Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Materials

Higher-Dose Primaquine to Prevent Relapse of *Plasmodium vivax* Malaria

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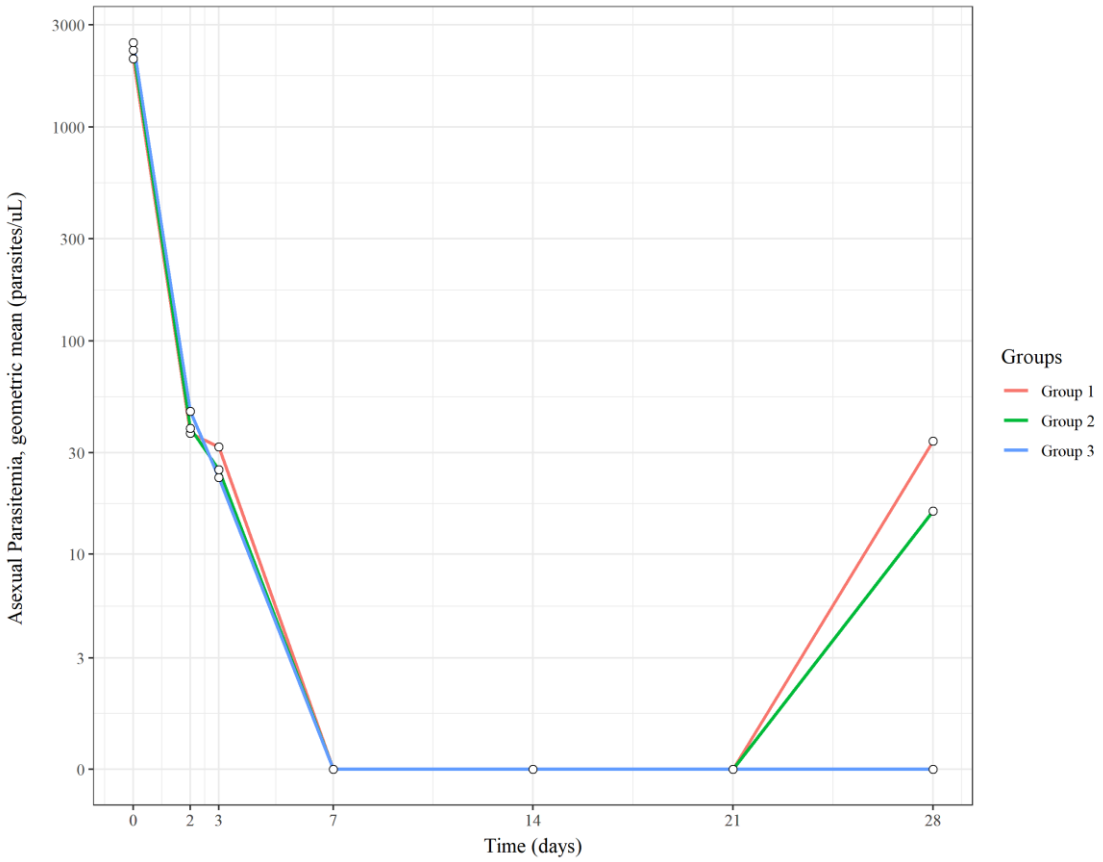
Figure S1. Geometric mean of asexual parasite density of *Plasmodium vivax* among patients per treatment group during the first 28 days of follow-up, Cruzeiro do Sul, 2018 (n= 254).*



Note:

*Mean including only non-zero values

Table S1. Selected sociodemographic data of the population affected by *Plasmodium vivax* in Cruzeiro do Sul, Acre State, and Brazil for 2018.[*,**] (Source: Brasil. Ministério da Saúde: Saúde de A a Z-Malária. [accessed on October 19, 2021]. Available at: https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/m/malaria)

