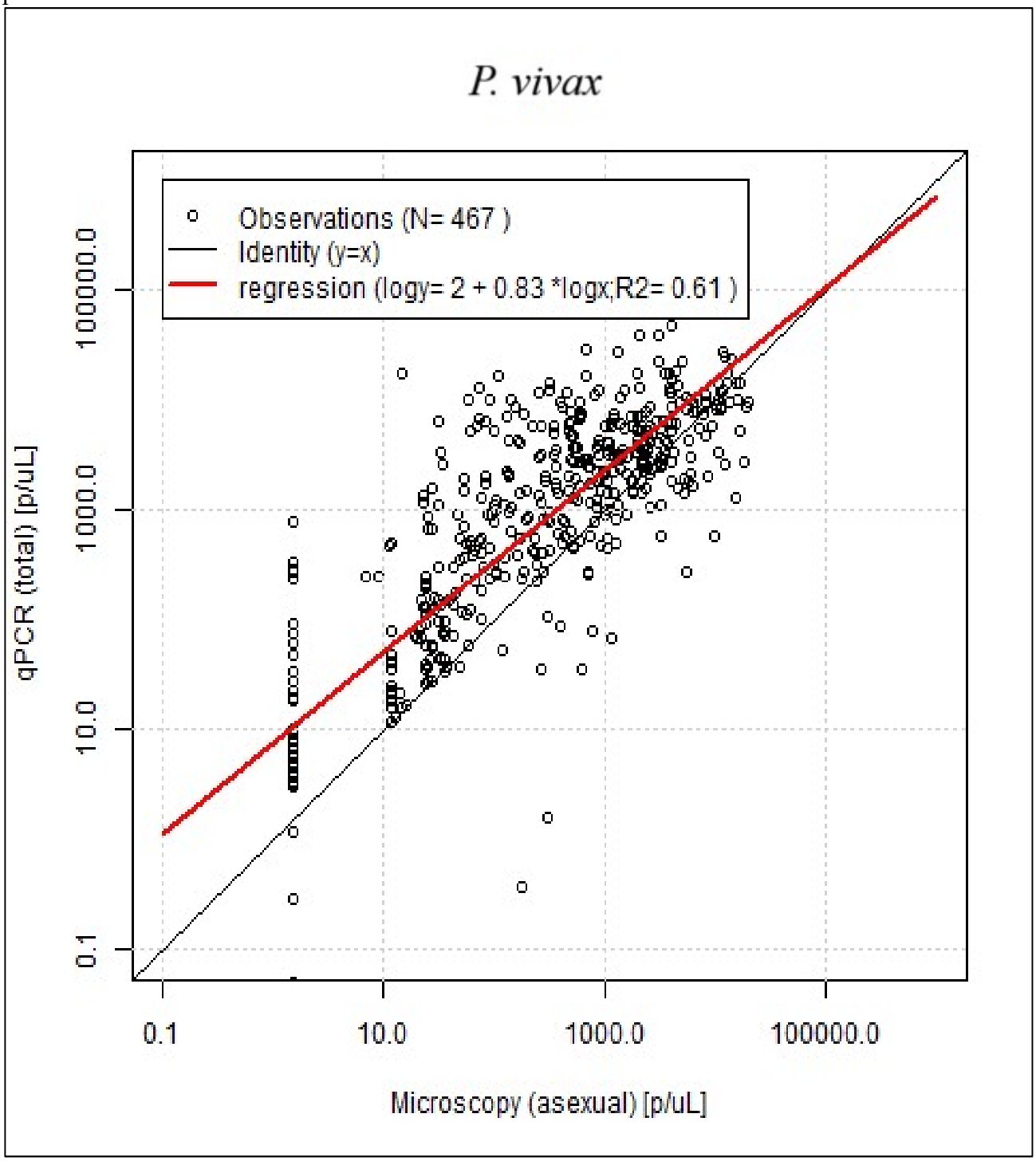


Figure S12: Count of total parasites by qPCR vs asexual parasites by microscopy for *P. vivax* patients



When the gametocyte microscopy count was added to the count of asexual parasites, the total count correlated relatively well to qPCR for *P. falciparum* (see **Figure S11**). For *P. vivax*, the correlation was improved when adding the gametocyte counts, but not to the same extent as for P. *falciparum* (see

Figure S12). Of note, the two methods of microscopy and qPCR had different limits of quantification (LOQ). Microscopy can detect parasitemias of as low as 3 parasites per µL and qPCR 0.1 parasite per µL. Values below the LOQ were set to half the LOQ, i.e. 1.5 and 0.05 parasite per µL for microscopy and qPCR respectively.

Figure S13: Count of total parasites by qPCR *vs* total parasites by microscopy (asexual+gametocytes) for *P. falciparum* patients

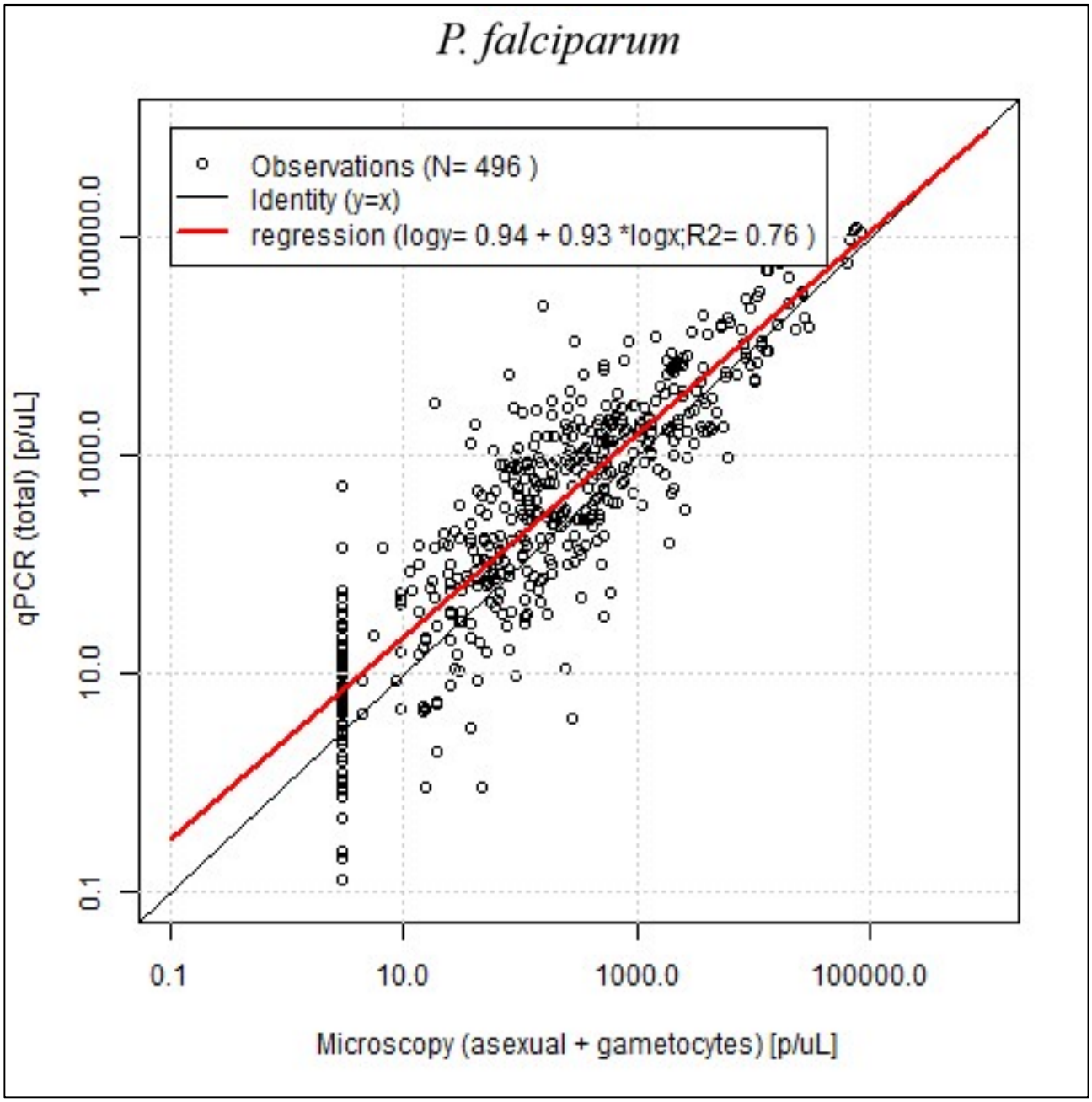
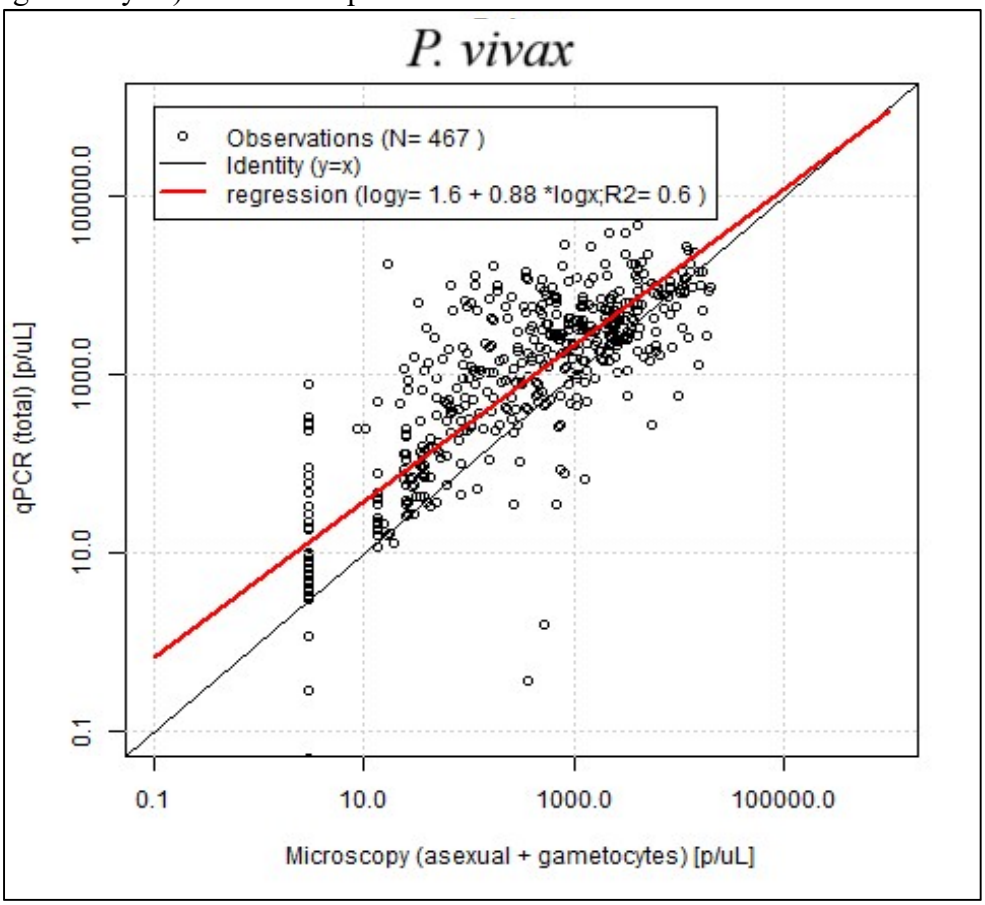

