Supplementary Data

Supplementary Table 1. Laboratory reference ranges with grading criteria

Parameter	Reference ranges	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Potentially life threatening)
HAEMATOLOGY – Adult Male					
Hemoglobin-Hb (Low) g/dL	10.6-17.0	10.0-<10.6	9-<10.0	7.0-< 9.0	<7.0 with clinical signs
Lymphocytes (Low) (×10^3 cells/µl)	1.2-3.5	0.6 - < 1.2	0.5-<0.6	0.35-<0.5	< 0.35
Neutrophils (× 10^3 cells/µl)	0.9-7.7	0.8-<0.9	0.6-0.799	0.4-0.599	<0.4
WBC (low) (× 10^3cells/µl)	3.0-10.1	2.0-<3.0	1.5-1.9	1.0-1.4	<1
PLT (× 10^3 cells/µl)	139-398	100-<139	50-<100	25-<50	<25
HAEMATOLOGY – Adult Female					
Hb (Low) g/dL	8.0-14.9	7-<8	6-<7	5- 6	<5 with clinical signs
Lymphocytes (× 10^3cells/µl)	1.3 – 3.5	0.6-<1.3	0.5-<0.6	0.35-<0.5	< 0.35
Neutrophils (× 10^3 cells/µl)	1.1-5.3	0.8-1.1	0.6-0.799	0.4-0.599	<0.4
WBC (× 10^3 cells/µl)	3.3-8.6	2.0-<3.3	1.5-1.9	1.0-1.4	< 1
PLT (× 10^3 cells/µl)	166-456	100-< 166	50-<100	25-<50	<25
BIOCHEMISTRY – Adult Male					
Sodium (mmol/L) Low	135-145	130 to < 135	125-<130	121-<125	≤ 120
Sodium (mmol/L) High	135-145	146-150	150-154	154-160	≥160
Potassium (mmol/L) Low	3.5-4.5	3.0-<3.4	2.5-<3.0	2.0-<2.5	<2.0
Potassium (mmol/L) High	3.5-4.5	4.5-<6.0	6.0-<6.5	6.5-<7.0	≥7.0
Urea (mmol/L) – Do not grade	2.1-7.1				
Creatinine (µmol/L) High	44-111	>111-144 1.1-1.3 X ULN	>144-199 >1.3-1.8 X ULN	199-<389 >1.8-<3.5 X ULN	≥ 388 ≥3.5 X ULN
ALT (IU/L)	11-80	>80-<200 1.25-<2.5 X ULN	200-<400 2.5-<5.0 ULN	400-<800 5.0-<10.0 ULN	≥ 800 ≥10.0 X ULN
BIOCHEMISTRY – Adult Female					
Sodium mmol/ (Low)	135-145	130-<135	125-<130	121-<125	≤ 120
Sodium mmol/L (High)	135 145	>145-<150	150-<154	154-<160	≥ 160
Potassium mmol/L	3.4-4.4	3.0-<3.4	2.5-<3.0	2.0-< 2.5	< 2.0
Urea (mmol/L) – Do not grade	2.1-7.1				
Creatinine (µmol/L) (High)	37-92	>92-<119 1-1.3 X ULN	>119-<166 >1.3-1.8 X ULN	>166-322 >1.8-<3.5ULN	≥ 322 ≥ 3.5 X ULN
ALT (IU/L)	8-55	>55-<138 1< 2.5 ULN	138-<275 2.5-<5.0 x ULN	275-<550 5.0-10 X ULN	≥ 550 ≥10.0 X ULN

'Low' refers to values below to the lower normal limit range; 'High' refers to values above the upper normal limit range

Supplementary Table 2. Overall Concomitant Medication Use based on location

	Kilifi North (N=34)	Kilifi South (N=112)	Ahero (N=15)	Total (N=161)
Amoxycillin	3 (8.8)	11 (9.8)	2 (13.3)	16 (9.9)
Amoxicillin/Clavulanate	0	1 (0.9)	0	1 (0.6)
Antacid	0	4 (3.6)	0	4 (2.5)
Antihistamine	2 (5.9)	4 (3.6)	2 (13.3)	8 (5)
Ceftriaxone	0	1 (0.9)	0	1 (0.6)
Clotrimazole	4 (11.8)	9 (8)	0	13 (8.1)
Diclofenac	0	1 (0.9)	0	1 (0.6)
Diclofenac Gel	0	7 (6.3)	0	7 (4.3)
Ibuprofen	10 (29.4)	13 (11.6)	1 (6.7)	24 (14.9)
Nystatin Oral Drops	1 (2.9)	1 (0.9)	1 (6.7)	3 (1.9)
ORS	0	3 (2.7)	0	3 (1.9)
Paracetamol	21 (61.8)	39 (34.8)	11 (73.3)	71 (44.1)

Data are presented as n (%) or otherwise specified. ORS, oral rehydration salts.