Variables	Region					
	Cruzeiro do Sul		Acre State		Brazil	
	n	%	n	%	n	%
Sex						
Male	6,099	44.3	11,465	43.6	76,891	39.7
Female	7,679	55.7	14,841	56.4	116,946	60.3
Age (years)						
≤18	6,523	47.3	12,641	48.1	81,907	42.3
19–59	6,761	49.1	12,713	48.3	102,811	53.0
≥60	494	3.6	952	3.6	9,119	4.7
Race						
White	450	3.3	1,155	4.4	10,481	5.4
Black	55	0.4	316	1.2	5,635	2.9
Asian	8	0.1	165	0.6	988	0.5
Brown	13,199	95.8	24,134	91.7	133,286	68.8
Indigenous	66	0.5	536	2.0	40,785	21.0
Site of diagnosis						
Rural	8,065	58.5	11,952	45.4	88,175	45.5
Urban	5,713	41.5	14,354	54.6	105,662	54.5
Parasite density (parasites/mm^3)						
200–300	3078	22.3	5230	19.9	25129	13.0
301–500	2973	21.6	5005	19.0	35066	18.1
501–10,000	2490	18.1	5157	19.6	65144	33.6
10,001–100,000	50	0.4	135	0.5	4814	2.5
>100,000	2	0.0	8	0.0	168	0.1
Symptom duration (hours)						
Not informed	653	4.7	1,126	4.3	8,696	4.5
≤24	1,318	9.6	3,025	11.5	29,289	15.1
24–48	7,687	55.8	13,500	51.3	80,781	41.7
49–72	2,056	14.9	3,793	14.4	29,403	15.2
≥73	2,064	15.0	4,862	18.5	45,668	23.6
Total	**13,778**	**100**	**26,306**	**100**	**193,837**	**100**

Notes: * Data by notification sites, cases of treatment verification slides excluded

** Due to missing values for some variables, sums not exact

Table S2. Overview on the representativeness of study participants compared to Cruzeiro do Sul municipality, 2018.

Category	Comment
Disease and condition under investigation	*Plasmodium vivax* malaria. Study on the therapeutic efficacy of chloroquine and primaquine to treat and prevent relapse of vivax malaria comparing lower (3.5 mg/kg) and higher (7.0 mg/kg) total doses of primaquine.
Overall considerations related to	
Sex	There was a slight majority of men (54.7%) in the study, but differences to trends in Cruzeiro do Sul and Brazil do not seem to be meaningful.
Age range (years)	The age median of the participants were 22.4 years, which also reflects trends in Cruzeiro do Sul and Brazil of malaria affecting younger populations.
Duration of fever	Study population had a mean duration of fever of 2 days, close to duration of malaria symptoms at presentation in Cruzeiro do Sul.
Place of residence	The majority of the participants (70.9%) were from urban areas, likely reflecting the urban nature of our study design. We do not expect this to have caused an issue with representativeness or generalizability of our findings. In fact, since malaria transmission is higher in rural areas, focusing antirelapse efficacy evaluations in urban settings help diminish the contribution of reinfection to recurrence rates during follow-up.
Background information of the population affected by malaria in Brazil	In Brazil, malaria occurs mainly in the Amazon Basin, which concentrates >99.0% of malaria cases in the country. Most cases in the Amazon are autochthonous and caused by *P. vivax*. The estimated relapse rate for the Brazilian Amazon region is 20.8%, which is likely underreported due to limitations of passive surveillance. We believe our study showed relapse rates closer to reality. Adequate antirelapse treatment is of utmost importance to prevent malaria morbidity and mortality.
Other relevant considerations of this malaria efficacy trial	We believe our study population is similar to that most affected by vivax malaria in Brazil. Our study followed recommendations for antimalarial efficacy trials and had a long follow-up, which allows for most relapses to occur and adequately support our findings.

Table S3. Plasma levels of chloroquine and desethylchloroquine on day 7 and day of recurrence in patients with vivax recurrence on or before day 28, Cruzeiro do Sul, 2018 (n= 3).

Patient	Plasma levels (ng/mL)		
	Chloroquine	Desethylchloroquine	Chloroquine plus desethylchloroquine
B069			
Day 7	58.8	69.7	128.5
Day 28	12.3	25.3	37.6
B076			
Day 7	61.0	84.3	145.3
Day 28	4.7	11.2	15.8
A114			
Day 7	103.1	126.5	229.6
Day 28	10.5	18.9	29.4

Table S4. Patient's status in the per-protocol analysis per study group on day 28 and day 168, Cruzeiro do Sul, 2018 (n = 254).