P. falciparum
Observations (N= 496)
Identity (y=x)
regression (logy= 0.94 + 0.93 *logx;R2= 0.76)
qPCR (total) [p/uL]
0.1
10.0
1000.0
100000.0
0.1
10.0
1000.0
100000.0
Microscopy (asexual + gametocytes) [p/uL]

Figure S14: Count of total parasites by qPCR vs total parasites by microscopy (asexual+gametocytes) for *P. vivax* patients



MIC and MPC

Only a few patients recrudesced. Initially, the *P. falciparum* parasitaemia declined relatively fast and reached the limit of quantification (LOQ). The parasitaemia levels remained below the LOQ over a number of days before recrudescence was observed. It was thus difficult to identify precisely the nadir. For *P. vivax*, the parasitaemia decline was slower and did not always fall below the LOQ. In the event that the parasitaemia dropped below the LOQ, the time interval to recrudescence was shorter compared to *P. falciparum.* **Table S15** shows the time of the last quantifiable observation before the parasitaemia fell below the LOQ and the time when the parasitaemia rose above the LOQ. The nadir was within this interval. The concentrations at these times are also shown. They provide an indication of where the MIC lies.
Assuming there is no variability among patients, the MIC for *P. falciparum* would be between 520 ng/mL (the highest value of the lower bound) and 1,510 ng/mL. (The lowest value of the upper bound) and the MIC for *P. vivax* between 546 ng/mL and 2,491 ng/mL. In the human challenge study where *P. falciparum* was inoculated to 7 healthy volunteers, recrudescence was observed in all subjects above the LOQ of qPCR and the MIC was found to be within the range of 552 ng/mL and 1,500 ng/mL with a median value of 1040 ng/mL. The findings in patients

are consistent with the analysis of the challenge study. Further studies are required to determine whether the MIC is the same for *P. vivax* and *P. falciparum*.

Table S15: Nadir (microscopy)

Patient ID	Lower bound for Nadir time (h)	Upper bound for Nadir time (h)	Lower bound DBS concentration (ng/mL)	Upper bound DBS concentration (ng/mL)
F1001	48	667.15	359	5500
F2001	42	495.25	14.6	4044
F2005	54	663.50	6.7	1510
F2009	72	330.40	520	2590
V1001	96	325.20	546	2540
V2002	60	399.00	243	12471
V2003	66	398.50	443	3049
V3001	138	327.50	91	2491

The MPC is the concentration at which the decline in parasitaemia loses its log-linearity. Since this happens when parasitaemia is below the LOQ, it is not possible to determine the MPC.

PK/PD relationships

The effect of DSM265 exposure on the cure of the patients was investigated along with the effect of baseline parasitaemia in **Figure S15** and

Figure S16 for *P. falciparum* and *P. vivax* patients respectively. Weexcluded from this analysis the subjects who dropped out before completion of the study, i.e. before Day 28, for *P. falciparum* or before Day 14 for *P. vivax* for reasons other than the lack of DSM265 efficacy. For *P. falciparum*, patients F1008, F1009 and F1010, who failed the initial success criterion, were excluded. patient F2009 who took another antimalarial drug and patient F2010, who withdrew consent three days after drug administration, were also excluded from the analysis. patient F1004, who took a prohibited concomitant drug, was included - if there was an interaction with DSM265, this would have been reflected in the DSM265 exposure parameter

(AUC over 168 hours). For *P. vivax*, Patients V1005, V2001, V2004, V2005, V2006, V2008, V2009, V3002, V3004, V3005 and V3006 were excluded.

Figure S15: Effect of baseline parasitaemia and AUC_{168h} on treatment success of *P. falciparum* patients at Day 28

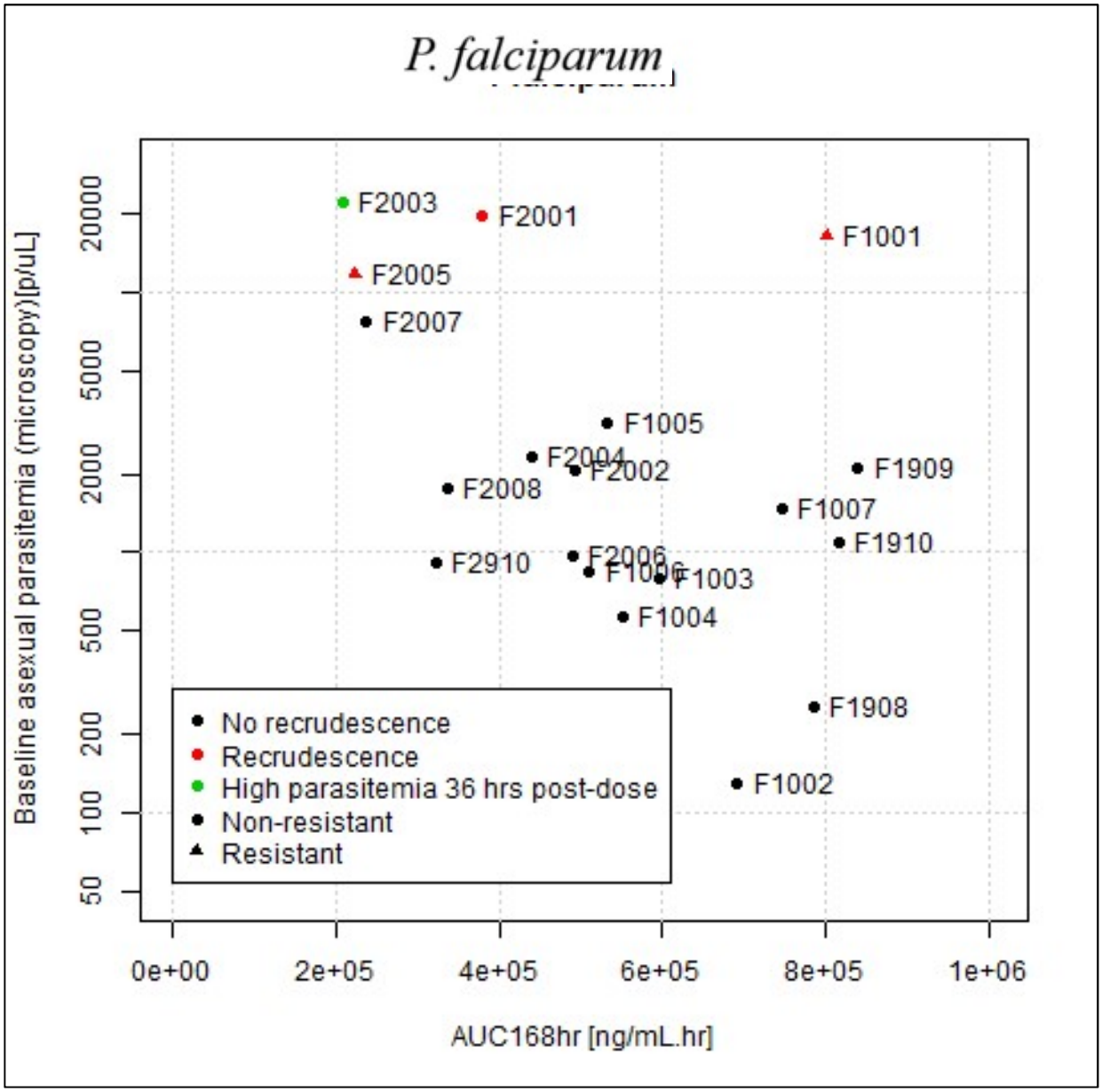
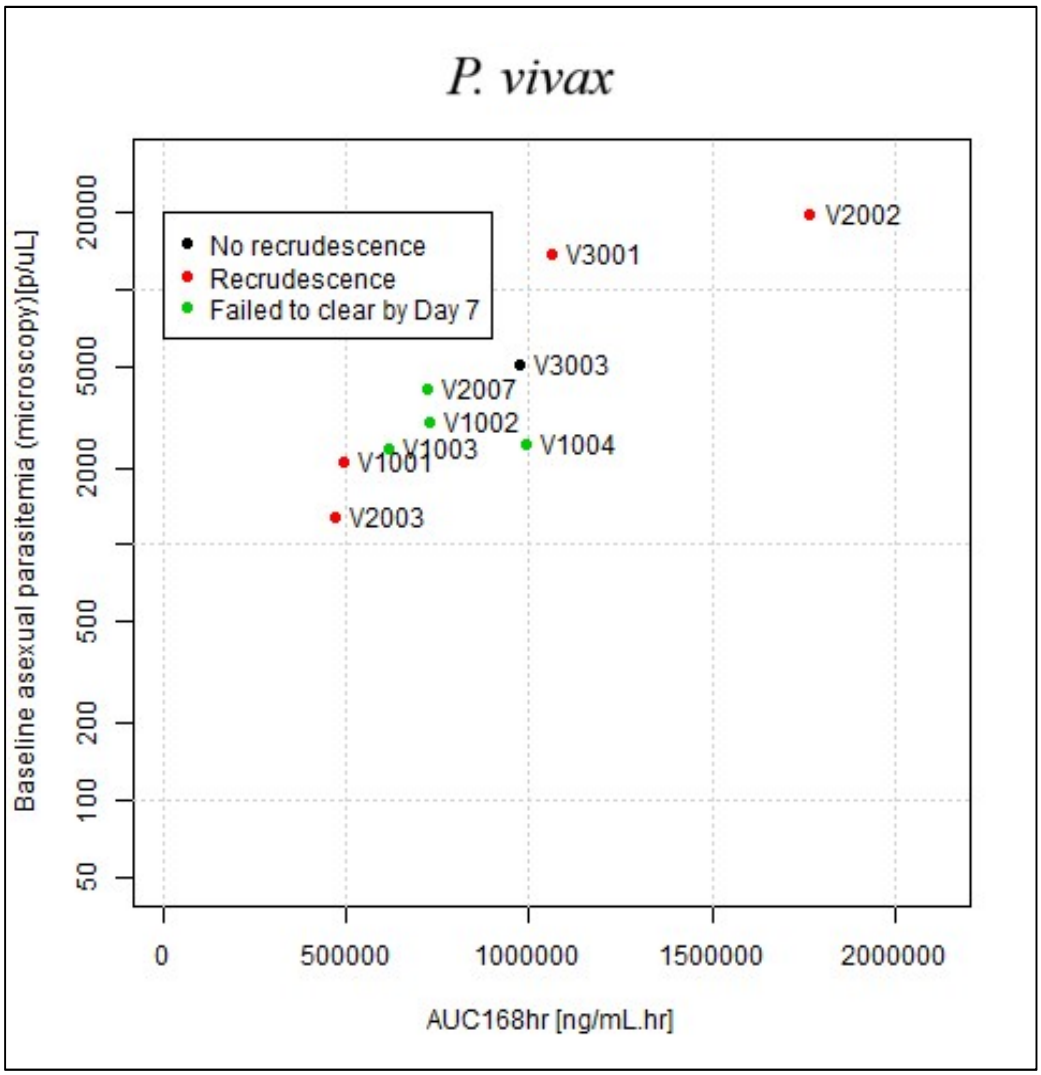


