Supplementary appendix 4

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Supplementary information 1. Schizonticidal antimalarial treatment dosing

A. Dihydroartemisinin-Piperaquine (DP)

Participants in the DP or DP-PQ arm were treated with standard doses of DP. DP treatment Tablets containing 160/320 mg piperaquine w. 20/40 mg dihydroartemisinin tablets (Eurartesim, Sigma Tau) were administered according to weight as per manufacturer guidelines shown below:

| Body weight (kg) | Total daily dose (mg) (1x/day for 3 days) | Tablet strength and number of tablets per dose |
|------------------|------------------------------------------|---------------------------------------------|
|                  | Piperaquine     | DHA                                       |                                              |
| 5 to <7          | 80              | 10                                        | ½ x 160mg / 20mg                             |
| 7 to <13         | 160             | 20                                        | 1 x 160mg / 20mg                             |
| 13 to <24        | 320             | 40                                        | 1 x 320mg / 40mg                             |
| 24 to <36        | 640             | 80                                        | 2 x 320mg / 40mg                             |
| 36 to <75        | 960             | 120                                       | 3 x 320mg / 40mg                             |
| 75 to 80         | 1,280           | 160                                       | 4 x 320mg / 40mg                             |
| >80              | Not eligible     |                                            |                                              |

Participants in the DP and PA arms received a full course of DP at baseline and a second full course at day 21, to ensure prophylaxis for the duration of follow-up.

B. Pyronaridine-Artesunate

Participants in the PA or PA-PQ arm were treated with standard doses of PA. PA granules containing 60 mg pyronaridine-tetraphosphate/20mg artesunate (Pyramax, Shin Poong Pharmaceutical Co.) were administered to children <20kg, and PA tablets containing 180 mg pyronaridine-tetraphosphate/60mg artesunate were administered to children and adults >20kg, according to weight as per manufacturer guidelines shown below:

| Granules (Children <20kg) |
|---------------------------|
| Body weight (kg)          | Total daily dose (mg) (1x/day for 3 days) | Sachet strength and number of tablets per dose |
|                           | Piperaquine     | Pyronaridine-tetraphosphate | Artesunate |                                            |
| 5 - <8kg                  | 60              | 20                          | 1 x 60mg/20mg                             |
| 8 - <15kg                 | 120             | 40                          | 2 x 60mg/20mg                              |
| 15 - <20kg                | 180             | 60                          | 3 x 60mg/20mg                              |

| Tablets (Children and adults >20kg) |
|-------------------------------------|
| Body weight (kg)                    | Total daily dose (mg) (1x/day for 3 days) | Tablet strength and number of tablets per dose |
|                                    | Piperaquine     | Pyronaridine-tetraphosphate | Artesunate |                                            |
| 20 - <24kg                         | 180             | 60                          | 1 x 180mg/60mg                             |
| 24 - <45kg                         | 360             | 120                         | 2 x 180mg/60mg                             |
| 45<-65kg                           | 540             | 180                         | 3 x 180mg/60mg                             |
| >65kg                              | 720             | 240                         | 4 x 180mg/60mg                             |
Supplementary figure 1. Schematic representation of sample collection and analysis pipeline

| DP & PA            | DP-PQ & PA-PQ                                      |
|--------------------|----------------------------------------------------|
| Day 0: Enrolment   | Day 0: Enrolment                                   |
| Day 1              | Day 1                                              |
| Day 2              | Day 2                                              |
| Day 7              | Day 7                                              |
| Day 10             | Day 10                                             |
| Day 14             | Day 14                                             |
| Day 21             | Day 21                                             |
| Day 28             | Day 28                                             |
| Day 35             | Day 35                                             |
| Day 42             | Day 42                                             |
| Day 49             | Day 49                                             |

Venous collection (EDTA vacutainer) • qRT-PCR gametocyte quantification
Venous collection (Heparin vacutainer) • Mosquito feeding assay
Finger-prick collection • Thick film microscopy • Haemoglobin density assay (Hemocue)
Sample collected but qRT-PCR not conducted
Mosquito feeding assay conducted IF either of previous TWO assays infected any mosquitoes
Supplementary table 1. Primer sequences and qPCR conditions for PfMGET CCp4 assay

**PfMGET Primer/Probe Sequences**

| Primers     | Sequence                                |
|-------------|-----------------------------------------|
| Primer-FW (5’-3’) | CGGTCCAAATATAAAATCCTG                   |
| Primer-RV (5’-3’) | TGTG TAACG TATG ATTCAATTTC              |
| Probe (5’-3’)     | FAM-CAGCTCCAG CATTTAAACAC-BHQ1          |

**CCp4 Primer/Probe Sequences**

| Primers     | Sequence                                |
|-------------|-----------------------------------------|
| Primer-FW (5’-3’) | CACATGAATATGAGATAAAATTG                 |
| Primer-RV (5’-3’) | TAGGCGAACATGTGGAAAG                    |
| Probe (5’-3’)     | TexasRed-AGCAACAACGTTATGTGCCCTAAAACG-BHQ2 |

Male and female gametocyte quantification was performed as described previously, using a multiplex RT-qPCR assay (1). Assays were run using commercial RT-qPCR mixes (Luna® Universal Probe One-Step RT-qPCR Kit, New England Biolabs, Ipswich, MA, USA). FW = Forward primer. RV = Reverse primer.
Supplementary information 2. Primary endpoint and sample size

