

# BMJ Open A study protocol for a randomised open-label clinical trial of artesunate-mefloquine versus chloroquine in patients with non-severe *Plasmodium knowlesi* malaria in Sabah, Malaysia (ACT KNOW trial)

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## ABSTRACT

**Introduction:** Malaria due to *Plasmodium knowlesi* is reported throughout South-East Asia, and is the commonest cause of it in Malaysia. *P. knowlesi* replicates every 24 h and can cause severe disease and death. Current 2010 WHO Malaria Treatment Guidelines have no recommendations for the optimal treatment of non-severe knowlesi malaria. Artemisinin-combination therapies (ACT) and chloroquine have each been successfully used to treat knowlesi malaria; however, the rapidity of parasite clearance has not been prospectively compared. Malaysia's national policy for malaria pre-elimination involves mandatory hospital admission for confirmed malaria cases with discharge only after two negative blood films; use of a more rapidly acting antimalarial agent would have health cost benefits. *P. knowlesi* is commonly microscopically misreported as *P. malariae*, *P. falciparum* or *P. vivax*, with a high proportion of the latter two species being chloroquine-resistant in Malaysia. A unified ACT-treatment protocol would provide effective blood stage malaria treatment for all *Plasmodium* species.

**Methods and analysis:** ACT KNOW, the first randomised controlled trial ever performed in knowlesi malaria, is a two-arm open-label trial with enrolments over a 2-year period at three district sites in Sabah, powered to show a difference in proportion of patients negative for malaria by microscopy at 24 h between treatment arms (clinicaltrials.gov #NCT01708876). Enrolments started in December 2012, with completion expected by September 2014. A total sample size of 228 is required to give 90% power ( $\alpha$  0.05) to determine the primary end point using intention-to-treat analysis. Secondary end points include parasite clearance time, rates of recurrent infection/treatment failure to day 42, gametocyte carriage throughout follow-up and rates of anaemia at day 28, as determined by survival analysis.

**Ethics and dissemination:** This study has been approved by relevant institutional ethics committees in Malaysia and Australia. Results will be disseminated to inform knowlesi malaria treatment policy in this region through peer-reviewed publications and academic presentations.

## Strengths and limitations of this study

- All age demographics known to acquire *Plasmodium knowlesi* malaria will be eligible for enrolment, at relevant district referral sites where treatment is normally given.
- The primary intention-to-treat analysis will appropriately evaluate rapidity of parasite clearance and associated health economic benefits; however, secondary analyses may not show a clinically useful difference in treatment failure at 42 days in the absence of known resistance to the study drugs.
- This trial addresses a knowledge gap in *knowlesi* malaria, with no previous randomised controlled trials in *P. knowlesi* infection to determine optimal treatment. If ACT is shown to be superior to chloroquine, it would support a national policy change to a unified ACT treatment protocol which would provide effective blood stage malaria treatment for all *Plasmodium* species.

**Trial registration number:** NCT01708876.

## INTRODUCTION

Naturally acquired infections with *Plasmodium knowlesi*, the fifth human malaria, are growing.<sup>1</sup> Since 2004, increasing numbers of cases have been reported from residents and returned travellers predominantly from Malaysia and throughout South-East Asia.<sup>2–11</sup> Cases coincide with the geographic distribution of its natural simian hosts (long-tailed and pig-tailed macaques) and *Anopheles leucosphyrus* group mosquito vector.<sup>12–14</sup> Despite the increase in reported cases, difficulties with microscopic diagnosis and a lack of PCR-based epidemiological surveillance studies

throughout South-East Asia mean the true disease burden is underestimated. *P. knowlesi* is microscopically misidentified as *P. malariae* and *P. falciparum* due to morphological similarities in the late trophozoite and early ring stages, respectively,<sup>14</sup> and is also misreported as *P. vivax*.<sup>15</sup> In Eastern Malaysia, multiple studies have shown that for cases microscopically reported as '*P. malariae*', <1% of these are confirmed when tested by PCR, with the vast majority being *P. knowlesi*.<sup>6 16–21</sup> The rise in zoonotic *P. knowlesi* cases has occurred at the same time that notifications of *P. falciparum* and *P. vivax* are decreasing due to an effective national Malaria Control Program in Malaysia.<sup>22</sup> *P. knowlesi* is now the most common cause of malaria in Malaysia, with minimum estimates of around 2268 cases (including those reported as '*P. malariae*' in Eastern Malaysia) from public health microscopy notifications in 2012 alone.<sup>23</sup>

Misdiagnosis of *P. knowlesi* has concerning treatment implications, as unlike *P. malariae*, knowlesi malaria has a rapid 24 h replication rate and can cause hyperparasitaemia, severe complications and fatal outcomes.<sup>21 24–26</sup> The inadvertent use of chloroquine (CQ) for widely CQ-resistant *P. falciparum* or *P. vivax* in this region may lead to treatment failure, with a consequent higher risk of severe disease or death. In addition, misreporting with *P. vivax* may have implications for the correct initiation of liver-stage *P. vivax* eradication.