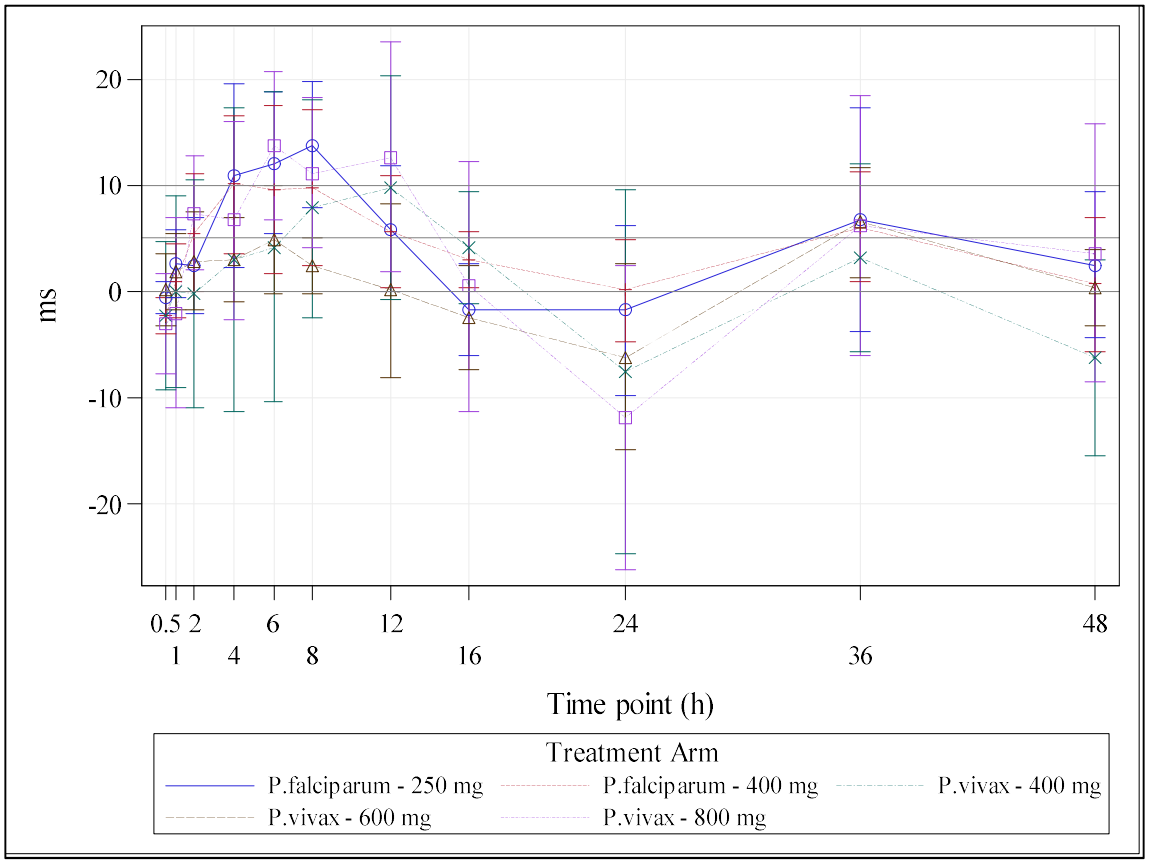
Figure S16: Effect of baseline parasitaemia and AUC_{168h} on treatment success of *P. vivax* patients at Day 14



For *P. falciparum* patients, the baseline parasitaemia seem to determine the rate of cure: the four patients with recrudescing parasites or insufficient clearance had the highest levels of parasitaemia, above 10,000 parasites/uL.
For *P. vivax* patients, DSM265 was not sufficiently effective and no trend could be found. Of the nine *P. vivax* patients reaching Day 14, only one cleared parasites by Day 7 without recrudescing by Day 14. For this patient, neither the baseline parasitaemia, nor the DSM265 exposure, stood out compared to the other patients.

ECG safety analysis

Figure S17: Analysis of central tendency of QTcF - Mean changes from baseline and two-sided 90% CI by type of infection and dose.



Central tendency analysis of other ECG parameters

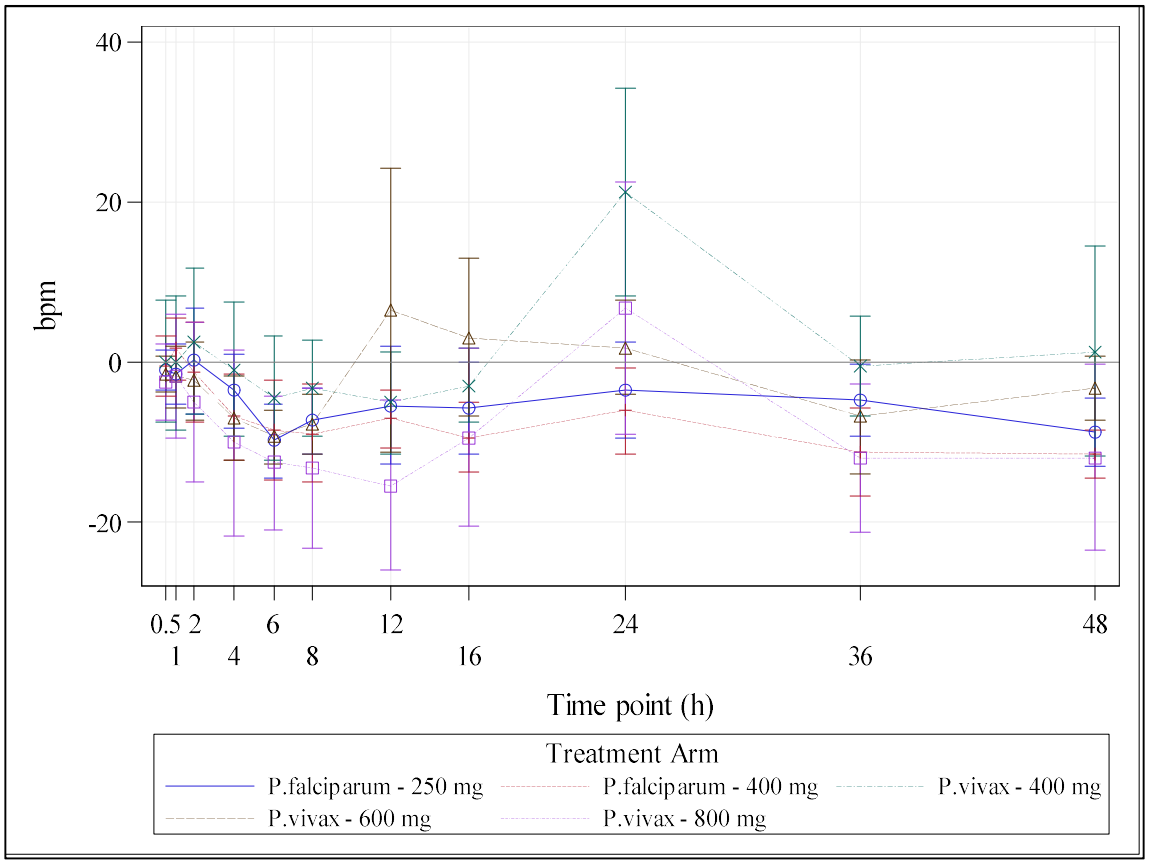
Graphical presentations of the descriptive statistics are shown in **Figure S18**,