The primary endpoint of the current trial is the median within person percent reduction in mosquito infection rate (the percentage of mosquitoes surviving to dissection that each participant infected) at day 2 compared to baseline. Sample size was informed by previous trials (2, 3). In those studies, ~79% of individuals infected at least one mosquito before treatment, and among those who infected at least one mosquito, an average of 24% of mosquitoes became infected, giving an estimated probability of mosquito infection of $0.79 \times 0.24 = 0.190$. Using a standard deviation of 0.24 for the change in proportion of infected mosquitoes before and after treatment (estimated from the same data), with 25 participants per arm, we would have 80% power to detect a 95% or greater reduction in infectivity (from 0.190 to 0.009) as statistically significant at the 0.05 level. With 25 participants per group, we would have 80% power to detect an 80% or greater reduction in the proportion of infectious individuals after treatment (a secondary outcome) as significant at the 0.05 level. Sample sizes were designed to assess reduction in mosquito infectivity over time within arms, not between arms. A formal comparison between arms would have necessitated larger sample sizes that affect the logistic feasibility of the study by the need to find sufficient gametocyte carriers in a single transmission season, and the required logistical intensity of mosquito membrane feeding assays (in terms of mosquito husbandry and labour). As in previous studies, we included between arm comparisons as secondary outcomes and report these data.
Supplementary table 2. Infectivity to mosquitoes (≤7 days post treatment)

**A. Infectious individuals**

| Day of follow-up | Treatment arm | Infectious individuals* % (n/N) | p-value\(\alpha\) | p-value\(\beta\) |
|------------------|---------------|----------------------------------|------------------|------------------|
| Day 0            | Overall       | 66 (66/100)                      | -                | -                |
|                  | DP            | 64 (16/25)                       | ref              | ref              |
|                  | DP-PQ         | 60 (15/25)                       | ref              | 0.7              |
|                  | PA            | 68 (17/25)                       | ref              | ref              |
|                  | PA-PQ         | 72 (18/25)                       | ref              | 0.76             |
| Day 2            | DP            | 56 (15/25)                       | 0.36             | ref              |
|                  | DP-PQ         | 4.17 (1.24)                      | 0.0004           | 0.0002           |
|                  | PA            | 64 (16/25)                       | 0.77             | ref              |
|                  | PA-PQ         | 0 (0/25)                         | -                | -                |
| Day 7            | DP            | 68 (17/25)                       | 0.77             | ref              |
|                  | DP-PQ         | 4.17 (1.24)                      | 0.0084           | 0.0005           |
|                  | PA            | 56 (14/25)                       | 0.39             | ref              |
|                  | PA-PQ         | 0 (0/24)                         | -                | -                |

**B. Mosquito infection (infection rate and oocyst intensity)**

| Day of follow-up | Treatment arm | Infectious individuals** | Median % (IQR)*** | p-value\(\alpha\) | p-value\(\beta\) | Infectious individuals** | Median % (IQR)*** | p-value\(\alpha\) | p-value\(\beta\) |
|------------------|---------------|--------------------------|-------------------|------------------|------------------|--------------------------|-------------------|------------------|------------------|
| Day 0            | Overall       | 15.77 (5.41-31.94)       | -                 | -                | 0.24 (0-0.68)    | -                        | 4.73 (0-23.59)    | -                | 0.06 (0-0.47)    |
|                  | DP            | 23.39 (7.95-37.78)       | ref               | ref              | 0.47 (0.1-1.01) | ref                       | 0.76 (0-29.31)    | ref              | 0.07 (0-0.3)     | ref              |
|                  | DP-PQ         | 22.89 (7.81-30.98)       | ref               | 0.94             | 0.33 (0.08-2.37) | ref                       | 7.38 (0-27.12)    | ref              | 0.47 (0-0.63)    | ref              |
|                  | PA            | 6.15 (2.99-24.97)        | ref               | ref              | 0.08 (0.03-0.49) | ref                       | 2.99 (0-13.73)    | ref              | 0.03 (0-0.32)    | ref              |
|                  | PA-PQ         | 9.89 (6.06-29.17)        | ref               | 0.27             | 0.15 (0.07-0.57) | ref                       | 7.14 (0-17.53)    | ref              | 0.37 (0-0.47)    | ref              |
| Day 2            | DP            | 13.59 (5.45-23.81)       | 0.0052            | ref              | 0.27 (0.03-0.86) | 0.036                      | ref              | 4.13 (1.33-21.88) | 0.028            | ref              | 0.04 (0-0.25)    | ref              |
|                  | DP-PQ         | 8.03 (4.00-16.00)        | <0.0001           | <0.0001          | 0.06 (0.01-0.48) | 0.0009                    | <0.0001          | 0.0002           | <0.0001          | <0.0001          |
|                  | PA            | 9.46 (1.64-23.44)        | 0.52              | ref              | 0.1 (0.02-0.4)   | 0.44                      | ref              | 4 (0.12-5)       | 0.34             | ref              | 0.04 (0-0.21)    | 0.30             |
|                  | PA-PQ         | 0 (0.0)                  | 0.0002            | <0.0001          | 0 (0.0)          | 0.0002                    | <0.0001          | 0 (0.0)          | 0.0001           | <0.0001          |
| Day 7            | DP            | 6.31 (2.06-27.78)        | 0.017             | ref              | 0.07 (0.03-0.43) | 0.0061                    | ref              | 0.03 (0.17-19)   | 0.14             | ref              | 0.03 (0-0.27)    | 0.051            |
|                  | DP-PQ         | 0 (0.0)                  | 0.0007            | <0.0001          | 0 (0.0)          | 0.0007                    | <0.0001          | 0 (0.0)          | 0.0004           | <0.0001          |
|                  | PA            | 2.5 (0.24-66)            | 0.19              | ref              | 0.03 (0.03-0.37) | 0.068                     | ref              | 1.52 (0.4-4.41)  | 0.48             | ref              | 0.02 (0-0.04)    | 0.21             |
|                  | PA-PQ         | 0 (0.0)                  | 0.0003            | 0.0001           | 0 (0.0)          | 0.0003                    | 0.0001           | <0.0001          | 0 (0.0)          | 0.0001           | <0.0001          |