### Treatment of non-severe knowlesi malaria

Initial observational and retrospective studies have suggested that both CQ and artemisinin-combination therapy (ACT) are effective therapies for non-severe *P. knowlesi* infection.<sup>16 21 25 27 28</sup> Case reports predominantly from returned travellers to South-East Asia also document non-severe knowlesi malaria responding well to conventional antimalarials such as CQ, mefloquine (MQ), atovaquone with proguanil, doxycycline and quinine.<sup>2 4 29–35</sup> However, to date there have been no prospective randomised trials to determine the optimal treatment of non-severe *P. knowlesi* infection in Malaysia, and there are no current recommendations on how to treat non-severe *P. knowlesi* infection in the current 2010 WHO Malaria Treatment Guidelines.<sup>36</sup>

### Artesunate-mefloquine

Artesunate (AS)-MQ is a common and widely available ACT, and along with artemether-lumefantrine (A-L) is one of only two first-line WHO recommended options for the treatment of non-severe *P. falciparum* infection that are registered in Malaysia and produced according to international good manufacturing practice standards. ACTs are the current mainstay of malaria elimination efforts,<sup>36</sup> with a mechanism of action resulting both in a rapid reduction in parasite mass and resolution of clinical features, while the long-acting component eliminates residual parasites and delays the development of de novo resistance.<sup>37 38</sup> Safety and tolerability of all ACTs are dependent on their partner drug<sup>39</sup> and while

gastrointestinal and self-limiting neuropsychiatric adverse events (AESs) have been reported with MQ, multiple safety and efficacy trials have recommended its use in adults and children for non-severe falciparum malaria.<sup>40–42</sup> Owing to concerns over the safety of MQ in the first trimester of pregnancy<sup>43</sup> and its use in patients with pre-existing psychiatric disorders or those who have previously had cerebral malaria, it is currently not advised for these groups.<sup>39</sup>

The initial reported use of ACT for knowlesi malaria was from our retrospective study at a tertiary referral hospital in Sabah, where a small sample of 8 of 34 patients with PCR-confirmed non-severe *P. knowlesi* infection were treated with oral A-L. Median microscopic parasite clearance time was 1 day (range 0–3), which was significantly faster than those receiving CQ (median 2.5 days, range 1–3,  $p=0.01$ ).<sup>25</sup> Our subsequent prospective study at the same site documented 109 knowlesi malaria patients successfully treated with A-L, with no recurrences identified. Unpublished data from this study showed that of the patients with non-severe *P. knowlesi* malaria enrolled, 51 received A-L monotherapy, with a median parasite clearance time of 2 days.<sup>21</sup> There are no published series of other ACTs used in the treatment of *P. knowlesi* infection, although currently both AS-MQ and A-L are being used in Sabah for non-severe *P. knowlesi* and *P. falciparum* malaria, including in children >5 kg, as recommended by local guidelines. In vitro studies using MQ have not been conclusive.<sup>44</sup> Clinically, however, MQ as a single agent has been used in the successful treatment of a Swedish traveller returning from Sarawak, Malaysia with PCR-confirmed non-severe *P. knowlesi* malaria and 0.1% infected erythrocytes in 2009.<sup>29</sup> However, oral MQ was not effective when used as an initial monotherapy in a case reported from Peninsular Malaysia with severe disease and 1% infected erythrocytes.<sup>45</sup> Current WHO 2013 management of severe malaria guidelines recommend intravenous AS and not oral therapy (with any drug) for patients meeting severe malaria criteria due to any *Plasmodium* species.<sup>46</sup> The long half life of MQ of around 14 days also means when used as the partner drug in an ACT for non-severe malaria caused by other *Plasmodium* species such as *P. vivax*, there is a significant reduction compared to A-L in the day 42 treatment failure rate.<sup>44 45</sup>