Figure S24, **Figure S23**,

Figure S24, **Figure S25**and **Figure S26**, respectively.
At baseline, the HR in the different cohorts varied from 68.2 to 80.9 bpm. Following administration of DSM265, HR decreased in all cohorts and this decrease appeared dose-dependent in the *P. vivax* but not in the *P. falciparum* patients. On Day 1, the mean maximum observed decreases in HR were 9.8 (250 mg *P. falciparum*), 9.3 (400 mg *P. falciparum*), 5.0 (400 mg *P. vivax*), 9.3 (600 mg P. *vivax*) and 15.3 (800 mg *P. vivax*) bpm. Although HR tended

to return to baseline 24 h after DSM265 administration, in most cohorts a decrease in HR was maintained on Days 2 and 3 (**Figure S18**).

Figure S18: Analysis of central tendency of HR - Mean changes from baseline and two-sided 90% CI over time by type of infection and dose



Administration of DSM265 caused an increase from baseline in PR interval, which were maximal 6 to 8 h after administration but had returned to baseline 12 h after drug intake. The

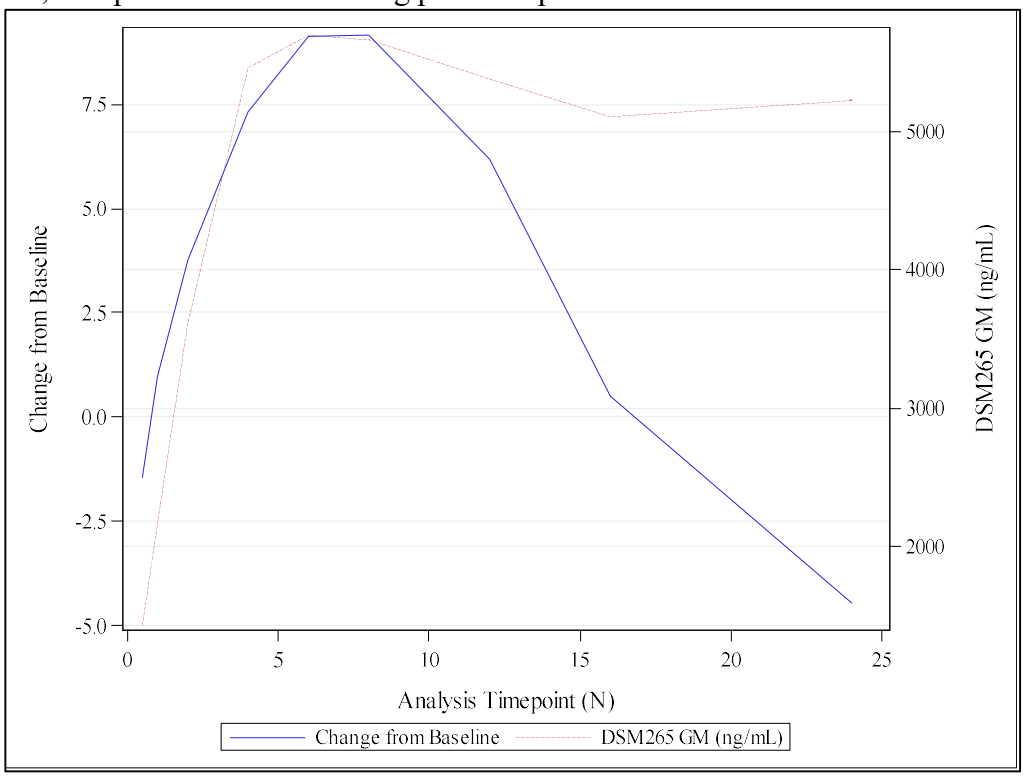
mean maximum increases varied from 4.6 to 10.5 ms between cohorts and appeared dose-dependent in the *P. vivax* but not *P. falciparum* cohorts (**Figure S23**).
Administration of DSM265 did not appear to affect the QRS interval (

Figure S24).
Although in the 250-mg *P. falciparum* cohort some incidental increases from baseline in QTcB were observed, administration of DSM265 had no consistent effect on this parameter (**Figure S26**).

Concentration-response analysis

Figure S19 indicates that during the initial increase in QTcF and decrease in HR, there was a relationship between DSM265 concentration and effect. However, in the presence of continued high DSM265 concentrations, effects on QTcF and HR dissipated indicating a loss of the relationship between concentration and response. Nevertheless, the trend curves on the plots of individual change from baseline in QTcF and HR versus DSM265 concentration did indicate a relationship for *P. falciparum* patients for both parameters but only for HR in the *P. vivax* patients (**Figure S20**).

Figure S19: Mean change in QTcF (upper panel, solid line) and HR (lower panel, solid line), and DSM265 geometric mean concentration (dotted line) over time in the overall population, *i.e.*, irrespective of the infecting parasite species and the dose.



