Supplementary Appendix

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

Supplement to: Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 2014;371:411-23. DOI: 10.1056/NEJMoa1314981

Spread of artemisinin resistance in falciparum malaria SUPPLEMENTARY APPENDIX

1. Supplementary METHODS 2
   1.1 Laboratory procedures: 2
   1.2 Parasitemia calculation 2
   1.3 Sequencing of the *P. falciparum kelch13* gene (PF3D7_1343700) 2
   1.4 Table S1 – PCR primers 3
2. Supplementary Figures: 4
   2.1 Figure S1 Trial profile 4
   Figure S2a Forest plot to show the effect of the higher artesunate dose on parasite clearance half-life 5
   2.2 Figure S2b. Change in $PCt_{1/2}$ with the higher (4mg/kg) artesunate dose 5
   2.3 Figure S3a Relationship of prevalence of admission patent gametocytemia and $PCt_{1/2}$ by study site 6
   2.4 Figure S3b Relationship of development of patent gametocytemia in the week after starting treatment to $PCt_{1/2}$ by study site. 6
3. Supplementary RESULTS Tables 7
   3.1 Table S2 Gametocytemia detected by microscopy 7
   3.2 Table S3 Change in hematocrit (Hct) and prevalence of anemia 7
   3.3 Table S4 Patients who vomited study drugs during first 3 days of treatment 8
   3.4 Table S4 Adverse events up to day 7 9
   3.5 Table S5 Serious Adverse events occurring at any time during follow-up 11
4. Study Drugs and doses: 12
   4.1 Table S6 Artesunate dosing table: 13
   4.2 Study Artesunate certificates of analysis 14
5. List of Ethics Committees/Institutional Review Boards which approved the protocol: 16
6. References 17

1. SUPPLEMENTARY METHODS

1.1 Laboratory procedures:

Hematocrit was estimated using the Hawksley® microhematocrit centrifuge method.
Slides were stained with Giemsa or Field's stain and parasite counts expressed per 1000 red blood cells or 500 white blood cells (WBC).

1.2 Parasitemia calculation

Parasitemia = N parasites per 1000 red blood cells on the thin smear x Hct x 125.6

Or

Parasitaemia- N parasites per N White blood cells on the thick smear by WBC count (or 8000 if unavailable) / N

1.3 Sequencing of the *P. falciparum kelch13* gene (PF3D7_1343700)

Admission blood samples were anticoagulated in EDTA, washed in PBS and filtered through a cellulose CF11 column to deplete host leucocytes (Venkatesan et al. 2012). Genomic DNA was extracted using QIAamp® DNA Mini Kit (QIAGEN, Germany), following the manufacturer's instructions. Eluted genomic DNA samples were quantified by PicoGreen analysis and quantitative real-time PCR using the Applied Biosystems StepOne RT-PCR system and frozen at -80°C. Samples with more than 50ng DNA and less than 80% human DNA contamination entered Illumina sequencing (Illumina Genome Analyzer II) following the manufacturer's standard protocols. Definition of single-nucleotide polymorphisms (SNPs) was based on analytical approaches described elsewhere (Manske et al. 2012). Filtering was adapted to the heteroallelic nature of relevant polymorphisms in the *kelch13* gene (Ariey et al. 2014), with calls at SNPs requiring one sequence read for the reference allele and two reads for the alternative allele (three if there were 50 or more total reads covering that position). In addition, potential SNPs where the alternative allele was not supported by at least 5 reads in at least one sample, SNPs that were not biallelic and polymorphisms in the *kelch13* low-complexity region (corresponding to amino acid positions 133-143) were not included. Because of the complex effect on phenotype, samples with heterozygosity at one or more positions were also excluded from genotype-phenotype analyses, as were samples with missingness at any of the *kelch13* SNP positions.