### Chloroquine

CQ with primaquine was initially suggested to have favourable treatment outcomes for non-severe *P. knowlesi* human infections after a retrospective review of patients from Kapit Hospital in Sarawak in 2004.<sup>16</sup> Following this, a prospective observational study conducted at the same site between 2006 and 2007 administered CQ as a total base dose of 25 mg/kg and primaquine as a gametocidal agent to 73 patients with non-severe PCR-confirmed *P. knowlesi* malaria. This resulted in a median fever clearance of 26 h, mean times to 50% and 90% microscopic parasite clearance of 3.1 and 10.3 h, respectively, and a

median PCR-adjusted clearance time of 3 days. None of the 60 patients who completed the 28-day follow-up demonstrated any evidence of resistance, reinfection or recrudescence.<sup>27</sup> A further 11 patients with non-severe *P. knowlesi* malaria confirmed by PCR (including seven microscopically misdiagnosed initially as *P. vivax*) from a prospective study at a tertiary site in Sabah were successfully treated with CQ with or without primaquine.<sup>21</sup>

### Rationale for the trial

While ACT and CQ have each been successfully used to treat knowlesi malaria, the rapidity of parasite clearance has not been prospectively compared. Malaysian Ministry of Health national policy in the malaria pre-elimination phase involves mandatory hospital admission for all patients with confirmed malaria with hospital discharge only permitted after two negative blood films.<sup>47</sup> Use of a more rapidly acting antimalarial agent would therefore allow earlier hospital discharge and have health cost benefits. A switch to a unified ACT-treatment protocol would avoid ineffectual treatment with CQ in the event of *P. knowlesi* being misdiagnosed as *P. falciparum* or *P. vivax*, both CQ-resistant in Malaysia. As ACTs are already being used for treating *P. falciparum* and are recommended for the increasingly CQ-resistant *P. vivax* found in South-East Asia,<sup>48</sup> the potential benefit of a unified treatment policy to facilitate prompt and effective treatment of all *Plasmodium* species requires evidence for ACT also being optimal treatment for *P. knowlesi* infection.

### AIM AND OBJECTIVES

We aim to test whether the fixed combination of AS-MQ is superior to CQ in order to define the optimal treatment for non-severe *P. knowlesi* infection in adults and children.

The primary end point is the therapeutic efficacy of AS-MQ versus CQ, as defined by the assessment of microscopic *P. knowlesi* parasite clearance 24 h after initiation of treatment. Secondary end points will include parasite clearance time, including at days 2 and 42. True treatment failure rates require a 42-day follow-up period as recommended by WHO antimalarial efficacy and resistance monitoring guidelines.<sup>49</sup> Prevalence of anaemia at day 28 will also be assessed in order to enable comparisons over a similar time frame to studies looking at the use of ACT for other malaria species and the impact on anaemia-associated morbidity.<sup>42</sup> Presence of gametocytes at follow-up will also be assessed to determine any post-treatment transmission benefit as seen in other malarial species treated with ACT.<sup>50</sup>

Specific aims include:

- ▶ Parasite clearance time of *P. knowlesi* infection.
- ▶ Length of hospital inpatient stay and estimated cost to health sector.
- ▶ Rates of recurrent infection/treatment failure at day 42.
- ▶ Occurrence of anaemia at day 28 when using AS-MQ versus CQ.
- ▶ *P. knowlesi* gametocyte carriage throughout follow-up when using AS-MQ versus CQ.
- ▶ Frequency of complications throughout follow-up when using AS-MQ versus CQ.
- ▶ Rates of *P. knowlesi* recurrence in a 1-year follow-up period.
- ▶ Utility of rapid diagnostic tests (RDTs) in the diagnosis of *P. knowlesi* infection.

### TRIAL DESIGN

#### Design and study sites

This is a two-arm, randomised, open-label trial to be conducted at Kudat, Kota Marudu and Pitas District Hospitals in Sabah, Malaysia (see figure 1) over a 3-year period, which will be powered to determine the optimal treatment for non-severe *P. knowlesi* infection.