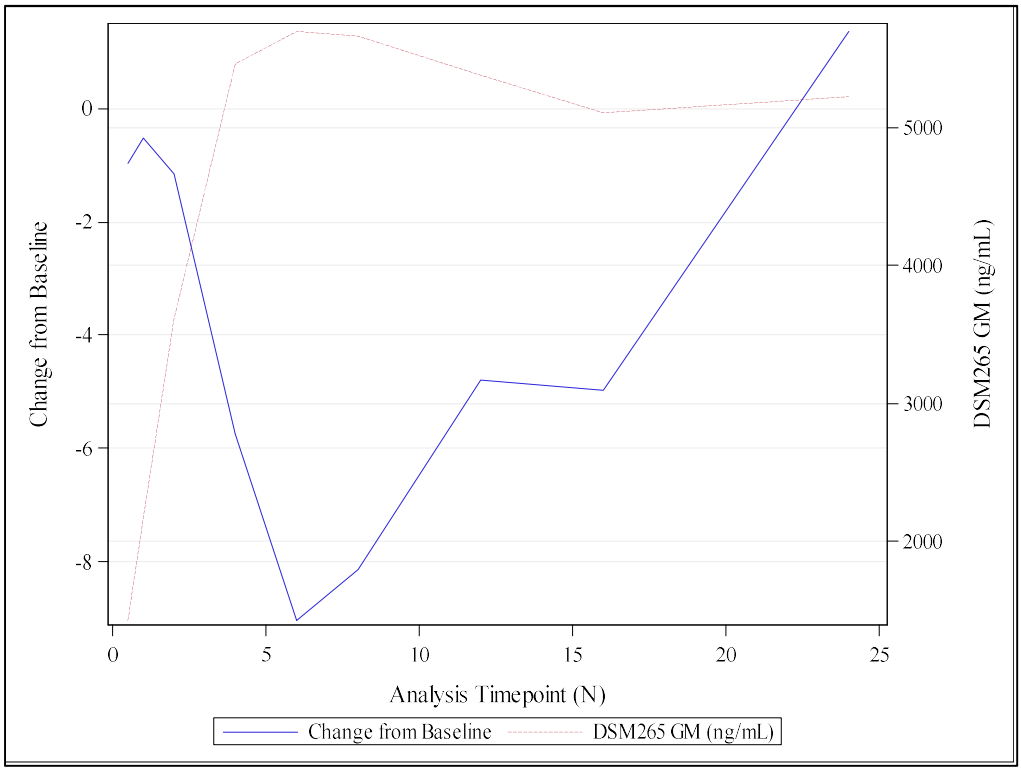
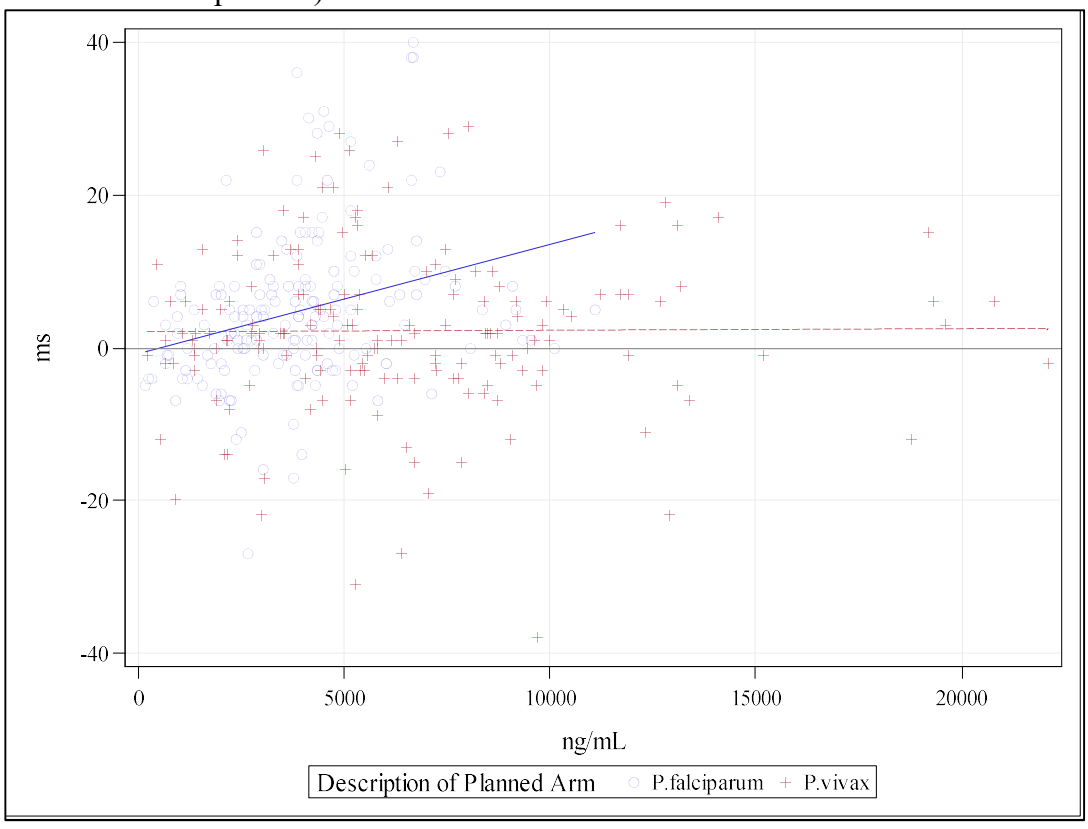
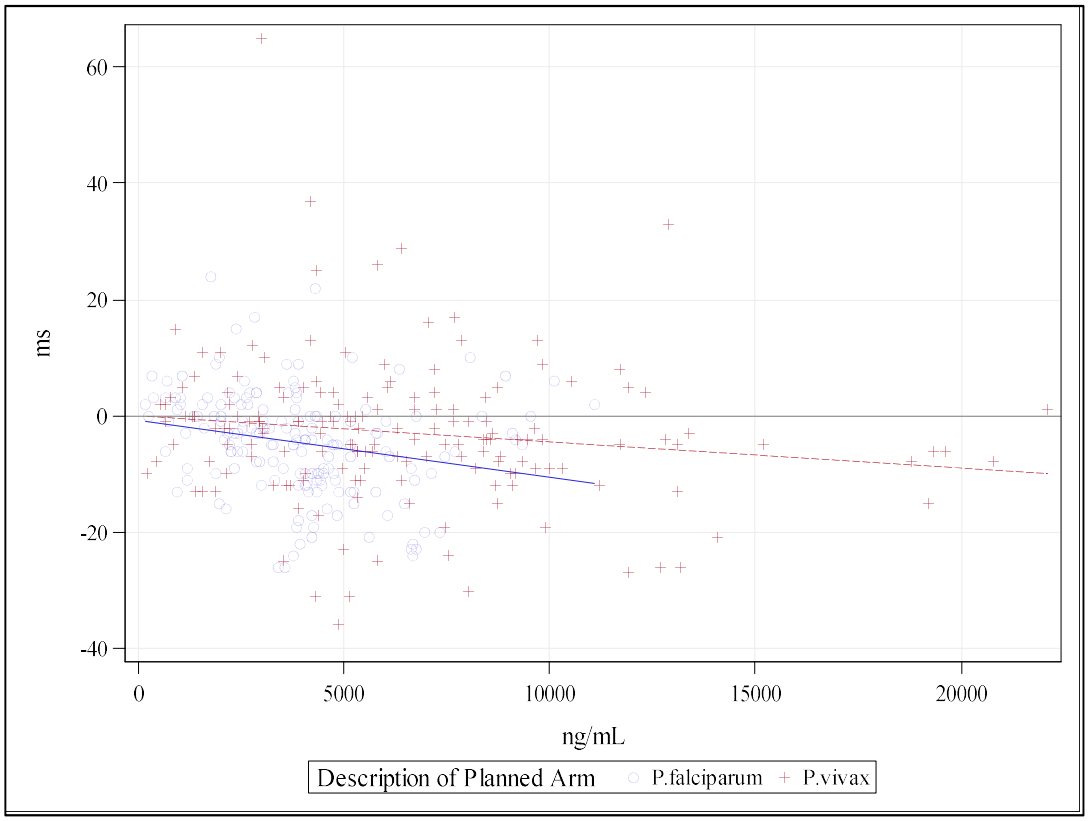


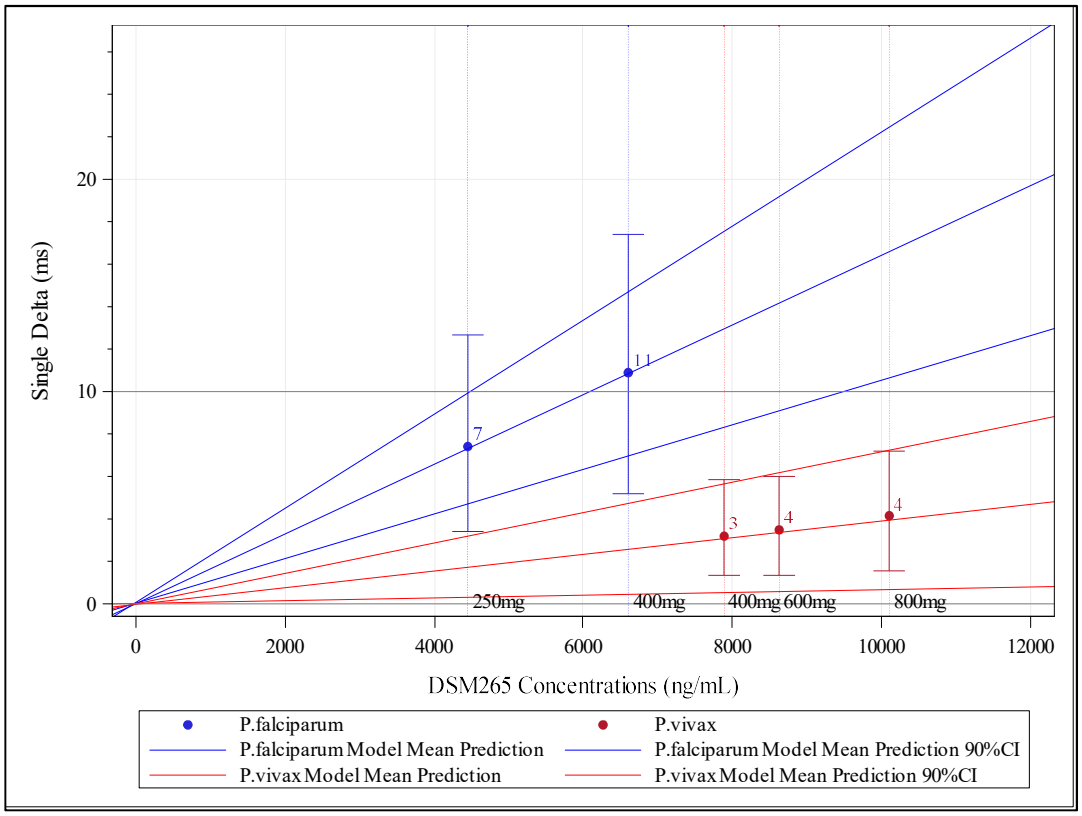
Figure S20: Scatter plots of individual changes in QTcF (upper panel) and HR (lower panel), and DSM265 concentration with trend curves (solid blue line for *P. falciparum* and dotted red line for *P. vivax* patients).