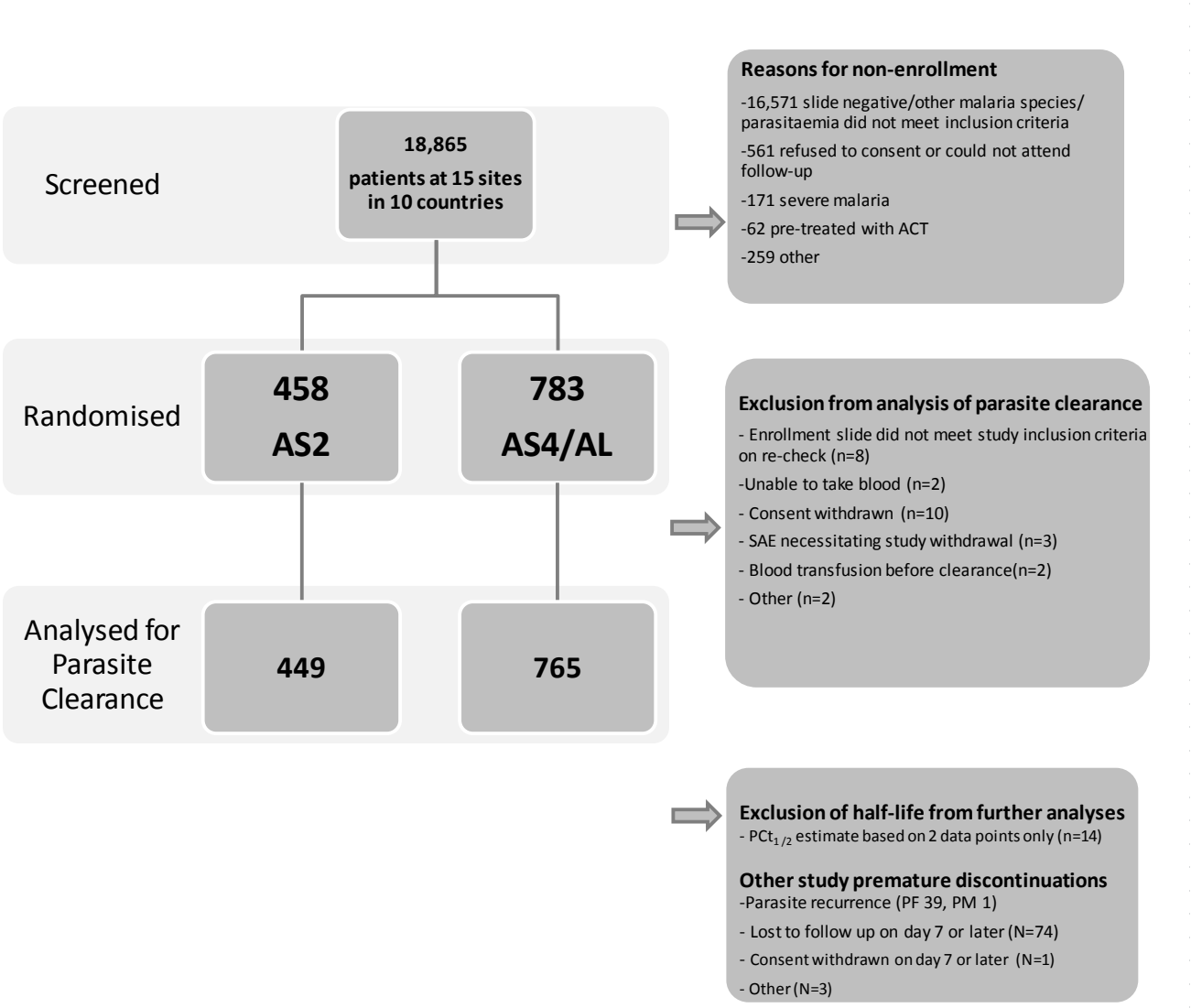
Samples not meeting the Illumina sequencing entry criteria were sequenced by standard dideoxy sequencing of PCR products. These were obtained by nested PCR, the initial PCR (nest 1) amplifying the whole gene (spanning 2438 bases) and three nested PCR reactions (nest 2, fragments a-c) each amplifying a fragment (approximately 850 – 950 bases) of the *kelch13* gene. PCR reaction conditions consisted of a final reaction volume of 100µl containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 2mM $MgCl_2$ (3mM for fragment c), 125µM 4-deoxynucleotide triphosphate (dNTPs), 250nM oligonucleotide primers (see Supplementary Table), 2µl of each genomic DNA template, and 0.4 units Platinum®*Taq* DNA polymerase (Invitrogen, USA). The cycling parameters were pre-denaturation 95°C for 5 min, followed by 25 (nest 1) or 35 (nest 2) PCR cycles involving denaturation at 94°C for 1 min, annealing at 58°C for 2 minutes and extension at 72°C for 2 minutes, with post-extension at 72°C for 7 min, using a MyCycler™ thermal cycler (Bio-Rad Laboratories, U.S.A.). Purified PCR products were sequenced at Macrogen, Republic of Korea and analysed using BioEdit version 7.1.3.0. using the 3D7 *kelch13* sequence as reference (Accession: XM_001350122.1).

1.4 Table S1 – PCR primers

Reaction	Fragment	Primer name	Sequence (5'>3')	Product (bp)
Nest 1	Whole gene	K13_c.-155F K13_c.2283R	AACAAGGCGTAAATATTCGTGT TGTGCATGAAAATAAATATTAAAGAAG	2438
Nest 2	Fragment a	K13_c.-155F K13_c.719R	AACAAGGCGTAAATATTCGTGT TCTCGAATAAAATTCATTTGTGTCTT	874
Nest 2	Fragment b	K13_c.614F K13_c.1464R	TTGAAACGGAATTAAGTGATGC CAATACAGCACTTCCAAAATAAGC	851
Nest 2	Fragment c	K13_c.1344F K13_c.2283R	AGGTGGATTTGATGGTGTAGAA TGTGCATGAAAATAAATATTAAAGAAG	940

2. SUPPLEMENTARY FIGURES:

2.1 Figure S1 Trial profile



Notes:

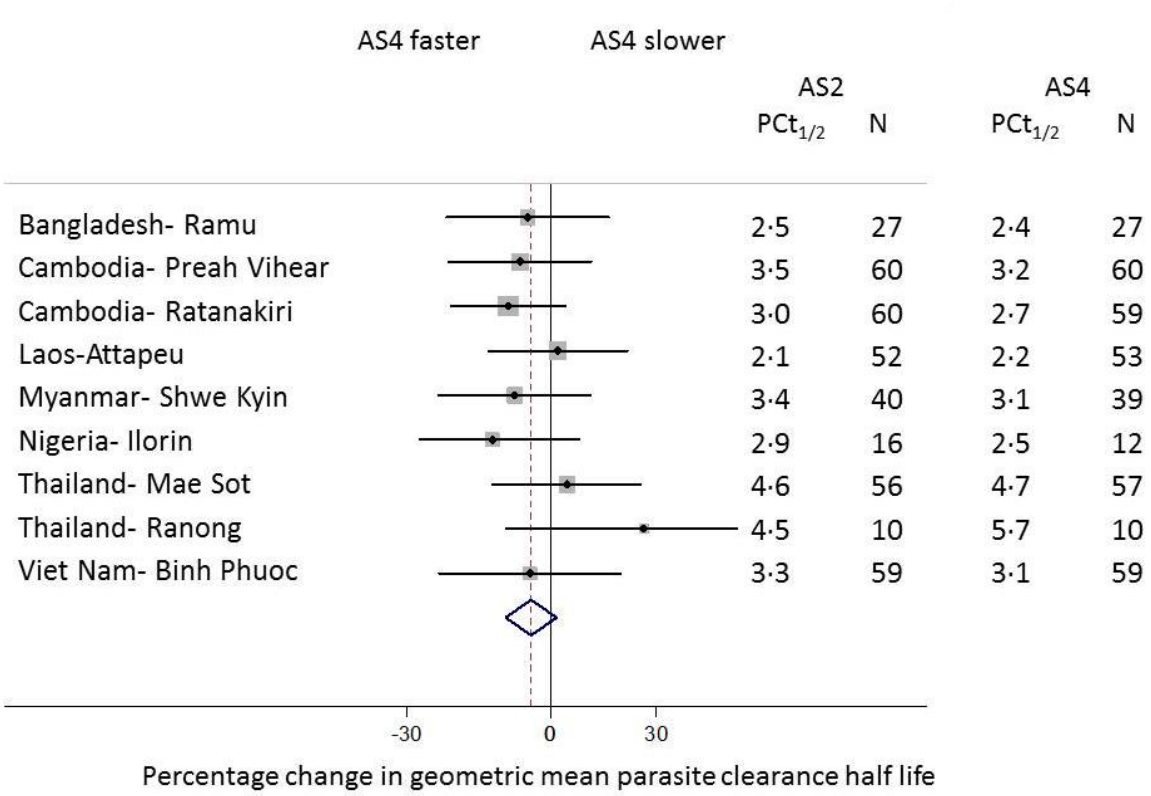
AS2= Artesunate 2mg/kg/day dosing arm; AS4=Artesunate 4mg/kg/day dosing arm; AL= artemether lumefantrine (Kinshasa site only)