A. Percentage of infectious individuals. *Individuals were classed as infectious if direct membrane feeding assays (DMFA) resulted in at least one mosquito with any number of oocysts.

B. Mosquito infection measures are presented for i. all participants who were infectious at baseline, and ii. all participants regardless of baseline infectivity. **Average percent infection = Median percentage of mosquitoes infected by each participant. Each participant’s mosquito infection rate was calculated as the number of mosquitoes infected as a percentage of all mosquitoes surviving to dissection. Oocyst densities are the median of all individuals’ average oocyst densities.  nc = not calculable, no positive observations. - = not tested. P-value\(\alpha\) = Within group comparison. P-value\(\beta\) = Between ACT matched group comparison (i.e. DP with DP-PQ, PA with PA-PQ). ref = reference group.
### Supplementary table 3. Median percent reduction in mosquito infection rate for individuals infectious before treatment (with 95% CI)

|                | Median % reduction (95% CI) | p-value | n     | Median % reduction (95% CI) | p-value  |
|----------------|-----------------------------|---------|-------|-----------------------------|----------|
| Day 2          |                             |         |       |                             |          |
| DP            | 16  | 50.44 (26.35–74.54)       | 0.013†  | 16  | 34.20 (6.76–67.65)         | 0.141†   |
| DP-PQ         | 15  | 100 (nc)                  | <0.0001† | 15  | 100 (nc)                  | <0.0001† |
| Difference     | -   | -8.67 (73.22–55.87)      | 0.85†   | 17  | 38.94 (19.59–57.87)       | 0.1328†  |
| PA-PQ         | 18  | 100 (nc)                  | <0.0001† | 17  | 100 (nc)                  | <0.0001† |
| Difference     | -   | -108.67 (170.01–39.34)   | <0.0001† | -   | -61.06 (121.38–0.74)      | 0.0601†  |

Median % reduction is the median percent reduction (relative to baseline) in mosquito infection rate at the given timepoints. Positive values denote reductions in percent infected, negative values denote increases. 95% Confidence intervals (CI) were calculated by bootstrapping with 1000 repetitions. All values are for individuals who were infectious to mosquitoes before treatment (i.e. infected any number of mosquitoes). [nc] = not tested. P-value¥ = Within group comparison by Wilcoxon signed rank test (day 0 as reference). P-value† = Between ACT matched group comparison (i.e. DP with DP-PQ, PA with PA-PQ) by Wilcoxon rank-sum test. Full details of mosquito feeding assay outcomes are in Appendix pp 6 & 8. nc = Not calculable due to total/near total transmission reduction.
Supplementary table 4. Infectivity to mosquitoes (PA and DP arms only, ≥10 days post treatment)

**A. Infectious individuals**

| Day of follow-up | Treatment arm | Infectious individuals (all)* | p-valueΔ | Individuals infectious (tested)* | p-valueΔ |
|------------------|---------------|-------------------------------|----------|---------------------------------|----------|
| Day 10           | DP            | 54.17 (13/24)                | 0.49     | 54.17 (13/24)                  | 0.49     |
|                  | PA            | 37.50 (9/24)                 | 0.036    | 37.50 (9/24)                   | 0.036    |
| Day 14           | DP            | 41.67 (10/24)                | 0.12     | 41.67 (10/24)                  | 0.12     |
|                  | PA            | 20 (4/25)                    | 0.0012   | 20 (4/25)                      | 0.0012   |
| Day 21           | DP            | 31.43 (9/29)                 | 0.0047   | 33.33 (5/15)                   | 0.0064   |
|                  | PA            | 9.52 (2/21)                  | 0.0005   | 25 (2/8)                       | 0.045    |
| Day 28           | DP            | 4.35 (1/23)                  | 0.0009   | 3.33 (1/12)                    | 0.0084   |
|                  | PA            | 4.35 (1/22)                  | 0.0006   | 16.67 (1/7)                    | 0.045    |
| Day 35           | DP            | 0 (0/23)                     | nc       | 0 (0/23)                       | nc       |
|                  | PA            | 0 (0/22)                     | nc       | 0 (0/22)                       | nc       |
| Day 42           | DP            | 0 (0/23)                     | nc       | 0 (0/23)                       | nc       |
|                  | PA            | 0 (0/22)                     | nc       | 0 (0/22)                       | nc       |