#### Trial population

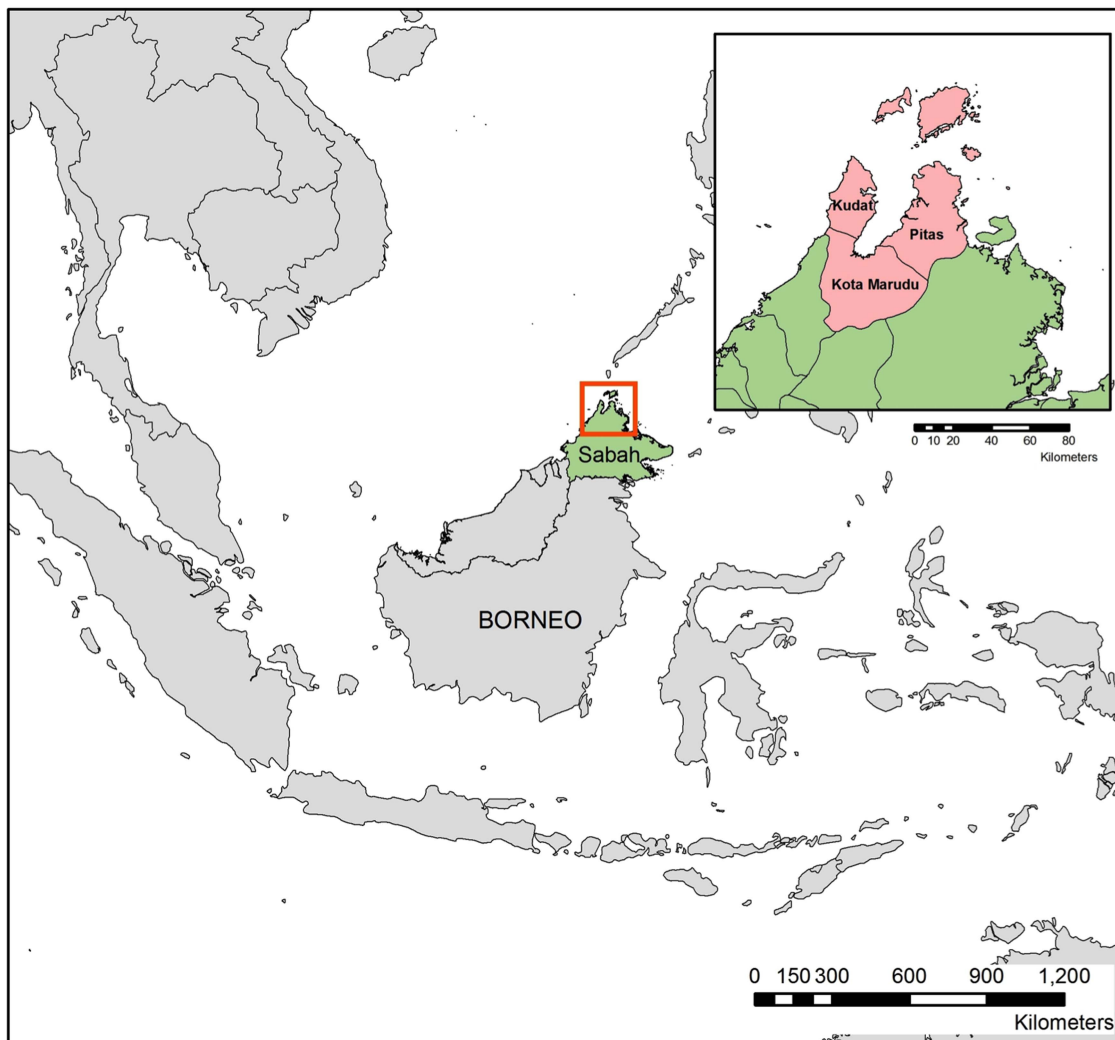
Male and female patients with acute, non-severe *P. knowlesi* malaria who present to Kudat, Kota Murudu and Pitas District Hospitals.

#### Inclusion criteria

- ▶ Male and female patients at least 1 year of age and weighing more than 10 kg.
- ▶ Microscopic diagnosis of *P. knowlesi* (including microscopy result of *P. malariae*).
- ▶ Negative *P. falciparum* malaria RDT (histidine-rich protein 2).
- ▶ Fever (temperature  $\geq 37.5^{\circ}\text{C}$ ) or history of fever in the past 48 h.
- ▶ Able to participate in the trial and comply with the clinical trial protocol.
- ▶ Written informed consent to participate in the trial.
- ▶ Subsequent PCR confirmation of *P. knowlesi* mono-infection; including patients initially enrolled and randomised in concurrent *P. vivax* randomised controlled trial using same study design (clinicaltrials.gov #NCT01708876).