ACT= Artemisinin based combination therapy

$PCt_{1/2}$ = parasite clearance half-life in hours

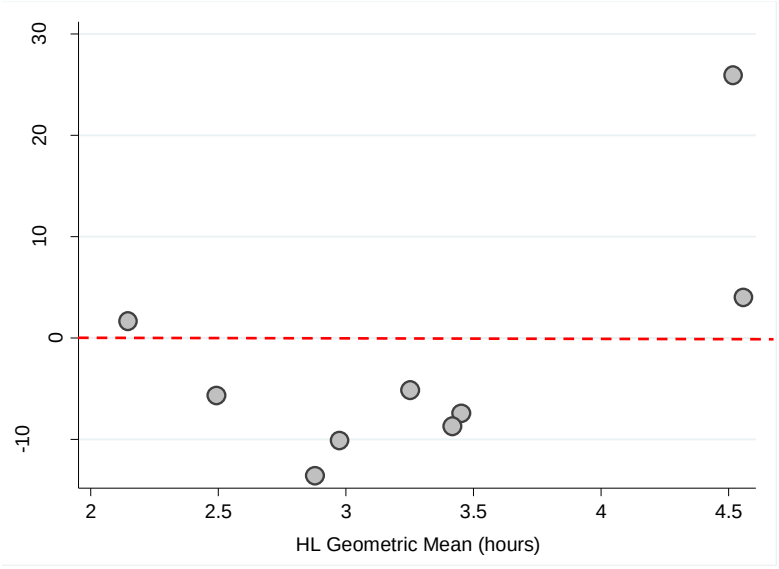
PF= *Plasmodium falciparum*; PM = *Plasmodium malariae*

Figure S2a Forest plot to show the effect of the higher artesunate dose on parasite clearance half-life



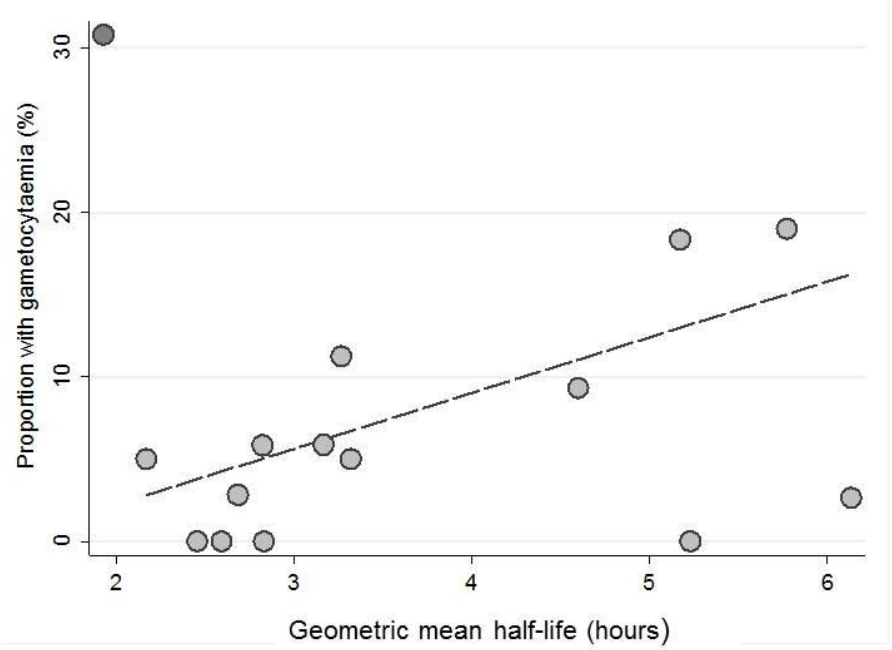
Note: $PCt_{1/2}$ = parasite clearance half-life in hours. AS2 : artesunate 2mg/kg, AS4 : artesunate 4mg/kg