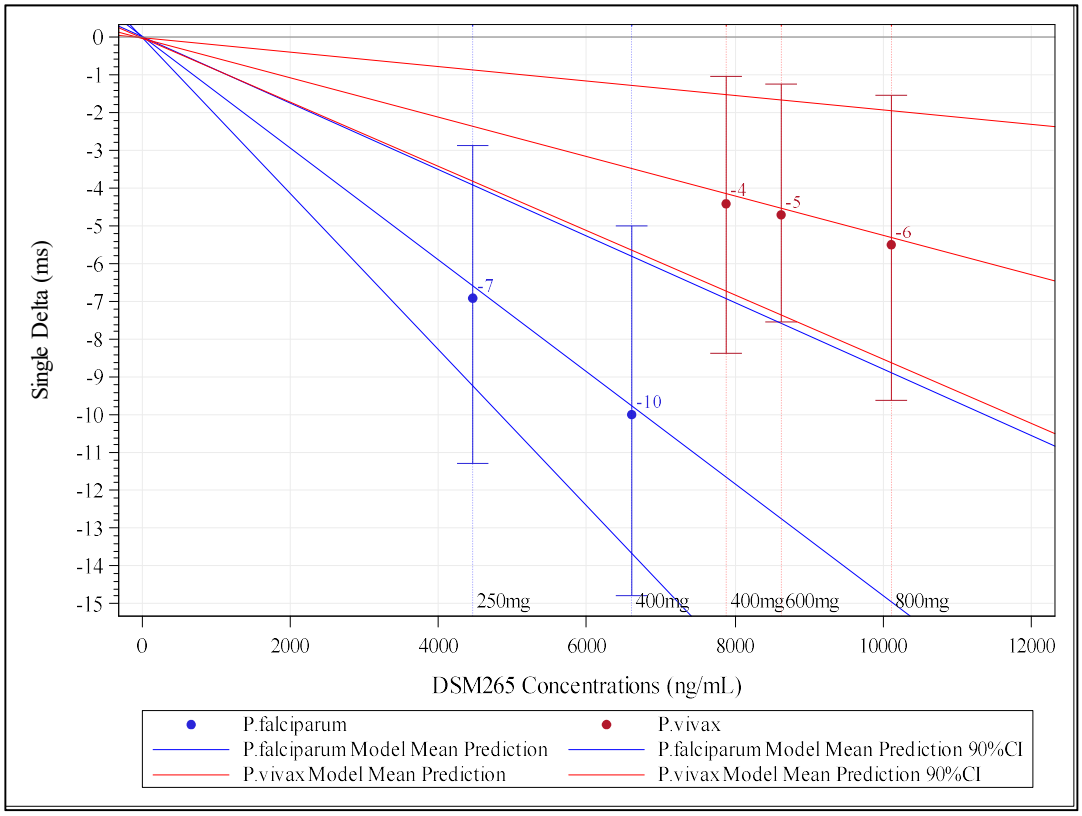




Results of the modelling of the concentration-response analysis are summarised in **Figure S21**.

Figure S21: Graphical display of model predictions for the maximum effect of DSM265 on QTcF (upper panel) and HR (lower panel) by infection type





Significant correlations between DSM265 plasma concentration and ΔQTcF and ΔHR were found in that with increasing DSM265 concentration an increase in QTcF and a decrease in HR

were predicted. According to the model, patients with *P. falciparum* infection appear to be more susceptible to these effects of DSM265 than *P. vivax* patients (**Figure S21**).

Categorical analysis of QTcF and other ECG parameters

Table S16 shows the number of patients with abnormal ECG parameter values. There were no values of QTcF >500 ms or changes from baseline exceeding 60 ms recorded in the present study. In a few patients the values for QTcF >450 ms but ≤500 ms were recorded. There appeared to be more patients with *P. falciparum* infection in whom a changes from baseline of >30 ms but ≤60 ms was recorded when compared to *P. vivax* patients. No abnormal PR and QRS interval values were recorded during the study (**Table S16**).

Table S16: Number (%) of patients presenting at least one abnormality on treatment

	P. falciparum		*P. vivax*		
Category	**250 mg (N=9)**	**400 mg (N=10)**	**400 mg (N=5)**	**600 mg (N=9)**	**800 mg (N=6)**
PR Interval (ms)					
PR>220 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Δ_{Rel}PR>25%	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
QRS Interval (ms)					
QRS>120 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Δ_{Rel}QRS>25%	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
QTcF (ms)					
QTc>450 ms	1 (11.1)	1 (10.0)	0 (0.0)	0 (0.0)	2 (33.3)
QTc>500 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ΔQTc>30 ms	3 (33.3)	2 (20.0)	0 (0.0)	0 (0.0)	1 (16.7)
ΔQTc>60 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
QTcB (ms)					
QTc>450 ms	1 (11.1)	1 (10.0)	1 (20.0)	1 (11.1)	2 (33.3)
QTc>500 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ΔQTc>30 ms	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ΔQTc>60 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Δ = Change from Baseline (Value - Baseline) - Δ_{Rel} = Relative Change from baseline (100x(Value-Baseline)/Baseline)

Morphological analysis

A summary of treatment-emergent abnormalities that occurred during the study by infection and dose is provided in **Table S17**.

During the study, 2 clinically significant ECG abnormalities occurred: one case of sinus tachycardia and one case of flat T wave, both occurred in the *P. vivax* 600 mg cohort. For 4 of 19 (21%) *P. falciparum* patients and 8 of 20 (40%) *P. vivax* patients, at least one abnormal but not clinically significant treatment-emergent ECG abnormality was reported. All of these abnormalities were incidental, *i.e.*, occurred in 1 or 2 patients, and for none could a relationship to dose be discerned (**Table S17**).

Table S17: Summary of treatment-emergent findings from the morphological analysis

			Number (%) of patients presenting at least one treatment-emergent abnormality				
			P .falciparum		*P. vivax*		
Conclusion	**Category**	**Abnormality nature**	**250 mg (N=9)**	**400 mg (N=10)**	**400 mg (N=5)**	**600 mg (N=9)**	**800 mg (N=6)**
Abnormal clinically significant	All	All				1 (11.1)	
	Rhythm	Sinus tachycardia, rate >130 bpm				1 (11.1)	
	T/U wave abnormalities	Flat T wave localised in widespread				1 (11.1)	
Abnormal non clinically significant	All	All	4 (44.4)		1 (20.0)	5 (55.6)	2 (33.3)
	Rhythm	Sinus tachycardia, rate 100-130 bpm	1 (11.1)				
		Sinus bradycardia, rate 40-49 bpm	1 (11.1)				1 (16.7)
		Premature atrial complexes conducted or non conducted	1 (11.1)				
		Premature ventricular complexes	2 (22.2)				
		Premature ventricular complexes, bigeminy pattern	1 (11.1)				
		Premature ventricular complexes, trigeminy pattern	1 (11.1)				
	T/U wave abnormalities	T wave inversion localized in antero-septal leads (v1 - v4)				1 (11.1)	

Table S17: Summary of treatment-emergent findings from the morphological analysis

			Number (%) of patients presenting at least one treatment-emergent abnormality				
			P .falciparum		*P. vivax*		
Conclusion	**Category**	**Abnormality nature**	**250 mg (N=9)**	**400 mg (N=10)**	**400 mg (N=5)**	**600 mg (N=9)**	**800 mg (N=6)**
		T wave inversion, nonspecific localised in antero-septal leads (v1 - v4)				1 (11.1)	1 (16.7)
	Intraventricular conduction defects	Lafb				1 (11.1)	
	T/U wave abnormalities	Flat T wave localized in lateral leads (i, avl, v5, v6)				1 (11.1)	
		Flat T wave localized in widespread				2 (22.2)	
		Bifid T wave localized in antero-septal leads (v1 - v4)			1 (20.0)		
		Bifid t wave localised in precordial leads (v1-v6)			1 (20.0)		
		Prolonged QT or QTcF interval above max QT/QTcF threshold	1 (11.1)				1 (16.7)
		QTcF increase from baseline, >30 msec and <60 msec	1 (11.1)				
Unable to evaluate but measurements provided are correct	All	All		1 (10.0)			1 (16.7)
	Technical	suspect arm lead reversal, interpretation assumes reversal		1 (10.0)			
		incomplete ECG, <9 leads present					1 (16.7)

Table S17: Summary of treatment-emergent findings from the morphological analysis			**Number (%) of patients presenting at least one treatment-emergent abnormality**				
			P .falciparum		***P. vivax***		
Conclusion	**Category**	**Abnormality nature**	**250 mg (N=9)**	**400 mg (N=10)**	**400 mg (N=5)**	**600 mg (N=9)**	**800 mg (N=6)**
N=Number of patients from the population							

Safety discussion and conclusions

The cardiac safety of single-dose oral administration of DSM265 was investigated in adult patients with acute, uncomplicated *P. falciparum* or *P. vivax* malaria mono-infection.
In this study, treatment of malaria patients with DSM265 appeared to cause a decrease in HR. However, in the acute phase of the disease, stress, anxiety and discomfort may lead to an increase in HR whereas during the recovery phase and after starting treatment, HR decreases and the QT interval lengthens. The observed effect of DSM265 on HR may well be an indirect effect due to its parasite-killing properties and thereby treating the disease rather than a direct effect on the heart.
Administration of DSM265 resulted in an increase in QTcF of approximately 10 ms depending on dose and type of infection. The maximum effect occurred 6 to 8 h after administration, *i.e.*, at the same time when DSM265 plasma concentrations peaked. Following the maximum effect, QTcF values returned to baseline whereas DSM265 concentrations remained high.
The concentration-response analysis did indicate a relationship between effects on QTcF and HR and DSM265 concentrations. This relationship appeared much stronger in *P. falciparum* patients when compared to *P. vivax* patients, an observation that is difficult to explain.
DSM265 treatment caused an increase in PR interval whereas no effect on QRS interval and, interestingly, QTcB was observed.
Since the clinical condition of patients with malaria may induce changes in HR and the QT interval, the effects of antimalarial treatments on cardiac repolarisation in patients are to be interpreted with caution and are better studied in healthy subjects.
In conclusion:
Single-dose administration of DSM265 appeared to cause increases in QTcF and PR interval, and a decrease in HR without affecting QRS interval and QTcB, which in part were dependent on the infecting *Plasmodium* species.
The observed effects of DSM265 on the ECG may be confounded by the parasite-killing effect of DSM265 that decreases disease symptoms.