**B. Mosquito infection (infection rate and oocyst intensity)**

| Day of follow-up | Treatment arm | Average percent infection** | p-valueΔ | Oocyst density Median % (IQR)*** | p-valueΔ | Average percent infection** | p-valueΔ | Oocyst density Median % (IQR)*** | p-valueΔ |
|------------------|---------------|----------------------------|----------|---------------------------------|----------|----------------------------|----------|---------------------------------|----------|
| Day 10           | DP            | 5.36 (0.17-17.8)           | 0.0018   | 0.08 (0.03-0.25)                | 0.0018   | 1.62 (0.11-11.55)          | 0.0016   | 0.02 (0.01-0.15)                | 0.0091   |
|                  | PA            | 0.34 (0.0499)              | 0.0001   | 0.01 (0.01)                     | 0.0004   | 0 (0-0.05)                 | 0.0046   | 0 (0-0.05)                      | 0.046    |
| Day 21           | DP            | 0.01 (0.0-0.00)            | 0.0010   | 0 (0.0-0.0)                     | 0.0001   | 0 (0-0.00)                 | 0.0035   | 0 (0-0.00)                      | 0.0035   |
|                  | PA            | 0 (0.0-0.0)                 | 0.0005   | 0 (0.0)                         | 0.0001   | 0 (0-0.00)                 | 0.0035   | 0 (0-0.00)                      | 0.0035   |
| Day 28           | DP            | 0.00 (0.0-0.00)            | 0.0005   | 0 (0.0)                         | 0.0001   | 0 (0-0.00)                 | 0.0035   | 0 (0-0.00)                      | 0.0035   |
|                  | PA            | 0 (0.0-0.0)                 | 0.0001   | 0 (0.0)                         | 0.0001   | 0 (0-0.00)                 | 0.0035   | 0 (0-0.00)                      | 0.0035   |

A. Percentage of infectious individuals. *From day 7 onwards, individuals in the ACT arms (DP and PA) were only tested for infectivity in the DMFA if they were infectious to mosquitoes at one of the previous two time-points. Failure to infect mosquitoes at two subsequent time points resulted in removal from the DMFA follow-up. The number of participants infectious to mosquitoes is presented both as a total percentage of individuals still in the trial (‘Infectious individuals [all]’) and a percentage of the decreasing sample size being tested by DMFA as the trial progressed (‘Infectious individuals [tested]’). Participants were treated with DP at day 21 of follow up to prevent re-infection and were followed-up with clinical and parasitological assessments for 49 days after treatment. Feeding was not conducted at day 49 for any participant, as no mosquito infections were observed after day 28 post-treatment. B. Mosquito infection measures are presented for i. all participants who were infectious at baseline, and. ii. all participants regardless of baseline infectivity. **Average percent infection = Median percentage of mosquitoes infected by each participant.. Oocyst densities are the median of all individuals’ average oocyst densities. nc = not calculable, no positive observations. - = not tested. P-valueΔ = Within group comparison. ref = reference group.
| Treatment group | Total gametocytes (CCP 4 & PfMGET) | p-value | Female gametocytes (CCP 4) | p-value | Male gametocytes (PfMGET) | p-value | p-value △♀ |
|----------------|---------------------------------|---------|---------------------------|---------|--------------------------|---------|------------|
| Circulation time Days (95% CI) | | | | | | | |
| DP | 6.48 (5.31-7.65) | ref | 5.61 (4.61-6.63) | ref | 6.36 (5.41-7.32) | ref | 0.29 |
| DP-PQ | 2.07 (1.89-2.24) | <0.0001 | 1.99 (1.79-2.19) | <0.0001 | 1.81 (1.61-1.97) | <0.0001 | 0.16 |
| PA | 6.46 (5.25-7.67) | ref | 6.33 (5.35-7.32) | ref | 5.79 (4.59-6.63) | ref | 0.48 |
| PA-PQ | 1.97 (1.79-2.16) | <0.0001 | 1.88 (1.67-2.08) | <0.0001 | 1.86 (1.69-2.03) | <0.0001 | 0.89 |
| AUC | | | | | | | |
| Median (IQR) gametocytes per μL/day | | | | | | | |
| DP | 27.21 (18.33-36.14) | ref | 17.64 (11.17-28.91) | ref | 7.90 (5.63-23.36) | ref |-0.0002 |
| DP-PQ | 7.38 (2.16-21.62) | <0.0001 | 3.06 (1.52-11.76) | <0.0001 | 2.6 (0.74-9.15) | <0.0001 | 0.011 |
| PA | 24.4 (9.92-42.02) | ref | 14.32 (7.79-23.67) | ref | 7.94 (2.62-13.95) | ref | 0.0011 |
| PA-PQ | 5.43 (2.36-19.93) | <0.0001 | 3.92 (1.59-12.52) | <0.0001 | 2.26 (0.83-5.23) | <0.0001 | 0.0003 |