2.2 Figure S2b. Change in $PCt_{1/2}$ with the higher (4mg/kg) artesunate dose



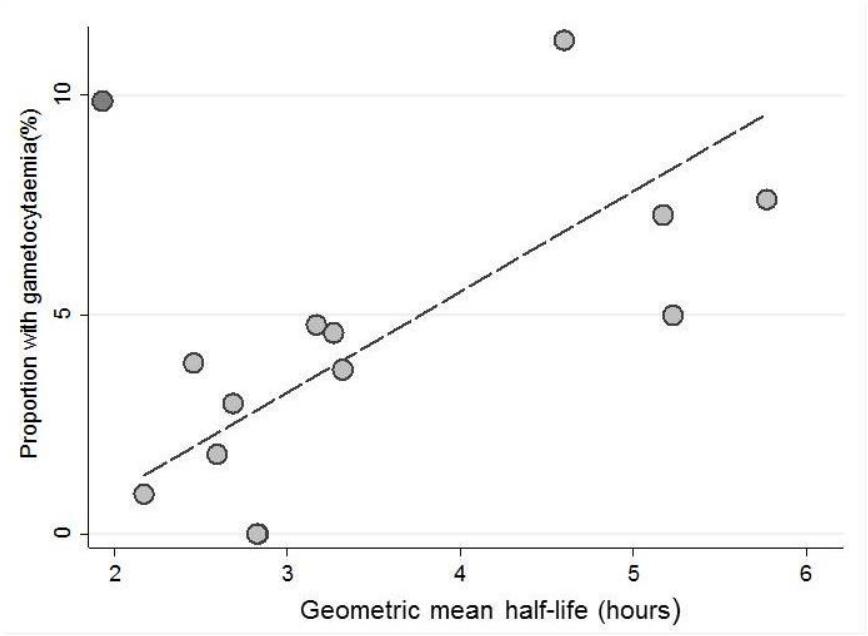
For intermediate geometric mean $PCt_{1/2}$ values (2.5 to 3.5 hours for 2mg/kg artesunate) the higher dose (4mg/kg) accelerated parasite clearance, but there was no overall significant difference in geometric mean $PCt_{1/2}$ between artesunate 2mg/kg and 4mg/kg groups. Dots represent from left to right: Attapeu (Laos), Ramu (Bangladesh), Ilorin (Nigeria), Ratanakiri (Cambodia), Binh Phuoc (Viet Nam), Shwe Kyin (Myanmar), Preah Vihear (Cambodia), Ranong (Thailand), Mae Sot(Thailand).

2.3 Figure S3a Relationship of prevalence of admission patent gametocytemia and $PCt_{1/2}$ by study site



Note: One dot represents one study site. Dark gray dot = Kinshasa DRC (excluded from the correlation)

2.4 Figure S3b Relationship of development of patent gametocytemia in the week after starting treatment to $PCt_{1/2}$ by study site.



Note: Dark gray dot = Kinshasa DRC (excluded from the correlation). One dot represents one study site. Patients in Srisaket, Thailand were excluded from the analysis as they also received single dose primaquine.

3. SUPPLEMENTARY RESULTS TABLES

3.1 Table S2 Gametocytemia detected by microscopy

	Country	Site	Gametocytemia Day 0 N (%)	Day 7	Day 14
1	India	Jalpaiguri	0/1 (0)	0	0
2	Bangladesh	Ramu	0/56 (0)	1/47 (2)	0/45 (0)
3	Myanmar	Shwe Kyin	9/80 (11)	6/80 (8)	2/80 (3)
4	Thailand	Mae Sot	11/118 (9)	7/118 (6)	2/112 (2)
5	Thailand	Srisaket	1/38 (3)	*a*	*a*
6	Laos	Attapeu	6/120 (5)	1/105 (1)	0/71 (0)
7	Cambodia	Pailin	19/100 (19)	10/95 (11)	7/84 (8)
8	Cambodia	Preah Vihear	6/120 (5)	6/118 (5)	4/119 (3)
9	Cambodia	Ratanakiri	7/120 (6)	5/112 (4)	0/101 (0)
10	Cambodia	Pursat	22/120 (18)	17/109 (16)	12/100 (12)
11	Vietnam	Binh Phuoc	7/119 (6)	7/110 (6)	4/101 (4)
12	Thailand	Ranong	0/23 (0)	1/20 (5)	1/19 (0)
13	Nigeria	Ilorin	1/35 (3)	1/20 (5)	0/19 (0)
14	DRC	Kinshasa	36/117 (31)	18/116 (16)	6/96 (6)
15	Kenya	Pingilikani	0/58 (0)	1/47 (2)	0/45 (0)

a Srisaket not included as patients were given primaquine according to local guidelines

3.2 Table S3 Change in hematocrit (Hct) and prevalence of anemia

	Country	Site	Day3-Day0 Change in Hct (%)	Day 14-Day 0 Change in Hct (%)	Day 0 Anemia n/N (%)	Day 7 Anemia	Day 14 Anemia
1	India	Jalpaiguri	-	-3	1/1 (100)	1/1 (100)	1/1 (100)
2	Bangladesh	Ramu	-3·7(2·7)	-2 (2·8)	5/56 (9)	11/53 (21)	11/53 (21)
3	Myanmar	Shwe Kyin	-2·9(3·8)	-1·4 (4·2)	21/80 (26)	32/80 (40)	21/79 (27)
4	Thailand	Mae Sot	-4·7(4·3)	-3·4 (5·7)	19/117 (16)	41/115 (36)	40/112 (36)
5	Thailand	Srisaket	-2·9(5·4)	0·1 (6·2)	4/38 (11)	3/33 (9)	3/36 (8)
6	Laos	Attapeu	-3·0(3·1)	-2·4 (3·4)	17/120 (14)	47/116 (41)	34/110 (31)
7	Cambodia	Pailin	-2·8(3·2)	-2·1 (3·8)	19/100 (19)	27/97 (28)	22/91 (24)
8	Cambodia	Preah Vihear	-3·1(3·6)	-1·8 (4·2)	44/120 (37)	61/120 (51)	43/120 (36)
9	Cambodia	Ratanakiri	-4·0(2·9)	-2·8 (4·2)	31/120 (26)	67/119 (56)	51/114 (45)
10	Cambodia	Pursat	-3·5(3·8)	-3·4 (5·2)	25/120 (21)	49/118 (42)	37/115 (32)
11	Vietnam	Binh Phuoc	-4·5(2·8)	-3·2 (3·4)	10/120 (8)	41/117 (35)	31/114 (27)
12	Thailand	Ranong	-4·5(2·7)	-2·8 (3·1)	2/23 (9)	4/22 (18)	2/22 (14)
13	Nigeria	Ilorin	-1·1(3·2)	0·3(3·8)	18/36 (50)	17/27 (63)	12/27 (44)
14	DRC	Kinshasa	-2·3(3·6)	2·4(4·9)	80/118 (68)	84/118 (72)	48/112 (43)