Supplementary Table 3 Abnormal laboratory result summaries and severity grades

	Grade	C-1*	8 to DoD*	DoD to Exit*	LTFU**
ALT (Low)	1	0	1 (0.6)	2 (1.2)	0
ALT (High)	1	2 (1.2)	45 (28)	34 (21.1)	0
	2	0	7 (4.3)	5 (3.1)	0
Creatinine (Low)	1	0	1 (0.6)	1 (0.6)	0
Creatinine (High)	1	9 (5.6)	33 (20.5)	23 (14.3)	3 (8.6)
Bilirubin (Low)		5(3.1)	18(11.2)	10(6.2)	2(5.7)
Bilirubin (High)		3(1.9)	2(1.2)	1(0.6)	1(2.9)
Albumin (Low)		3(1.9)	10(6.2)	9(5.6)	0
Albumin (High)		21(13)	16(9.9)	25(15.5)	0
Potassium (Low)	1	22 (13.7)	21 (13.0)	16 (9.9)	8 (5.0)
	2	2 (1.2)	0	0	0
Potassium (High)	1	4 (2.5)	16 (9.9)	9 (5.6)	0
Sodium (Low)	1	0	25 (15.5)	33 (20.5)	11 (31.4)
Urea (Low)		6 (3.7)	2 (1.2)	3 (1.9)	1 (2.9)
Hb (Low)	1	1 (0.6)	1 (0.6)	0	0
	3	1 (0.6)	0	0	0
Hb (High)		4 (2.5)	2 (1.2)	4 (2.5)	0
Platelets (Low)	1	7 (4.3)	11 (6.8)	20 (12.4)	8 (24.2)
	2	0	1 (0.6)	2 (1.2)	1 (3)
	3	0	1 (0.6)	1 (0.6)	0
	4†	0	1 (0.6)	1 (0.6)	0
Platelets (High)		6 (3.7)	5 (3.1)	6 (3.7)	0
WBC (Low)	1	1 (0.6)	4 (2.5)	6 (3.7)	1 (3)
WBC (High)	0	4 (2.5)	18 (11.2)	19 (11.8)	0

C-1 refers to a day before challenge; 8-DoT refer to days after challenge; DoT-Exit refers to period of observed treatment to in-patient exit; LTFU refers to long term follow up; *Percentages expressed over total subjects challenged (N=161); **Percentages expressed over number of subjects followed up for each respective parameter – chemistry (N=35) and haematology (N=33); Duration to normal ALT levels: median 14 days (min 2 days and max 67 days); †Grade 4 low platelet reading was 22x10^3 cells/µl on days 22 and 24 with duration to normal levels: median 11 days (min 2 days and max 71 days). 'Low' refers to values below to the lower normal limit range; 'High' refers to values above the upper normal limit range

Supplementary Table 4: qPCR outcomes by location after exclusion of all volunteers with any detectable lumefantrine levels

	Nairobi (N=28)	Ahero (N=7)	Kilifi North (N=27)	Kilifi South (N=40)
PCR (-)	0% (0/28)	14.3% (1/7)	7.4% (2/27)	17.5% (7/40)
Treated, febrile	10.7% (3/28)	14.3% (1/7)	51.9% (14/27)	7.5% (3/40)
Treated, non-febrile	85.7% (24/28)	14.3% (1/7)	37.0% (10/27)	5.0% (2/40)
Untreated but PCR (+)	3.6% (1/28)	57.1% (4/7)	3.7 (1/27)	70.5% (28/40)

Data are presented as % and (n/N) or otherwise specified. PCR (-) refers to PCR negative; PCR (+) refers to PCR positive.

Supplementary figure legends

Supplementary Figure 1 Plasma ALT concentration over time based on gender.