Gametocyte circulation time was calculated using a deterministic compartmental model (4), and is presented as the model estimate (mean days) with 95% CI. Area under the curve (AUC) of gametocyte density per participant over time was calculated using the linear trapezoid method (5), and is presented as the median and IQR of individual AUC values by treatment arm. P-values are for differences in the t-statistic between ACT matched treatment groups. Ref = reference group. Circulation time and AUC were calculated using the first 28 days of observation only.
Supplementary table 6. Total gametocyte density, prevalence and sex ratio

| Day of follow-up | Treatment arm | Density gametocytes/μL (IQR) | p-value | Prevalence % (n/N) | p-value | Proportion male Median p (IQR) | p-value |
|------------------|---------------|-----------------------------|---------|-------------------|---------|-------------------------------|---------|
|                  |               |                             |         |                   |         |                               |         |
|                  | Overall       | 71.97 (31.01-149.53)        |         | 100 (96/96)       | 0.34    | 0.26-0.44                     |         |
|                  |               |                             |         |                   |         |                               |         |
|                  | Day 0         |                             |         |                   |         |                               |         |
|                  | DP            | 74.48 (37.56-126.44)        |         | 100 (25/25)       | 0.34    | 0.29-0.46                     |         |
|                  | PA            | 86.13 (22.87-189.92)        | 0.08    | 100 (24/24)       | 0.34    | 0.25-0.44                     | 0.53    |
|                  | PA-QC         | 83.23 (36.07-123.21)        |         | 100 (25/25)       | 0.37    | 0.29-0.45                     | 0.3     |
|                  | Day 2         |                             |         |                   |         |                               |         |
|                  | PA-QC         | 58.95 (28.82-146.13)        | 0.76    | 100 (24/24)       | 0.34    | 0.21-0.44                     | 0.74    |
|                  | Day 7         |                             |         |                   |         |                               |         |
|                  | DP            | 67.86 (33.92-119.46)        |         | 100 (25/25)       | 0.35    | 0.26-0.45                     |         |
|                  | PA-QC         | 43.06 (8.42-116.59)         | 0.15    | 100 (23/23)       | 0.44    | 0.24-0.46                     | 0.37    |
|                  | Day 10        |                             |         |                   |         |                               |         |
|                  | PA-QC         | 57.14 (27.92-102.41)        |         | 100 (25/25)       | 0.29    | 0.24-0.42                     | 0.49    |
|                  | Day 14        |                             |         |                   |         |                               |         |
|                  | DP            | 31.34 (10.23-156.91)        | 0.42    | 100 (24/24)       | 0.4     | 0.22-0.48                     | 0.49    |
|                  | Day 21        |                             |         |                   |         |                               |         |
|                  | DP            | 41.63 (16.14-68.2)          |         | 100 (25/25)       | 0.37    | 0.28-0.45                     |         |
|                  | Day 28        |                             |         |                   |         |                               |         |
|                  | PA-QC         | 0.17 (0-1.95)               |         | <0.0001           | 68.57   | 0.0003                        | 0.0036  |
|                  | Day 35        |                             |         |                   |         |                               |         |
|                  | PA-QC         | 41.38 (17.66-60.06)         |         | 100 (24/24)       | 0.35    | 0.23-0.45                     |         |
|                  | Day 42        |                             |         |                   |         |                               |         |
|                  | PA            | 3.42 (2.02-18.05)           |         | 95.24 (20/21)     | 0.28    | 0.23-0.47                     |         |
|                  | Day 49        |                             |         |                   |         |                               |         |
|                  | PA            | 0.17 (0.0-0.6)              |         | <0.0001           | 60.87   | 0.0006                        | 0.19    |
|                  |               |                             |         |                   |         |                               |         |

P-values are for differences between ACT matched treatment groups. Density and proportion male were compared using Wilcoxon rank sum tests, and prevalence was compared using Fishers exact tests. For males and females, Proportion male is given for participants/time-points with total gametocyte densities of 0.2/μL and over, as described previously (3). For the calculation of gametocyte prevalence, samples were classified as negative for a particular gametocyte sex if the estimated density of in gametocytes of that sex was less than 0·01/μL (i.e. one gametocyte per 100 μL of blood sample). nc = not calculable, no observations/no observations over the threshold density for analysis. - = not tested. ref = reference group.
## Supplementary table 7. Female (CCP4) and male (PfMGET) gametocyte density and prevalence