#### Exclusion criteria

- ▶ Clinical or laboratory criteria for severe malaria, including warning signs, requiring parenteral treatment according to modified WHO 2010 criteria.<sup>21</sup>
- ▶ Parasitaemia  $>20\,000/\mu\text{L}$ , a parasite threshold associated with increased risk of severe malaria in *P. knowlesi* infection.<sup>21</sup>
- ▶ Inability to tolerate oral treatment.
- ▶ Concomitant infection with any other malaria species.
- ▶ Pregnancy or lactation.
- ▶ Unable or unwilling to use contraception during study period.
- ▶ Known hypersensitivity or allergy to artemisinin derivatives.



**Figure 1** Map of study sites.

- ▶ Serious underlying disease (cardiac, renal or hepatic).
- ▶ Received antimalarials in previous 2 months.
- ▶ Previous psychiatric illness or epilepsy.
- ▶ Previous episode of cerebral malaria.

### Sample size

We expect that 55% of study participants treated with CQ will remain parasitaemic 24 h after the start of treatment in contrast to 33% of study participants treated with AS-MQ.<sup>25 27</sup> A trial that will have 90% power to falsify the Null hypothesis: “there is no significant difference in parasite clearance between artesunate-mefloquine and chloroquine at 24 hours” with a significance level ( $\alpha$ ) of 0.05 requires 114 participants in each arm or 228 trial participants. It is assumed that 10% of study participants cannot be followed up for 42 days. Therefore, we plan to recruit 256 trial participants.

### Duration and size of trial

Total duration of the trial is 36 months, with an enrolment period of 24 months. Individual participation

requires follow-up clinic visits after discharge from hospital on days 7, 14, 28 and 42 post-treatment, and telephone contact, when possible, to assess for malaria recurrence up to a total period of 12 months. The number of trial participants required is 256 (each arm=128).

### Treatment allocation

An independent statistician will use computer-generated randomisation to generate blocks of patient numbers for each treatment in the separate arms. Each code will be given a sealed opaque envelope which will contain that patient's treatment group and which will only be opened by a study nurse when a patient has been allocated a code number.

### Test drugs

Both study drugs are currently registered and available in Malaysia, and approved for use by the Malaysian Ministry of Health. The choice of drugs, including their administration and dosing have all been chosen to mimic existing local protocols and conditions in order

to ensure the data gathered are relevant to the clinical setting in which the drugs will be used.

- ▶ *Artequin* (Mepha Ltd, Aesch, Switzerland) consisting of two different fixed dose tablet blister packs: 600/1500—three tablets of AS 200 mg, six tablets of MQ hydrochloride 275 mg equivalent to MQ 250 mg base; 300/750—three tablets of AS 100 mg, three tablets of MQ 250 mg base.
- ▶ *Artequin Paediatric*: a fixed dose formulation of granules containing: AS 50 mg and MQ 125 mg per dose.
- ▶ *CQ*: consisting of 155 mg tablets.

### AS-MQ dosing

The safety and efficacy of AS-MQ in the treatment of non-severe malaria due to other *Plasmodium* species is based on a dose of AS 4 mg/kg/day for 3 days<sup>51</sup> and a total MQ dose of 25 mg/kg split over either days 2 and 3, or equally over all 3 days, as this leads to increased bioavailability and reduces risk of vomiting.<sup>41 52</sup> The dosages of the AS-MQ combinations to be administered in this study are based on these target weight dependent calculations and are consistent with current WHO<sup>36</sup> and Malaysian Ministry of Health treatment guidelines (see [figure 2](#)).<sup>47</sup>

### CQ dosing

The total dose of CQ is 25 mg base/kg body weight over 3 days, as used for non-severe knowlesi malaria in other studies<sup>27</sup> and is also consistent with WHO and Malaysian Ministry of Health guidelines for non-severe vivax malaria. Each tablet contains 155 mg of CQ base.

Adult dose ( $\geq 35$  kg): 620 mg (4 tablets) at 0 h, and 310 mg (2 tablets) at 6, 24 and 48 h.

Child dose ( $< 35$  kg): 10 mg/kg at 0 h, and 5 mg/kg at 6–8, 24 and 48 h.

### Dosing schedule

The day a patient is enrolled and receives the first dose of medicine is designated 'day 0' ([figure 3](#)). All antimalarial treatment administration will be supervised and witnessed by a study team member. Enrolled patients will be observed for at least 30 min after treatment to ensure they do not vomit the medicine. Any concomitant

medications will be documented in the case record form (CRF).