Outcome	Study group			
	Group 1	**Group 2**	**Group 3**	**Total**
Per-protocol day 28 analysis				
Adequate clinical and parasitological response (day 28)	61	88	90	239
Any *Plasmodium vivax* recurrence	2	1	0	3
Homologous recurrences	1	1	0	2
Heterologous recurrences	0	0	0	0
Missing genotype	1	0	0	1
Patients considered in day 28 per-protocol analysis	**63**	**89**	**90**	**242**
P. falciparum infections	0	3	1	4
Loss to follow-up	0	4	4	8
Per-protocol day 168 analysis				
Recurrence free by day 168	29	44	67	140
Any *P. vivax* recurrence	24	34	12	70
Homologous recurrences	12	17	4	33
Heterologous recurrences	8	11	6	25
Missing genotype	4	6	2	12
Patients considered in day 168 per-protocol analysis	**53**	**78**	**79**	**210**
P. falciparum infections	2	4	4	10
Loss to follow-up	8	14	12	34
Total patients enrolled	**63**	**96**	**95**	**254**

Table S5. Day 28 and day 168 survival probabilities (day 28 adequate clinical and parasitological response [ACPR] and day 168 recurrence-free proportions) considering any vivax recurrence, 95% confidence interval (CI), and standard errors derived from the Kaplan–Meier survival analysis in the intention-to-treat population, Cruzeiro do Sul, 2018 (n = 254).

Study day and group	Survival probability (%)	95% CI	Standard error
Day 28			
Group 1	96.8%	91.1–99.7%	0.2
Group 2	98.9%	95.7–100%	0.1
Group 3	100%	NA	NA
Day 168			
Group 1	58.4%	44.5–70.0%	0.07
Group 2	58.8%	47.4–68.6%	0.05
Group 3	85.6%	76.0–91.6%	0.04

Notes:

CI= confidence interval

NA= not available

Rounding of percentages to whole numbers in the final (published) manuscript

Table S6. Differences in survival probabilities (day 28 adequate clinical and parasitological response [ACPR] and day 168 recurrence-free proportions) on days 28 and 168 considering any vivax recurrence, P value (Wald test), and 97.5% confidence interval (CI) derived from the Kaplan–Meier survival analysis in the intention-to-treat population, Cruzeiro do Sul (n=254).

Comparison per study day	**Difference in survival (%)**	**97.5% CI (%)**	**P value**
Day 28			
Group 3 vs. Group 1	3.2%	NA	NA
Group 3 vs. Group 2	1.1%	NA	NA
Day 168			
Group 3 vs. Group 1*	27.2%	10.2–44.3%	<0.001
Group 3 vs. Group 2*	26.8%	11.8–41.8%	<0.001

Notes:

CI= confidence interval

NA= not available

* Log rank comparison of respective survival curves: P <0.001

Rounding of percentages to whole numbers in the final (published) manuscript

Table S7. Day 28 adequate clinical and parasitological response (ACPR) and day 168 recurrence-free proportions in the per-protocol population per study group, Cruzeiro do Sul, 2018 (n= 254).

Group	Per-protocol analysis considering any vivax recurrence		Per-protocol analysis considering only homologous vivax recurrences
	Day 28 ACPR proportion, % (95% CI)	**Day 168 recurrence-free proportion, % (95% CI)**	**Day 168 recurrence-free proportion, % (95% CI)**
Group 1 (n=63)	96.8% (89.0–99.6%)	54.7% (40.4–68.4%)	70.7% (54.5–83.9%)
Group 2 (n=96)	98.9% (93.9–100%)	56.4% (44.7–67.6%)	72.1% (59.2–82.9%)
Group 3 (n=95)	100% (96.0–100%)	84.8% (75.0–91.9%)	94.4% (86.2–98.4%)

Note:

CI= confidence interval

Table S8. Haplotypes classification of paired samples *Plasmodium vivax* recurrences with valid microsatellite results, Cruzeiro do Sul, 2018 (n= 58).