| Day of follow-up | Treatment arm | Female gametocytes (CCP4) | p-value | Male gametocytes (PfMGET) | Density | p-value | Male gametocytes (PfMGET) | Density |
|------------------|--------------|--------------------------|---------|---------------------------|---------|---------|---------------------------|---------|
|                  |              | Density | Median gametocytes/µL (IQR) |         | Prevalence | % (n/N) | p-value | Density | Median gametocytes/µL (IQR) | Prevalence | % (n/N) | p-value |
| Day 0            | Overall      | 46.35 (19.01-93.39)     | -       | 100 (76/96)              | 22.44 (9.46-51.35) | -       | 100 (76/96)              |
|                  | DP           | 47.14 (28.36-79.86)     | ref     | 100 (25/25)              | 27.33 (16.87-37.53) | ref     | 100 (25/25)              |
|                  | DP, PQ       | 38.91 (14.54-154.59)    | 1.0     | 100 (24/24)              | 23.85 (7.68-69.07) | 0.53    | 100 (24/24)              |
|                  | PA           | 48.36 (23.28-75.39)     | ref     | 100 (23/23)              | 21.18 (8.21-33.33) | ref     | 100 (23/23)              |
|                  | PA, PQ       | 44.14 (15.58-100.96)    | 0.87    | 100 (24/24)              | 19.05 (6.3-50.3)  | 0.98    | 100 (24/24)              |
| Day 14           | DP           | 49.6 (13.61-76.79)      | ref     | 100 (25/25)              | 23.65 (9.92-52.24) | ref     | 100 (25/25)              |
|                  | DP, PQ       | 15.71 (6.14-45.09)      | 0.034   | 100 (23/23)              | 12.81 (4.38-31.8)  | 0.24    | 100 (23/23)              |
|                  | PA, PQ       | 35.42 (21.18-59.63)     | ref     | 100 (23/23)              | 18.07 (5.76-42.96) | ref     | 100 (23/23)              |
| Day 21           | DP           | 19.97 (6.64-80.3)       | 0.28    | 100 (24/24)              | 13.03 (1.21-40.71) | 0.59    | 100 (24/24)              |
|                  | DP, PQ       | 22.42 (13.84-36.33)     | ref     | 96 (24/25)               | 13.44 (6.3-39.11)  | ref     | 100 (25/25)              |
|                  | PA           | 24.61 (8.54-35.31)      | ref     | 100 (24/24)              | 11.21 (4.12-25.41) | ref     | 100 (24/24)              |
|                  | PA, PQ       | 0.0 (0.09)              | <0.0001 | 43.48 (10/23)            | <0.0001 | 0.05 (0.04-0.62)         | <0.0001 | 40.87 (14/23)            | 0.0006|
| Day 28           | DP           | 15.61 (4.50-34.32)      | -       | 91.3 (21/23)             | 9.26 (2.38-28.06)  | -       | 95.65 (22/23)            |
|                  | DP, PQ       | 15.61 (17.57-31.80)     | -       | 95.83 (27/24)            | 6.94 (2.98-23.83)  | -       | 95.83 (27/24)            |
|                  | PA           | 12.66 (7.82-18.9)       | ref     | 75 (20/25)               | 6.75 (2.01-21.3)   | ref     | 100 (25/25)              |
|                  | PA, PQ       | 0.0 (0.02)              | <0.0001 | 25 (6/24)                | <0.0001 | 0.0 (0.0)                | <0.0001 | 20.83 (6/24)             | <0.0001|
|                  | DP, PQ       | 0.0 (0.03)              | <0.0001 | 39.13 (9/24)             | <0.0001 | 0.0 (0.0)                | <0.0001 | 38.43 (7/24)             | <0.0001|
| Day 35           | DP           | 4.67 (1.31-12.22)       | ref     | 90.48 (19/21)            | 1.37 (0.3-5.09)    | ref     | 90.48 (19/21)            |
|                  | DP, PQ       | 0.0 (0.0)               | <0.0001 | 20.83 (6/24)             | <0.0001 | 0.0 (0.0)                | <0.0001 | 16.67 (4/24)             | <0.0001|
|                  | PA           | 3.19 (1.33-3.51)        | ref     | 90.48 (19/21)            | 1.12 (0.63-1.85)   | ref     | 90.48 (19/21)            |
|                  | PA, PQ       | 0.0 (0.03)              | <0.0001 | 32.82 (7/22)             | <0.0001 | 0.0 (0.0)                | <0.0001 | 13.64 (3/22)             | <0.0001|
| Day 42           | DP           | 0.43 (0.23)             | ref     | 60.87 (14/23)            | 0.41 (0.03-1.05)   | ref     | 86.96 (20/23)            |
|                  | DP, PQ       | 0.0 (0.0)               | 0.0001  | 13.04 (5/33)             | 0.0 (0.0)          | <0.0001 | 4.33 (1/23)              | <0.0001|
|                  | PA           | 0.54 (0.04-0.29)        | ref     | 80 (16/20)               | 0.0 (0.01-0.39)    | ref     | 78 (15/20)               |
|                  | PA, PQ       | 0.0 (0)                 | <0.0001 | 0.0 (0.0)                | <0.0001 | 4.76 (1/21)              | <0.0001 | 0.0 (0)                  |
| Day 49           | DP           | 0.03 (0.06)             | 0.04    | 52.17 (12/23)            | 0.04 (0.04-0.14)   | 65.22 (15/23) |
|                  | PA           | 0.09 (0.02-0.29)        | -       | 60 (12/20)               | 0.0 (0.08)         | -       | 40 (8/20)                |
|                  | PA, PQ       | 0.0 (0.07)              | -       | 25 (6/20)                | 0.0 (0.0)          | -       | 20 (4/20)                |
|                  | DP           | 0.0 (0.0)               | -       | 8.7 (2/23)               | 0.0 (0.0)          | -       | 4.33 (1/23)              |
|                  | PA           | 0.0 (0.0)               | -       | 9.52 (2/21)              | 0.0 (0.0)          | -       | 0.0 (0/21)               |

P-values are for differences between ACT matched treatment groups. Density was compared using Wilcoxon rank sum tests, and prevalence was compared using Fishers exact tests. For the calculation of gametocyte prevalence, samples were classified as negative for a particular gametocyte sex if the estimated density of in gametocytes of that sex was less than 0·01 gametocytes per µL (i.e. one gametocyte per 100 µL of blood sample). - = not tested. ref = reference group.
Supplementary figure 2. Proportion of gametocytes that were male