Anaemia defined as Hb<11g/dL or <10g/dL in children under 5y (WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Accessed 25th November 2013 at http://www.who.int/vmnis/indicators/haemoglobin.pdf.)

Hematocrit = 5·62 + 2·60 * Hemoglobin. (Lee SJ, Stepniewska K, Anstey N et al. The relationship between the haemoglobin concentration and the haematocrit in *Plasmodium falciparum* malaria. Malar J. 2008; 7: 149. doi:10.1186/1475-2875-7-149.)

3.3 Table S4 Patients who vomited study drugs during first 3 days of treatment

Day 0	Treatment	Day 1	Treatment
BD001-024	AS2	LA001-012	AS2
KE001-120	AS2	LA001-021	AS4
KE001-134	AS2	TH004-026	AS4
KE001-138	AS2	TH004-066	AS4
KH004-055	AS4	CD001-011	AL dose 3
LA001-067	AS2	CD001-112	AL dose 3
NG001-026	AS4		
NG001-036	AS2		
TH004-012	AS4		
CD001-042	AL dose 1		
CD001-002	AL dose 2		
CD001-012	AL dose 2		
CD001-112	AL dose 2		

Note:

AS2 Artesunate 2mg/kg
AS4 Artesunate 4mg/kg
AL Artemether-lumefantrine
BD001: Bangladesh-Ramu
KE001: Kenya- Pingilikani
LA001: Laos-Attapeu
NG001: Nigeria-Ilorin
TH004:Thailand-Mae Sot
CD001: DRC-Kinshasa

No subjects vomited their medication on day 2

3.4 Table S4 Adverse events up to day 7

Country	Site	Initial treatment	Abdominal pain	Anorexia	Blurred vision	Diarrhea	Dyspnea	hearing difficulty	Dizziness	Headache	Itch	Joint Pain	Muscle Pain	Nausea	Palpitations	Skin rash	Fatigue	Vomiting
Bangladesh	Ramu	AS 2	1/25 (4)	0/8	0/27	0/27	1/27	0/27	1/10 (10)	1/6 ()	0/27	0/16	1/10 (10)	0/17	0/26	0/27	0/2	1/21 (5)
Bangladesh	Ramu	AS 4	1/23 (4)	1/15 (7)	0/26	0/25	0/26	0/26	1/13 (8)	1/1 (100)	0/26	1/17 (6)	2/9 (22)	0/21	0/25	0/26	2/3 (67)	0/23
Myanmar	Shwe Kyin	AS 2	0/37	0/40	0/40	0/40	0/40	0/40	1/38 (3)	0/38	0/40	0/40	0/40	0/40	0/40	0/40	0/40	0/39
Myanmar	Shwe Kyin	AS 4	0/40	0/40	0/40	0/40	0/40	0/40	1/36 (3)	0/40	0/40	0/38	0/39	0/40	0/40	0/40	0/40	0/38
Thailand	Mae Sot	AS 2	11/49 (22)	7/23 (30)	0/53	3/57 (5)	0/54	1/57 (2)	6/15 (40)	0/2	2/57 (4)	4/15 (27)	7/22 (32)	8/36 (22)	7/40 (18)	0/59	7/37 (19)	4/43 (9)
Thailand	Mae Sot	AS 4	9/48 (19)	8/27 (30)	2/58 (3)	5/55 (9)	0/59	1/57 (2)	10/17 (59)	0/2	4/58 (7)	4/19 (21)	8/27 (30)	11/43 (26)	6/43 (14)	1/60 (2)	5/38 (13)	5/48 (10)
Thailand	Srisaket	AS 4	0/36	0/31	0/38	1/38 (3)	0/38	0/38	3/26 (12)	3/3 (100)	0/38	0/36	4/7 (57)	6/30 (20)	0/37	0/38	2/24 (8)	2/35 (6)
Laos	Attapeu	AS 2	0/28	1/4 (25)	0/31	1/47 (2)	0/33	0/39	3/6 (50)	-	0/58	2/16 (13)	2/14 (14)	1/22 (5)	0/17	0/59	-	0/39
Laos	Attapeu	AS 4	1/34 (3)	1/3 (33)	0/32	2/42 (5)	0/27	0/41	1/4 (25)	-	0/59	0/18	1/11 (9)	2/23 (10)	0/17	0/58	0/2	0/41
Cambodia	Pailin	AS 4	8/76 (11)	4/33 (12)	5/87 (6)	2/98 (2)	3/99 (3)	4/88 (5)	17/47 (36)	1/2 (50)	0/99	1/22 (5)	3/46 (7)	4/52 (8)	1/91 (1)	1/98 (1)	9/13 (69)	1/86 (1)
Cambodia	Preah Vihear	AS 2	5/33 (15)	6/24 (25)	5/29 (17)	2/54 (4)	0/47	2/42 (5)	2/21 (10)	2/6 (33)	0/59	1/39 (3)	3/39 (8)	3/36 (16)	2/33 (6)	0/58	2/5 (40)	1/38 (3)
Cambodia	Preah Vihear	AS 4	2/35 (6)	7/23 (30)	3/29 (10)	0/50	2/42 (5)	2/42 (5)	7/19 (37)	2/3 (67)	0/58	2/31 (6)	2/38 (5)	3/27 (11)	4/38 (11)	0/59	5/8 (63)	1/31 (3)
Cambodia	Ratanakiri	AS 2	2/60 (3)	2/50 (4)	0/60	1/60 (2)	0/60	0/60	4/48 (8)	-	0/60	0/60	0/57	1/44 (2)	0/60	0/60	1/57 (2)	0/42
Cambodia	Ratanakiri	AS 4	0/60	1/50 (2)	0/60	0/60	0/60	0/60	1/46 (2)	1/5 (20)	0/60	0/58	0/56	1/39 (3)	0/60	0/60	1/59 (2)	0/48
Cambodia	Pursat	AS 4	7/73 (10)	9/64 (14)	2/87 (2)	2/116 (2)	0/97	1/89 (1)	7/68 (10)	1/13 (8)	1/112 (1)	2/48 (4)	5/51 (10)	8/67 (12)	5/78 (6)	0/119	8/25 (32)	5/93 (5)