Patient	Haplotype classification	Study day	Microsatellite						
			MS2	MS6	Pv3.502	Pv11.162	Pv12.35	Pvms038	PV10.29
A003	Heterologous	D000	197	246	133	189	**165**	206	116
A003		D083	197	246	133	189	**161**	206	110
A004	Heterologous	D000	**197**	246	133	189	161	206	116
A004		D081	**200**	246	133	189	161	206	116
A007S	Heterologous	D000	**197**	246	133	189	161	206	116
A007S		D151	**200**	246	133	189	161	206	116
A009	Homologous	D000	210	211	133	193	165	194	110
A009		D049	210	211	133	193	165	194	110
A026	Heterologous	D000	**214**	211	133	217	165	206	126
A026		D056	**197**	211	133	217	165	202	120
A038	Heterologous	D000		**246**	133	189		206	110
A038		D051	218	**240**	133		165	206	110
A044	Homologous	D000	222	211	133	221	167	206	110
A044		D106	222	211	133	221	167	206	110
A048	Homologous	D000	197	246	133	189	161	206	116
A048		D112	197	246	133	189	161	206	116
A054	Heterologous	D000	197	240	133	**221**		206	**116**
A054		D041	197	240	133	**189**	161	206	**110**
A057	Homologous	D000	210	211	133	209	161	190	116
A057		D075	210	211	133	209	161	190	116
A061S	Heterologous	D000	200	**246**	133	**189**	161	206	116
A061S		D112	200	**211**	133	**221**	161	206	116
A067	Homologous	D000	214	211	133	221	171	206	116
A067		D084	214	211	133	221	171	206	116
A076	Homologous	D000	210	240	133	193	163	190	120
A076		D140	210	240	133	193	163	190	120
A092	Homologous	D000	197, 205	240, 243	133, 142	181, 185, 217	163, 167	198, 202, 206	110
A092		D061	197, 210	211, 243	133, 142	181	163, 175	198, 202, 206	110
A094	Homologous	D000	210	211	133	185	161	190	116
A094		D066	210	211	133	185	161	190	116

Patient	Haplotype classification	Study day	Microsatellite						
			MS2	MS6	Pv3.502	Pv11.162	Pv12.35	Pvms038	PV10.29
A095	Heterologous	D000	210	**240**	133	193	165	194	110
A095		D081	210	**211**	133	193	165		110
A100	Homologous	D000	197	246	133	189	161	206	116
A100		D040	197	246	133	189	161	206	116
A101S	Homologous	D000	210	211	133	189	161	198	116
A101S		D085	210	211	133		161	198	116
A110	Homologous	D000	210	240	133	185	165	190	110
A110		D076	210	240	133	185	165	190	110
A113	Heterologous	D000	**210**	**211**	**167**	**213**	**161**	**198**	**116**
A113		D084	**218**	**246**	**133**	**217**	**165**	**206**	**110**
A114	Homologous	D000	210	211	133	193	165	194, 206	110
A114		D028	210	211	133	193	165	206	110
A115	Homologous	D000	197	246	133	189	161	206	116
A115		D056	197	246	133	189	161	206	116
A119	Homologous	D000	200	246	133	193	161	206	116
A119		D063	200	246	133	193	161	206	116
A125S	Heterologous	D000	**197**	**246**	133	**193**	**161**	**206**	116
A125S		D077	**210**	**211**	133	**217**	**169**	**198**	116
A129	Homologous	D000	197	246	133	193	161	206	116
A129		D047	197	246	133	193	161	206	116
A130S	Homologous	D000	210	211	133	193	165	206	110
A130S		D137	210	211	133	193	165	206	110
A134	Homologous	D000	210	211	133	189	161	198	116
A134		D085	210	211	133	189	161	198	116
A141	Homologous	D000	197	246	133	193	161	206	116
A141		D112	197	246	133	193	161	206	116
A143	Heterologous	D000	197	**246**	133	**189**	161	206	116
A143		D138	197	**211**	133	**185**	161	206	116
A144	Homologous	D000	210	246	133	189	169	190	120
A144		D084	210	246	133	189	169	190	120
A147	Heterologous	D000	**200**	211	133	217	**161**	198	**110**
A147		D056	**218, 210**	211	133	217	**169, 171**	198, 206	**116**
A150	Heterologous	D000	**205**	**246**	133	**189**	**165**	**198**	**110**
A150		D056	**210**	**240**	133	**193**	**163**	**190**	**120**