The proportion of gametocytes that were male was calculated for all values with total gametocyte densities of 0.2/µL and over, as described previously (3). P-values (<0.05) for differences between ACT matched treatment groups were calculated using Wilcoxon rank sum tests and are indicated with asterisks (*).
| Day of follow-up | Treatment arm | Haemoglobin density Mean g/dL (range) | p-value<sup>3</sup> | p-value<sup>1</sup> | Percent change from day 0 Mean % (Lower/upper 95% CI) | p-value<sup>3</sup> | p-value<sup>1</sup> |
|------------------|---------------|---------------------------------------|---------------------|---------------------|--------------------------------------------------------|---------------------|---------------------|
| Day 0            | Overall       | 12.9 (9.8-15.8)                       |                     |                     | -                                                     |                     |                     |
|                  | DP            | 12.1 (10.4-15.1)                      |                     |                     | -                                                     |                     |                     |
|                  | DP-PQ         | 11.8 (9.9-14.4)                       |                     |                     | -                                                     |                     |                     |
|                  | PA            | 12.2 (10.2-15.8)                      |                     |                     | -                                                     |                     |                     |
|                  | PA-PQ         | 12.1 (9.8-14.3)                       |                     |                     | -                                                     |                     |                     |
| Day 1            | DP            | 11.6 (9.1-15.2)                       | 0.012               | 0.016               | -8.88 (-9.56/-8.81)                                     | 0.016               | ref                 |
|                  | DP-PQ         | 11.1 (9.2-12.9)                       | 0.007               | 0.16                | -0.04 (-0.52/-0.36)                                     | <0.0001             | 0.26                |
|                  | PA            | 12.9 (9.4-14.8)                       | 0.099               | ref                 | -2.14 (-3.47/-0.66)                                     | 0.12                | ref                 |
|                  | PA-PQ         | 11.2 (8.7-12.9)                       | <0.0001             | 0.046               | -4.85 (-5.81/-3.88)                                     | <0.0001             | 0.036               |
| Day 2            | DP            | 11.7 (9.6-13.1)                       | 0.009               | 0.006               | -2.53 (-3.3/-0.24)                                     | 0.072               | ref                 |
|                  | DP-PQ         | 11.2 (9.6-13.1)                       | <0.0001             | 0.006               | -3.22 (-3.52/-2.92)                                     | <0.0001             | 0.13                |
|                  | PA            | 12.1 (9.5-16.0)                       | 0.41                | ref                 | -1.36 (-4.52/-1.8)                                     | 0.38                | ref                 |
|                  | PA-PQ         | 11.6 (9.3-14.2)                       | 0.062               | 0.28                | -3.49 (-7.33/-0.36)                                     | 0.073               | 0.38                |
| Day 7            | DP            | 11.5 (9.6-13.5)                       | 0.021               | ref                 | -4.26 (-8.11/-0.42)                                     | 0.031               | ref                 |
|                  | DP-PQ         | 11.1 (7.6-12.7)                       | <0.0001             | 0.13                | -6.61 (-9.33/-3.89)                                     | <0.0001             | 0.31                |
|                  | PA            | 11.7 (9.6-15.8)                       | 0.019               | ref                 | -4.02 (-7.43/-0.66)                                     | 0.023               | ref                 |
|                  | PA-PQ         | 11.4 (9.3-13.8)                       | 0.064               | 0.43                | -2.77 (-6.67/-1.42)                                     | 0.12                | 0.03                |
| Day 10           | DP            | 11.1 (9.9-13.3)                       | 0.029               | ref                 | -3.37 (-6.3/-0.3)                                       | 0.048               | -                   |
|                  | PA            | 12.1 (10.3-16.1)                      | 0.41                | ref                 | -1.28 (-4.62/-2.1)                                     | 0.44                | -                   |
| Day 14           | DP            | 11.7 (10.4-16.1)                      | 0.13                | ref                 | -2.31 (-4.68/-1.42)                                     | 0.21                | ref                 |
|                  | DP-PQ         | 11.3 (9.9-13.3)                       | 0.013               | 0.20                | -4.06 (-7.43/-0.68)                                     | 0.021               | 0.48                |
|                  | PA            | 12.1 (10.3-16.6)                      | 0.35                | ref                 | -1.33 (-4.28/-1.61)                                     | 0.36                | ref                 |
|                  | PA-PQ         | 11.6 (9.7-13.3)                       | 0.13                | 0.22                | -2.23 (-6.29/1.82)                                     | 0.27                | 0.71                |
| Day 21           | DP            | 11.9 (10.3-15.8)                      | 0.56                | ref                 | -0.53 (-4.52/3.32)                                     | 0.80                | ref                 |
|                  | DP-PQ         | 11.7 (9.9-13.2)                       | 0.49                | 0.39                | -0.76 (-4.03/2.51)                                     | 0.56                | 0.92                |
|                  | PA            | 12.4 (10.15)                          | 0.73                | ref                 | -0.61 (-2.25/3.47)                                     | 0.66                | ref                 |
|                  | PA-PQ         | 11.8 (9.6-14.1)                       | 0.52                | 0.12                | -0.56 (-4.27/3.14)                                     | 0.76                | 0.61                |
| Day 28           | DP            | 12.2 (10.3-16.5)                      | 0.67                | ref                 | -0.36 (-4.94/4.23)                                     | 0.87                | ref                 |
|                  | DP-PQ         | 11.4 (9.13)                           | 0.11                | 0.13                | -2.59 (-6.27/1.09)                                     | 0.16                | 0.44                |
|                  | PA            | 12.3 (9.6-15.4)                       | 0.84                | ref                 | -0.27 (-4.46/3.92)                                     | 0.90                | ref                 |
|                  | PA-PQ         | 12.4 (10.19)                          | 0.34                | 0.85                | 4.62 (3.43/12.68)                                      | 0.25                | 0.27                |
| Day 35           | DP            | 11.9 (10.3-16.2)                      | 0.41                | ref                 | -1.18 (-3.28/0.92)                                     | 0.56                | ref                 |
|                  | DP-PQ         | 11.8 (10.13-2)                       | 0.87                | 0.88                | 0.79 (-2.79/4.36)                                      | 0.65                | 0.46                |
|                  | PA            | 12.4 (10.16)                          | 0.81                | ref                 | 0.56 (-2.83/3.95)                                      | 0.74                | ref                 |
|                  | PA-PQ         | 11.9 (9.5-13.4)                       | 0.65                | 0.13                | -0.12 (-4.24/4.01)                                     | 0.93                | 0.80                |
| Day 42           | DP            | 12.2 (10.5-15.3)                      | 0.47                | ref                 | -0.77 (-4.2-3.67)                                      | 0.33                | ref                 |
|                  | DP-PQ         | 12.1 (9.14-5)                         | 0.13                | 0.77                | 0.59 (-0.43/7.6)                                       | 0.078               | 0.63                |
|                  | PA            | 12.7 (10.18)                          | 0.20                | ref                 | 3.03 (1.39/-7.45)                                      | 0.17                | ref                 |
|                  | PA-PQ         | 12.3 (9.6-14.8)                       | 0.33                | 0.27                | 3.82 (2.41/10.04)                                      | 0.22                | 0.83                |
| Day 49           | DP            | 12.4 (10.4-16.4)                      | 0.18                | ref                 | 3.4 (2.99/7.8)                                         | 0.12                | ref                 |
|                  | DP-PQ         | 12.1 (9.13-4)                         | 0.39                | 0.25                | 2.41 (-2.19/7.7)                                       | 0.29                | 0.75                |
|                  | PA            | 12.8 (9.9-16.8)                       | 0.11                | ref                 | 3.01 (-0.76/6.77)                                      | 0.11                | ref                 |
|                  | PA-PQ         | 12.3 (9.4-13.6)                       | 0.23                | 0.29                | 3.76 (-3.57/8.3)                                       | 0.14                | 0.81                |