Plasma samples one day before challenge (C-1), days 10 or 22 or day of treatment (DoT), and day 36 after challenge including a sample taken after late follow up were analysed for ALT concentrations (IU/L) based on gender. Volunteers (N=142) were inoculated on C1 and followed over time. LFTU refers to long term follow up.

Supplementary Figure 2 Anti-malarial drug concentrations based on treatment outcome.

Plasma samples taken from volunteers one day before challenge (C-1) and seven days after challenge (C+8) were analysed for the anti-malarials: (A) chloroquine; (B) lumefantrine; (C) sulphadoxine; and (D) pyrimethamine. The red horizontal line represents the minimum inhibitory concentration for therapeutic efficacy for each respective drug. Treatment Required refers to whether volunteers were treated before C+22 (Yes) or on C+22 (No). Artemether and dihydroartemisinin levels were all undetectable.

Supplementary Figure 3 Individual qPCR results based on volunteer outcome and location

Blood samples from C+8 to C+22 after CHMI were used for qPCR from: (A) Nairobi – N=28; (B) Kilifi North – N=34; (C) Kilifi South – N=93; and (D) Ahero – N=15. Parasitaemia was determined by asexual *18S* ribosomal RNA gene qPCR for each volunteer (limit of detection 20 parasites/ml). Blue lines represent individuals who required treatment and reached treatment threshold (reached DoT); green lines represent individuals who did not meet criteria for treatment threshold but were qPCR positive; orange lines represent individuals who were qPCR negative throughout monitoring; and red dot denotes individuals were febrile and met treatment

criteria. Shown are each of the 142 volunteers with their respective subject identification numbers.

Supplementary Figure 4 Individual qPCR results based on volunteer outcome and cohort

Blood samples from C+8 after inoculation were used to determine estimated parasite density from each volunteer enrolled into the cohorts: (A) 2016 – N=36; (B) 2017 – N=53; and (C) 2018 – N=93. Parasitaemia was determined by asexual *Plasmodium 18S* ribosomal RNA gene qPCR for each volunteer. Blue lines represent individuals who required treatment and reached treatment threshold (reached DoT); green lines represent individuals who did not meet criteria for treatment threshold but were qPCR positive; orange lines represent individuals who were qPCR negative throughout monitoring; and red dot denotes individuals were febrile and met treatment criteria. Shown are each of the 142 volunteers with their respective subject identification numbers.

Supplementary Figure 5 Matrix correlation for qPCR and qRT-PCR assay comparisons

Blood samples from 20 volunteers from the 2018 cohort representing six time points (C+8 to C+10.5) after inoculation were used to estimate parasitaemia using *Plasmodium 18S* rRNA gene qPCR assays from Kilifi and Mahidol University (MU) and *18S* rRNA qRT-PCR from the University of Washington (UW). Shown are Spearman correlation comparing the respective assays. Units on both axes are estimated parasites/mL. Negative results are displayed at the LOD for each assay (Kilifi 20 parasites/mL, UW 1 parasite/mL, MU 10 parasites/mL).

Supplementary Figure 6 Individual qPCR and qRT-PCR post-challenge results

Blood samples from 20 volunteers from the 2018 cohort representing six time points (C+8 to C+10.5) after inoculation were used to determine parasitaemia using the *Plasmodium 18S*

rRNA gene qPCR assays from Kilifi (green) and Mahidol (orange), and the *Plasmodium 18S* rRNA qRT-PCR from Washington (blue). Shown are each of the 20 volunteers with their respective subject identification numbers. Negative results are shown at 10 (Kilifi), 0.05 (MU) and 0.005 (UW) parasites/ml in order to graph separate lines.

Supplementary Figure 7 ALT concentration over time based on qPCR status

Plasma samples a day before challenge (C-1), Days 10 or 22 or day of treatment (DoT), and Day 36 after challenge including a sample taken after late follow-up were analysed for ALT concentrations (IU/L) based on qPCR status. Volunteers were inoculated on C1 and followed over time and exited the study at C+36.

Data availability

Volunteer data will be deposited into the KWTRP Harvard Dataverse repository and made available including data dictionaries after de-identification of the volunteer data that represent the results reported in this article. The data will be available to researchers who will need to submit proposals to dgc@kemri-wellcome.org to gain access to the data following a signed data access agreement. The study protocol, informed consent forms, and all other associated documents have been previously published.

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