### Rescue medication

Should the patient vomit the first dose of each treatment within 1 h, this dose will be repeated. If a patient vomits again within 1 h, or for any patient subsequently meeting modified WHO 2010 criteria for severe malaria<sup>21</sup> or early treatment failure,<sup>36</sup> the trial medication will be discontinued and rescue medication will be initiated. Patients will receive intravenous AS 2.4 mg/kg initially, and again at 12 and 24 h. A full course of subsequent oral rescue treatment will be given that will be opposite to the trial medication administered initially.

### TRIAL PROCEDURES

Appropriately qualified research medical staff will conduct screening and enrolment of participants. This will include clinical examination, observations and blood sampling for laboratory investigations during admission, and at scheduled clinic follow-up visits at 7, 14, 28 and 42 days post-treatment (see [figure 4](#)). Research staff will also ensure patients receive proper allocated medication dosages, monitor for adherence and AEs and initiate rescue medication where appropriate. A standardised CRF will be used to collect clinical, biochemical and parasitological data including AEs until the end of the individual's study period.

### Temperature

Temperature will be measured six hourly during admission and then at all follow-up visits using a consistent method in order to evaluate fever clearance.

### Parasite counts

Thick and thin blood films for parasite counts will be obtained via finger prick six hourly postadministration of study medications until negative by microscopy consecutively over a minimum 24 h period, then at follow-up on days 7, 14, 28 and 42. A qualified microscopist will read all the slides independently and be blinded to treatment. Slides will be cross-checked by a second expert microscopist at a later date and parasite densities quantitated by the expert microscopist will be used. Blood

**Figure 2** Artesunate-mefloquine (AS-MQ) dosing guide.

Weight:	Formulation:	No. of tablets:	Daily dose:
> 40 kg	Artequin <sup>R</sup> 600/1500	AS = 1 tablet	AS = 200mg
		MQ = 2 tablets	MQ = 500mg
21 - 40 kg	Artequin <sup>R</sup> 300/750	AS = 1 tablet	AS = 100mg
		MQ = 1 tablet	MQ = 250mg
>10 - 20 kg	Artequin Paediatric <sup>R</sup>	AS / MQ granules	AS = 50mg
			MQ = 125mg

**Figure 3** Dosing schedule.

	Day 0 0 hrs	Day 0 6 hrs	Day 1 24 hrs	Day 2 48 hrs
Artesunate-mefloquine	X		X	X
Chloroquine	XX	X	X	X

smears with discordant results (differences between the two microscopists in species diagnosis, in parasite density of >50% or in the presence of parasites) will be re-examined by a third microscopist, and parasite density will be calculated by averaging the two closest counts.

### Blood sampling

Up to 65 mL of an adult participant's blood, or up to 50 mL for children, will be taken in total for research purposes during the standard study duration and schedule of activities. This will include initial samples processed for thick and thin blood films, assessment of glucose-6-phosphate dehydrogenase (G6PD) activity (Beutler fluorescent spot test), malaria RDTs, serological assays and other parasitological and immunological parameters. Haemoglobin will be assessed daily during admission and at follow-up visits. Confirmatory PCR speciation will be performed on all samples using established methods.<sup>53–55</sup> Drug levels for CQ and MQ using

high-pressure liquid chromatography with ultraviolet detection will be performed on samples from days 0, 7 and 28. Blood cultures will be done on all patients in order to exclude any concurrent blood stream infection. All samples will be labelled anonymously, and may be stored for a period of up to 10 years after publication of the study. Results of blood sampling, including any other standard laboratory or radiological investigations ordered by the study site hospital during admission, will be documented in the CRE.