Absolute haemoglobin density and percent change in haemoglobin density (relative to baseline) were compared within treatment arms using paired t-tests (with day 0 as reference for percent change) (p-value<sup>3</sup>), and between treatment arms using two-way t-tests (p-value<sup>1</sup>).
Supplementary figure 3. Percent change in haemoglobin density.

Box plots represent median within person percent change from baseline in each treatment arm. P-values (<0.05) from paired T-tests of the difference in mean percent change between time-points within each treatment arm are indicated with asterisks (*). Outliers were excluded from these plots to allow standardisation of axes.
Supplementary figure 4. Absolute haemoglobin concentration

Absolute haemoglobin density is given in grams per dL (y axis, from 8-20g/dL) and is indicated for each participant individually with grey lines. The single black line shows the mean absolute haemoglobin density. P-values (<0.05) from paired t-tests for the difference in mean haemoglobin density relative to baseline (day 0) within each treatment groups are indicated with asterisks (*).
Supplementary table 9. Prevalence of adverse events

| Description                  | All (n=100) | DP (n=25) | DP-PQ (n=25) | PA (n=25) | PA-PQ (n=25) |
|------------------------------|-------------|-----------|--------------|-----------|--------------|
| All                          | 75% (75/100) | 76% (19/25) | 72% (18/25) | 80% (20/25) | 72% (18/25) |
| **P-value**                  | 0.36*       | 0.50**    | 0.37**       |           |              |
| All (mild)                   | 73% (73/100) | 76% (19/25) | 68% (17/25) | 80% (20/25) | 68% (17/25) |
| **P-value**                  | 0.73*       | 0.38**    | 0.26**       |           |              |
| All (moderate)               | 11% (11/100) | 8% (2/25)   | 16% (4/25)  | 12% (3/25) | 8% (2/25)    |
| **P-value**                  | 0.90*       | 0.33**    | 0.50**       |           |              |
| All (related to treatment)   | 23% (23/100) | 32% (8/25)  | 16% (4/25)  | 24% (6/25) | 20% (5/25)   |
| **P-value**                  | 0.64*       | 0.16**    | 0.50**       |           |              |

P-values are from Fisher’s exact tests for differences in proportion of individuals with an AE between all groups* or between ACT matched treatment arms**. Classification as ‘related to treatment’ was defined as probably, possibly or definitely related to treatment, as described in the methods.
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