Vietnam	Binh Phuoc	AS 2	1/59 (2)	2/54 (4)	0/59	0/56	0/59	0/59	3/51 (6)	3/3 (100)	0/59	0/58	2/47 (4)	2/54 (4)	0/59	0/59	6/50 (12)	0/54
Vietnam	Binh Phuoc	AS 4	1/58 (2)	2/55 (4)	0/60	0/58	0/60	0/60	5/49 (10)	7/8 (88)	0/60	1/59 (2)	3/55 (5)	1/49 (2)	0/60	0/60	6/53 (11)	0/52
Thailand	Ranong	AS 2	2/10 (20)	1/9 (11)	0/10	0/10	0/10	0/10	1/9 (11)	3/4 (75)	0/10	1/10 (10)	1/2 (50)	1/9 (11)	0/10	0/10	8/8 (100)	1/8 (13)
Thailand	Ranong	AS 4	3/13 (23)	3/10 (30)	0/13	0/12	0/13	0/13	0/13	2/4 (50)	1/13 (8)	0/12	3/5 (60)	0/7	0/11	0/13	6/9 (67)	0/10
Nigeria	Ilorin	AS 2	0/16	0/17	0/18	0/17	0/18	0/18	0/18	0/17	0/18	0/18	0/18	0/15	0/18	0/18	0/16	1/11 (9)
Nigeria	Ilorin	AS 4	0/15	0/14	0/15	0/15	0/15	0/15	0/15	-	0/15	0/15	0/15	0/15	0/15	0/15	0/15	-
DRC	Kinshasa	AS 4	0/40	0/57	0/22	3/59 (5)	0/49	0/39	0/28	1/39 (3)	0/55	0/29	0/28	0/45	0/22	1/57	1/50 (2)	1/51 (2)
DRC	Kinshasa	AL	0/42	0/54	0/22	2/58 (3)	0/47	0/34	0/23	0/37	0/53	0/22	0/22	0/47	0/11	0/56	0/47	1/47 (2)
Kenya	Pingilikani	AS 2	3/44 (7)	1/44 (2)	0/56	2/53 (4)	0/53	0/56	0/56	0/28	1/56 (2)	0/49	0/55	1/56	0/56	3/54 (6)	0/56	2/38 (5)

Notes

An adverse event is defined as either a drug-related side effect or a new (or exacerbation of a pre-existing) symptom, sign or illness
Adverse events were solicited using both open questions and a symptom checklist.
AEs tabulated above are derived from data collected on days 0,1,2,3,7 when all patients were scheduled for a follow-up visit
Data presented as n/N (%) where n is number of subjects reporting the adverse event, N is number of evaluable subjects (i.e. did not present the adverse event on enrollment)
It was not possible to assess presence of certain AEs e.g. headache, dizziness, palpitations, vision or hearing disturbance in very young children.

3.5 Table S5 Serious Adverse events occurring at any time during follow-up

	Site	Subject ID	Description	Relationship to study drug
1	Cambodia-Pailin	KH004-008	Acute alcohol withdrawal resulting in prolongation of hospitalisation and study discontinuation	Not related
2	Thailand-Mae Sot	TH004-012	Cannula site infection requiring prolongation of hospitalisation	Not related
3	Thailand- Ranong	TH007-001	Fever after parasite clearance requiring prolongation of hospitalization. No source found. Treated with antimicrobials.	Not related
4	Vietnam- Binh Phuoc	VN001-024	Patient developed respiratory symptoms after parasite clearance. Responded to broad spectrum antibiotics.	Not related
5	Vietnam- Binh Phuoc	VN001-027	3 year old child presenting with uncomplicated malaria and high fever. Convulsion after enrollment. Switched to severe malaria treatment and made a complete recovery in <24hours. Likely febrile convulsion	Not related
6	Thailand- Mae Sot	TH004-061	Anemia requiring blood transfusion	Not related
7	Thailand- Mae Sot	TH004-066	Delerium secondary to acute alcohol withdrawal	Not related
8	Thailand- Mae Sot	TH004-067	Anemia requiring blood transfusion	Not related
9	Thailand-Srisaket	TH005-12	Acute upper gastrointestinal bleed – referred to another hospital	Not related
10	Vietnam- Binh Phuoc	VN001-024	Viral respiratory tract infection requiring prolongation of hospitalisation	Not related
11	Nigeria- Ilorin	NG001-012	Acute asthma attack requiring prolongation of hospitalisation	Not related
12	DRC-Kinshasa	CD001-023	Anaemia requiring blood transfusion	Not related

Notes:
Length of follow up varied between 14 and 42 days depending on the site (see Methods)

SAEs were first notified to the Study Medical Monitor (AMD) using an initial SAE reporting form. He made an assessment of the event and the report was then emailed to all members of the DSMB within 24h of the medical monitor being notified to make an independent assessment. A final SAE reporting form was submitted once the outcome of the SAE was known. The DSMB had the final say on judging whether the event was related to the study drug or not.