Figure S22: Analysis of central tendency of RR interval (ms) - Mean changes from baseline and two-sided 90% CI over time

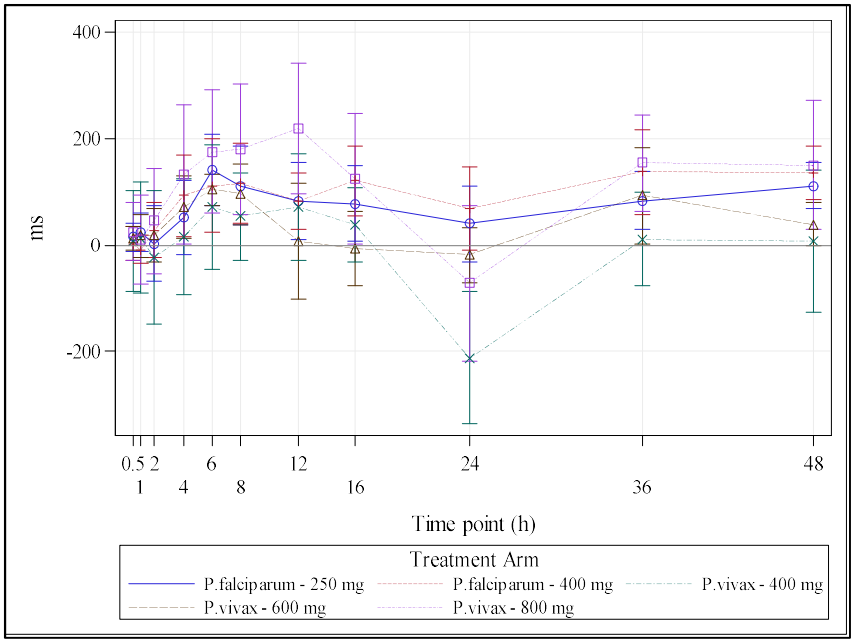


Figure S23: Analysis of central tendency of PR interval (ms) - Mean changes from baseline and two-sided 90% CI over time

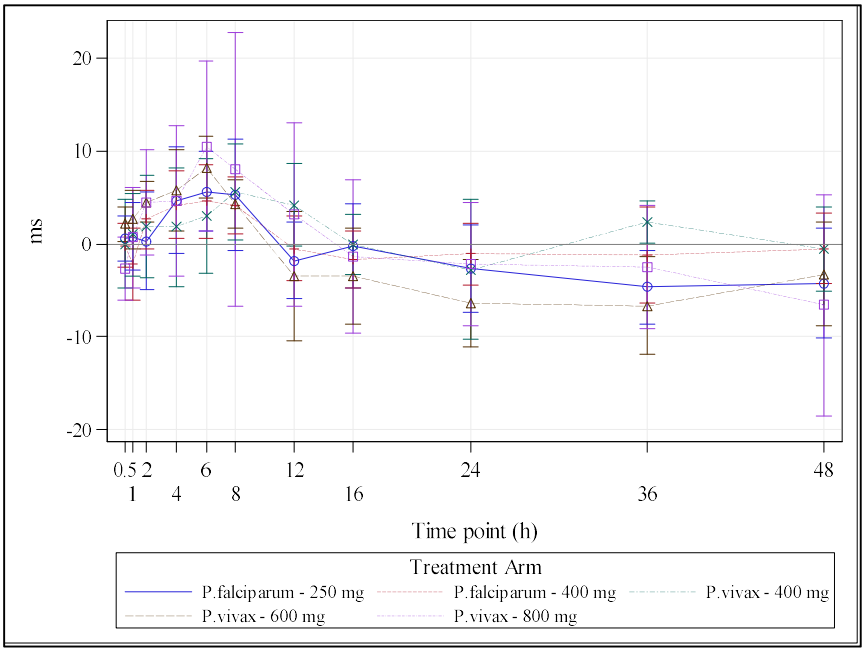


Figure S24: Analysis of central tendency of QRS interval (ms) - Mean changes from baseline and two-sided 90% CI over time

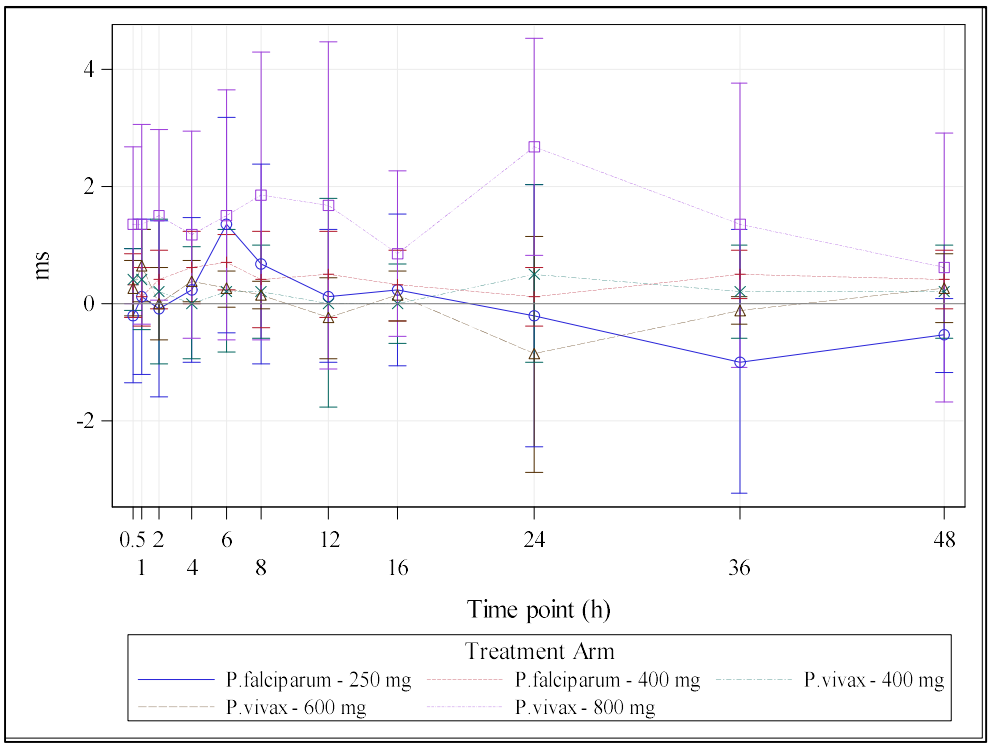


Figure S25: Analysis of central tendency of QT interval (ms) - Mean changes from baseline and two-sided 90% CI over time

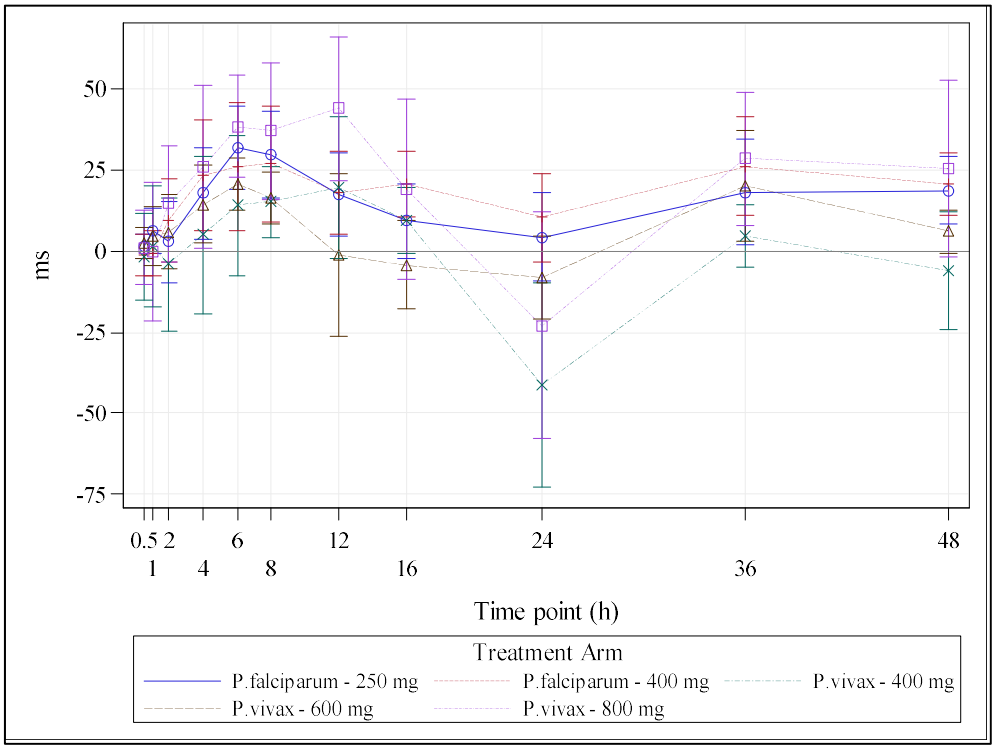
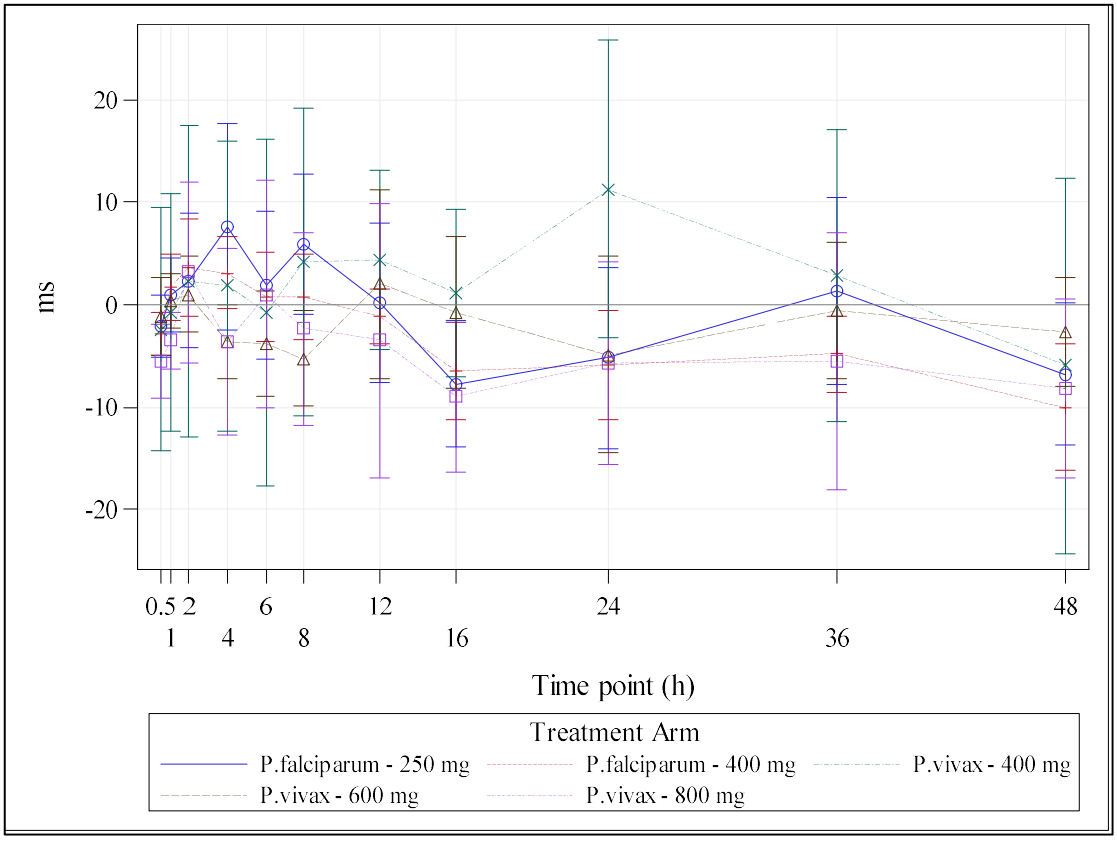