Patient	Haplotype classification	Study day	Microsatellite						
			MS2	MS6	Pv3.502	Pv11.162	Pv12.35	Pvms038	PV10.29
B004	Homologous	D000	197	246	133	189	161	206	116
B004		D091	197	246	133	189	161	206	116
B005S	Heterologous	D000	200	211	175	189	169	**198**	110
B005S		D064	200	211	175	189	169	**200**	110
B007	Homologous	D000	210	211	133	193	163	194	110
B007		D048	210	211	133	193	163	194	110
B008	Homologous	D000	197	240	133	181	161	206	110
B008		D084	197	240	133	181	161	206	110
B016	Homologous	D000	197	246	133	189	161	206	116
B016		D056	197	246	133	189	161	206	116
B021	Homologous	D000	197	246	133	189	161	206	116
B021		D056	197	246	133	189	161	206	116
B024	Homologous	D000	214	211	133	193	165	206	122
B024		D052	214	211	133	193	165	206	122
B032	Homologous	D000	197	246	133	185	161	206	116
B032		D084	197	246	133	185	161	206	116
B042	Heterologous	D000	**200**	246	133	189	161	206	116
B042		D140	**197**	246	133	189	161	206	116
B048	Heterologous	D000	197	**243**	**142**	**221**	**163**	**206**	**110**
B048		D098	197	**240**	**167**	**217**	**169**	**194**	**124**
B050	Heterologous	D000	210	240	133	193	163	**190**	**120**
B050		D096			133			**206**	**116**
B053T	Homologous	D000	210	246	133	189	175	194	116
B053T		D056	210	246				194	116
B061	Homologous	D000	205	240	133	189	161	206	110
B061		D125	205	240	133	189	161	206	110
B067	Heterologous	D000	**197**	**243**	**142**	**185**	**163**	206	110
B067		D056	**210**	**211**	**133**	**193**	**169**	206	110
B069	Homologous	D000	210	246	133	189	163	198	122
B069		D028	210	246	133	189	163	198	122
B070	Homologous	D000	197	211	167	217	161	206	110
B070		D078	197	211	167	217	161	206	110
B075	Heterologous	D000	197	240	133	185	165	206	**110**
B075		D141	197	240	133	185	165	206	**116**

Patient	Haplotype classification	Study day	Microsatellite						
			MS2	**MS6**	**Pv3.502**	**Pv11.162**	**Pv12.35**	**Pvms038**	**PV10.29**
B078	Heterologous	D000	**197**	240	133	185	**169**	**202**	120
B078		D084	**210**	240	133	185	**163**	**198**	120
B079	Homologous	D000	210	211	133	193	165	194, 206	110
B079		D140	210	211			165	206	110
B083	Homologous	D000	210	211	133	193	165	206	110
B083		D103	210	211	133	193	165	206	110
B088	Heterologous	D000	197	211	**167**	209	163	**198**	**112**
B088		D140		211	**133**		163	**190**	**116**
B091	Heterologous	D000	**214**	211	133	221	171	206	116
B091		D084	**218**	211	133	221	171	206	116
B097	Homologous	D000	197	211	133	217	165	206	126
B097		D088	197	211	133	217	165	206	126
B101	Heterologous	D000	**200**	211	**167**	**217**	161	206	110
B101		D133	**197**	211	**133**	**193**	161	206	110
B103	Heterologous	D000	**200**	246	133	189	161	206	116
B103		D133	**197**	246	133	189	161	206	116
B107	Heterologous	D000	**200**	246	133	189	161	206	116
B107		D056	**197**	246	133	189	161	206	116

Table S9. Cox proportional hazards regression model for freedom from any vivax recurrence by day 168 considering study group, age, weight, G6PD enzyme activity, place of residence, CYP2D6 phenotype, and day of primaquine initiation in the per-protocol population, Cruzeiro do Sul (n= 254).

Parameters	**Hazard ratio (95% CI)**
Study group (group 3 as reference)	
Group 1	3.9 (2.0–8.0)
Group 2	3.8 (1.9–7.5)
Age (≤25 years as reference)	0.9 (0.5–1.6)
Weight (≤60 kg as reference)	1.4 (0.8–2.4)
CYP2D6 phenotype (intermediate activity as reference)	0.7 (0.5–1.2)
Place of residence (urban as reference)	1.5 (0.9–2.5)
Day of primaquine start (start on or earlier than day 17 as reference)	1.1 (0.7–1.8)

Note:

CI= confidence interval