4. STUDY DRUGS AND DOSES:

Artesunate 50 mg tablets:

Pingilikani, Kenya study site. Manufactured by Guilin Pharmaceutical Co. Ltd, P.R. China, batch number:AS101001, analysed by the National Quality Control Laboratory of Kenya.

Srisaket and Ranong, Thailand sites. Manufactured by Guilin Pharmaceutical Co. Ltd, P.R. China, batch numbers:AS110102 and AS120702; repackaged by Atlantic Pharmaceutical Company, Thailand.

All other sites. Manufactured by Guilin Pharmaceutical Co. Ltd, P.R. China, batch numbers: AS101008 and AS111104. Provided by the World Health Organisation Global Malaria Programme (Who-GMP) after quality check at the Research Institute for Industrial Pharmacy® incorporating CENQAM®, South Africa.

Artemisinin-based combination therapies:

Viet Nam. Dihydroartemisinin-piperaquine (Arterakine®, Pharbaco, Viet Nam)

Cambodia. Dihydroartemisinin-piperaquine (Duo-cotecxin®, Holley-Cotec, China and Eurartesim®, Sigma-Tau, Italy, donated by the manufacturers)

Myanmar, Bangladesh, Nigeria, Laos and DRC. Artemether-lumefantrine (Coartem®, Novartis, Switzerland) provided by WHO-GMP or purchased directly from Novartis Switzerland.

India. Artesunate + sulphadoxine-pyrimethamine co-blister (Medicamen Biotech Ltd, Delhi, India)

Thailand. Artesunate (as above) + mefloquine (Mequin®, Atlantic laboratories Corporation Ltd, Thailand)

4.1 Table S6 Artesunate dosing table:

One tablet contains 50 mg of AS.

Weight (kg)	4 mg/kg (OD)		2 mg/kg (OD)	
	tablets	mL	tablets	mL
2		0.8		0.4
3		1.2		0.6
4		1.6		0.8
5		2.0		1.0
6	1/2	2.4	1/4	1.2
7	1/2	2.8	1/4	1.4
8	3/4	3.2	1/4	1.6
9	3/4	3.6	1/4	1. 6
10	3/4	4.0	1/2	2.0
11	1	4.4	1/2	2.2
12	1	4.8	1/2	2.4
13 - 14	1		1/2	
15 - 16	1 1/4		1/2	
17 - 20	1 1/2		3/4	
21	1 3/4		3/4	
22 - 23	1 3/4		1	
24 - 26	2		1	
27 - 28	2 1/4		1	
29	2 1/4		1 1/4	
30 - 32	2 1/2		1 1/4	
33 - 34	2 3/4		1 1/4	
35	2 3/4		1 1/2	
36 - 39	3		1 1/2	
40	3 1/4		1 1/2	
41 - 42	3 1/4		1 3/4	
43 - 45	3 1/2		1 3/4	
46	3 3/4		1 3/4	
47 - 48	3 3/4		2	
49 - 51	4		2	
52 - 53	4 1/4		2	
54	4 1/4		2 1/4	
55 - 57	4 1/2		2 1/4	
58 - 59	4 3/4		2 1/4	
60	4 3/4		2 1/2	
61 - 64	5		2 1/2	
65	5 1/4		2 1/2	
66 - 67	5 1/4		2 3/4	
68 - 70	5 1/2		2 3/4	

Instructions:

If you need to give a suspension of artesunate to young children who are unable to swallow tablets, crush 1 tablet in 5ml clean drinking water and give the volume specified in the table above. Discard any unused solution.

4.2 Study Artesunate certificates of analysis

PASP

CENQAM

Research Institute for Industrial Pharmacy® incorporating CENQAM®

Private Bag X6001 Potchefstroom
South Africa 2520
Tel +27 18 299 2268
Fax +27 18 299 2291
E-mail Ena.Swanepoel@nwu.ac.za

CLIENT COMPANY
Global Malaria Programme
World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland

Contact person: Dr P Ringwald

A SANAS Accredited Pharmaceutical Laboratory, No P0005

CERTIFICATE OF ANALYSIS

PRODUCT:	Artesunate 50 mg Tablets	**MANUFACTURING COMPANY:**	Guilin Pharmaceuticals Co Ltd (China)
FORM:	Tablets	**CLIENT COMPANY:**	WHO Switzerland
BATCH NO.:	AS111104	**METHOD REFERENCE:**	IPVCMET0356
SAMPLE CODE:	WHO-12-0007-ST	**WORKSHEET:**	10.05.2012
MANUFACTURING DATE:	11.2011	**DATE RECEIVED:**	10.05.2012
EXPIRY DATE:	11.2013	**DATE TESTED:**	20.06.2012
CONTAINER:	Blister 12's	**REPORT VERSION:**	1