Figure S26: Analysis of central tendency of QTcB (ms) - Mean changes from baseline and two-sided 90% CI over time



Resistance Analysis

Selective Whole-Genome Amplification (SWGA) of Clinical Samples

Primers for SWGA of *P. falciparum* and *P. vivax* from clinical samples were designed and SWGA was carried out as previously described[1,2]. Primer set 6A followed by primer set 8A were used to perform two rounds of SWGA on the *P. falciparum* samples. Primer set pv1920 was used to perform one round of SWGA on the *P. vivax* samples. All primers contain phosphorothioate bonds between the two nucleotides at the 3' end to prevent degradation by the phi29 enzyme as indicated by asterisks.

Primer set pv1920:
5'-AACGAAGC*G*A-3'
5'-ACGAAGCG*A*A-3'
5'-ACGACGA*A*G-3'
5'-ACGCGCA*A*C-3'
5'-CAACGCG*G*T-3'
5'-GACGAAA*C*G-3'
5'-GCGAAAAA*G*G-3'
5'-GCGAAGC*G*A-3'
5'-GCGGAAC*G*A-3'
5'-GCGTCGA*A*G-3'
5'-GGTTAGCG*G*C-3'
5'-AACGAAT*C*G-3'

Primer set 6A:
5′-TAAATAAAAA*A*A-3′
5′-CATAAAAAA*A*A-3′
5′-TAAATAATAA*T*A-3′
5′-ATCATAATA*A*T-3′
5′-TAACAAAAAA*A*A-3′
5′-TAATAAATAA*A*A-3′
5′-TAACATAGG*T*C-3′
5′-TAGTAGTAG*T*A-3′
5′-ATAATAAATA*A*T-3′
5′-CATAATAATA*A*T-3′)

Primer set 8A :
5′-TTTTTTTATT*T*A-3′
5′-TATTATTATT*T*A-3′
5′-TTTTTTTAT*G*T-3′
5′-ATTATTATG*A*T-3′
5′-TTTTTTTTGT*T*A-3′
5′-TATTTATTAT*T*A-3′
5′-GACCTATG*T*TA-3′
5′-TACTACTAC*T*A-3′

5′-TATTATTTAT*T*A-3′
5′-TATTATTATT*G*T-3′

For the SWGA reactions, 8-23 ng of input DNA was added to a 50 µl reaction containing 3.5 µM SWGA primers, 4mM dNTPs, 30 U phi29 DNA polymerase enzyme (New England BioLabs), 1x phi29 buffer (New England BioLabs), 1% bovine serum albumin, and water. The reaction was carried out on a thermocycler consisting of a ramp down from 35°C to 30°C (10 minutes per degree), 16 hours at 30°C, 10 minutes at 65°C, and hold at 4°C. The samples were diluted 1:1 with DNase-free, RNase-free water and purified with Ampure XP beads (Beckman-Coulter) at a 1:1 ratio per the manufacturer's protocol. When performed, a second round of SWGA contained 100-200 ng of the Ampure XP purified product from the first reaction.

Whole-genome sequencing

Sequencing libraries of the SWGA products were prepared using the Nextera XT DNA preparation kit (Illumina) per the manufacturer's protocol. Samples were pooled and clustered on a Hiseq 2500 (Illumina) in Rapid Run mode with 100 base pair paired end reads. Raw fastq files were aligned to the Sal-1 reference genome (PlasmoDB version 13, http://plasmodb.org/common/downloads/release-13.0/PvivaxSal1/fasta/data/) or the 3D7 reference genome (PlasmoDB version 13, http://plasmodb.org/common/downloads/release-13.0/Pfalciparum/fasta/data/) using the Burroughs-Wheeler Aligner (version 0.7.8) and samtools (version 0.1.19) as previously described[3]. Picard (version 2.0.1) was used to remove unmapped reads and the Genome Analysis Toolkit (GATK)[4] was used to realign the sequences around the indels.

Variant Calling and Analysis

We followed the GATK's best practices to call variants[5,6]. The aligned sequences were run through GATK's HaplotypeCaller in "reference confidence" mode to create genomic GVCF files for each sample, then samples were joint genotyped using the GenotypeGVCFs tool. Variants were further filtered based on quality scores and sequencing bias statistics based on default parameters from GATK. SNPs were filtered out if they met any of the following criteria: Quality Depth (QD) < 2.0, Mapping Quality (MQ) < 50.0, Phred-scaled p-value using Fisher's exact test to detect strand bias (FS) >60.0, Symmetric Odds Ratio (SOR) >4.0, Z-score from Wilcoxon rank sum test of Alternative vs. Reference read mapping qualities (MQRankSum) < -12.5, ReadPosRankSum (RPRS) < -8.0. Variants were annotated using snpeff (version 4.2)[7]. Custom

scripts were used to perform further sample comparisons. DoR refers to the sample collected at day of recrudescence.

Table S 18: Read coverage for SWGA.

Sample	Total reads	% aligned reads (P. fal)	mean coverage	% bases > 5 reads
DSM265-F1001-D1-SWGA	20168104	92.5	65.5	89.8
DSM265-F1001-DoR-SWGA	21506102	28.1	18.2	37.9
DSM265-F1008-D1-SWGA	22813366	82	64.7	89.5
DSM265-F1008-D DoR -SWGA	20100780	63.2	32.2	69.1
DSM265-F1009-D1-SWGA	22534472	67.7	55.05	88.3
DSM265-F1009-D DoR -SWGA	19945758	48	29.35	70.5
DSM265-F1010-D1-SWGA	19740674	91.3	62.4	89.3
DSM265-F1010-D DoR -SWGA	24291660	66	56.22	87
DSM265-F2001-D1-SWGA	27765874	87	76.32	89.3
DSM265-F2001-D DoR -SWGA	33893106	87.8	19	2.6
DSM265-F2005-D1-SWGA	22747604	93.7	65.3	74.9
DSM265-F2005-D DoR -SWGA	21309272	71.4	46.1	72.9
DSM265-F2009-D1-SWGA	24195342	88.3	72.1	90.5
DSM265-F2009-D DoR -SWGA	24596752	54.3	36.9	44.6
Average	23154778	72.95	49.95	71.16

Table S 19: Read coverage for C276Y and G181S dihydroorotate reductase alleles for subjects on Day 1 and either Day 28 or the day of recrudesence. The high read coverage in F2005 is suggestive of an amplification event. Ref: Reference reads; Alt: Alternative reads.

Chr	Pos.	All.		F1001		F1008		F1009		F1010		F2001		F2005		F2009	
				1	28	1	20	1	10	1	11	1	21	1	28	1	14
6	131,212	C276Y	Ref C	46	0	50	18	53	10	48	45	59	-	26	113	48	-
			Alt T	0	58	0	0	0	0	0	0	0	-	0	0	0	-
6	131,498	G181S	Ref C	37	11	50	6	36	-	40	14	41	-	5	111	25	4
			Al T	0	0	0	0	0	-	0	0	0	-	0	17	0	0

Table S 20: Mutants were obtained in drug selection studies *in vitro* with cultured *P. falciparum* asexual blood-stage parasites (the Dd2 B2 clone) an d the listed mutants had the same mutations as those found in patient samples. The fold shift for the C276F mutant was previously reported[8] and was based on mean IC_{50} values. This mutant Dd2 line remained fully sensitive to atovaquone and artemisinin (data not shown)[8].

AA mutations	**Parasite**	**Drug Pressure [DSM265]**	**Number of parasites**	**Fold IC_{50} change**
C 276 F	Dd2 R1A.Clb	60 nM	2×10^9	32
C 276 Y	Dd2 DSM265 R2 H9	28 nM	2×10^9	18

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