TEST	SPECIFICATION	RESULT	COMPLIANCE STATEMENT	APPROVED BY **
APPEARANCE: (Package insert)	Round white flat bevelled edge uncoated tablets. Scoreline on one side of the tablet with AS engraved on the one side of the score-line and 50 on the other side. AS 50 engraved on the other side of the tablet.	Round white flat bevelled edge uncoated tablets. Scoreline on one side of the tablet with AS engraved on the one side of the score-line and 50 on the other side. AS 50 engraved on the other side of the tablet.	Complies	
IDENTIFICATION: (IR)	The IR absorption spectrum of the precipitate exhibits maxima at the same wavelengths as that of the standard	The IR absorption spectrum of the precipitate exhibits maxima at the same wavelengths as that of the standard	Complies	
ASSAY: (HPLC)				
Artesunate:	90.0 - 110.0 %		Complies	
Sample 1:		98.1%		
Sample 2:		98.7%		
Sample 3:		99.3%		
Average: (%RSD)		98.7% (0.6%)		
DISSOLUTION: (HPLC)				
Artesunate:	NLT 80 % (Q) within 45 minutes		Complies	
Vessel 1:		90%		
Vessel 2:		92%		
Vessel 3:		101%		
Vessel 4:		96%		
Vessel 5:		101%		
Vessel 6:		95%		
Average (%RSD):		97% (3.7)		

QC VERIFICATION AND RELEASE

Date: 10 August 2012

Signature:

National Quality Control Laboratory

Hospital Road, KNH Complex, P.O. Box 29726, 00202 Nairobi, Kenya
Telephone: 2726953, +254 - 020 - 3544525/30 • Fax: 2718073
Email: info@nqcl.go.ke Website: www.nqcl.go.ke

CERTIFICATE OF ANALYSIS

CERTIFICATE No: CAN/2010/796

PRODUCT: ARTESUNATE TABLETS **REF. NO:** NDQD201011963

DATE RECEIVED: 24.11.2010	**LABEL CLAIM:**	Each tablet contains Artesunate 50 mg
BATCH NO: AS101001	**PRESENTATION:**	White coloured, circular shaped, bevel edged tablets, single scored on one face and embossed 'AS 50' on both faces, packed in a white multi dose plastic tin containing 1000 tablets.
MFG. DATE: 08 Oct. 2010	**MANUFACTURER:**	Guilin Pharmaceutical Co., Ltd.
EXP. DATE: 07 Oct. 2012	**ADDRESS:**	Not Indicated.
CLIENT REF NO. -	**CLIENT:**	Steffen Borrmann, c/o Rosa Chilengi c/o KEMRI-Wellcome Trust Programme, P.O. Box 230 - 80108, Kilifi.
	TEST(S) REQUESTED:	Identification, Friability, Dissolution and Assay.

RESULTS

TEST	METHOD	COMPENDIA	SPECIFICATION	DETERMINED	REMARKS
Uniformity of Weight	Weight	B.P. 2007 Vol. IV App. XII G.	≤ 2 tablets deviate by more than 5% from mean weight	None Deviates	COMPLIES
Identification	IR	Manufacturer's In-House Method	Component HPLC peak in assay sample super-imposable with standard preparation	Super-imposable peaks at RT value: 4.9 ± 0.1 min	COMPLIES
	HPLC	Manufacturer's In-House Method	Sample IR absorption spectrum is concordant with the spectrum obtained from Artesunate ICRS	Sample IR absorption spectrum concordant with Artesunate ICRS	
Friability	Weight	B.P. 2007 Vol. IV App. XVII G.	Not more than 1.0%	0.2%	COMPLIES
Dissolution	HPLC	Manufacturer's In-House Method	No tablet less than 80.0% [n=6]	83.6% (n=6; RSD=2.1%)	COMPLIES
Assay	HPLC	Manufacturer's In-House Method	95.0 – 105.0%	102.6% (n=9; RSD=1.8%)	COMPLIES

CONCLUSION: The product complies with the specifications for the tests performed.

ANALYST:	MR. E. KIPRONOH	**DATE:**	31/12/2010
ANALYST:	DR. G. WANGANGA	**DATE:**	31/12/2010
ANALYST:	DR. N. MWAURA	**DATE:**	31/12/2010
DIRECTOR:	DR. H. K. CHEPKWONY	**DATE:**	31/12/2010

Quality Medicines Protect

5. LIST OF ETHICS COMMITTEES/INSTITUTIONAL REVIEW BOARDS WHICH APPROVED THE PROTOCOL:

Thailand: Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Rd., Rathchadewee, Bangkok 10400, Thailand and Tak Province Community Ethics Advisory Board (T-CAB)

India: National Institute of Malaria Research Institutional Ethics committee, Indian Council of Medical Research, Sector 8, Dwarka, New Delhi.

Kenya: The Pharmacy and Poisons Board's Expert Committee on Clinical Trials Kenya Medical Research Institute Ethics Review Committee

Cambodia: National Ethics Committee for Health Research, Ministry of Health, Kingdom of Cambodia Institutional Review Board and National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

Myanmar: The Government of the Republic of the Union of Myanmar, Ministry of Health, Department of Medical Research (Lower Myanmar)

Laos: Ministry of Health. National Ethics Committee for Health Research, Lao Peoples' Democratic Republic.

Nigeria: Ethical Review Committee, University of Ilorin Teaching Hospital, Ilorin, Nigeria

Bangladesh: National Research Ethics Committee, Bangladesh Medical Research Council

Democratic Republic of the Congo: Republique Democratique du Congo, Ministere de l'Enseignement Superieur, Universitaire et Recherche Scientifique, Universite de Kinshasa, Ecole de Sante Publique. Comite d'Ethique.

Viet Nam: Ethics Committee for biomedical research of the Ministry of Health, Institute of Malariology-Parasitology-Entomology, Ho Chi Minh City

6. REFERENCES

Ariey, F., et al. (2014). "A molecular marker of artemisinin-resistant Plasmodium falciparum malaria." Nature **505**(7481): 50-55.

Manske, M., et al. (2012). "Analysis of Plasmodium falciparum diversity in natural infections by deep sequencing." Nature **487**(7407): 375-379.

Venkatesan, M., et al. (2012). "Using CF11 cellulose columns to inexpensively and effectively remove human DNA from Plasmodium falciparum-infected whole blood samples." Malar J **11**: 41.