### Malaria RDTs

Pretreatment blood will be used for different malaria RDTs in order to assess for *P. falciparum* histidine-rich protein 2 (HRP2) as an exclusion criterion and to evaluate different monoclonal antibodies against *Plasmodium* parasite-lactate dehydrogenase (LDH) for *P. knowlesi* detection.<sup>56–58</sup> RDTs will include: CareStart Pf/VOM, Humasis Pf/Pv Ag, Bionote Pf/Pv Ag, OnSite Pf/Pv Ag, CareStart PAN. RDTs will be transported,

TIMEPOINT (Day)	STUDY PERIOD											
	Enrolment	Allocation	Post allocation									
	0	0	1	2	3	4	7	14	28	42	≤ Wk 52	
<b>ENROLMENT</b>												
Eligibility screen	X											
Pregnancy test	X											
Malaria rapid diagnostic test	X											
Informed consent	X											
Drug allocation		X										
<b>INTERVENTIONS</b>												
Artesunate-mefloquine		X										
Chloroquine		X										
<b>ASSESSMENTS</b>												
Medical history, previous medication	X											R
Physical examination, vital signs	X		X	X	X	(X)	X	X	X	X	X	R
Axillary temperature (6 hourly)	X		X	X	X	(X)	X	X	X	X	X	R
Blood slide for microscopy (6 hourly)	X		X	X	X	(X)	X	X	X	X	X	R
Concomitant medication reporting			X	X	X	(X)	X	X	X	X	X	R
Adverse event reporting			X	X	X	(X)	X	X	X	X	X	R
Haemoglobin / haematocrit	X	X	X	X	X	(X)	X	X	X	X	X	R
PCR speciation	X						R	R	R	R	R	R
Immunological/parasitological markers	X						X			X	R	R
Antimalarial drug level	X						X			X	R	R
Phone call (fever history reporting)												X

x = once daily; (x) = patients without parasite clearance; R = if recrudescence

**Figure 4** Timeline of activities.

stored and conducted according to manufacturer's instructions.

### Pregnancy testing

Female patients of childbearing age, defined as those who menstruate or are aged over 12 years, will be asked to take a screening urine pregnancy test before enrolment in the study. CQ has limited prospective published data in the first trimester but is generally regarded as safe.<sup>36</sup> However, due to previous concerns from studies of MQ of monotherapy in pregnant women in Thailand,<sup>43</sup> AS-MQ is currently not recommended throughout pregnancy and any patients found to be pregnant will be excluded from the study.

## SAFETY EVALUATION

### Treatment failure classification and management

While no treatment failures have been reported for non-severe *P. knowlesi* infection, as prospective data is limited, the conservative framework modelled on WHO definitions for treatment failure in *P. falciparum* infection will be used.<sup>36</sup> These treatment outcomes are classified on the basis of an assessment of both the parasitological and clinical outcome of antimalarial treatment. All patients will be classified as having early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response.<sup>36</sup>

### Adverse events

AEs and serious AEs (SAEs) are defined according to International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP).<sup>59</sup> At each patient encounter, a standardised system for assessing, reporting and following up AEs and SAEs will be adhered to as per guidelines developed by the World Wide Antimalarial Resistance Network (WWARN).<sup>60</sup> All SAEs occurring during the study will have a separate notification form and be reported by the principal investigator to the sponsor and the study monitor.

### Removal of patients from trial

Participants will be removed from the trial if informed consent is withdrawn, or if they fail to complete treatment (ie, inability to tolerate oral medication, non-adherence to treatment regime, or develop one or more SAEs necessitating cessation of study medicine before completion).

### Suspension or termination of the trial

The safety of both these medications is well established and they are currently first-line recommended Malaysian Ministry of Health treatments.<sup>47</sup> In addition, as these are not new administration regimens, a safety and monitoring board will not be used. Strict reporting of AEs and SAEs will be done, with an experienced study monitor to conduct visits before, during and at the end of the trial. Any decision on suspending or terminating the

trial will be based on an evaluation of SAE reporting and monitoring visits.

## ANALYSIS

Clinical, biochemical and parasitological information from standardised CRFs will be double entered into an electronic database (EpiData, V.2.2, Denmark) to ensure accuracy. The data will be transferred and analysed in STATA, V.12 (StataCorp Ltd, Texas, USA).

### Primary variable

The difference in the proportion of patients who are parasite free at 24 h after treatment with AS-MQ compared with CQ. This will be compared by intention-to-treat analysis.

### Secondary variables

- ▶ Days 28 and 42 cure rate (with and without PCR correction).
- ▶ Proportion of patients with a negative slide at days 2 and 3.
- ▶ Parasite clearance: proportion of parasites/ $\mu$ L at days 1, 2 or 3 after treatment compared with baseline.
- ▶ Time to resolution of fever, other symptoms and clinical signs.
- ▶ Haematological recovery as shown by incremental change in haematocrit or haemoglobin from days 0 to 28 and days 0 to 42.
- ▶ Gametocyte carriage throughout 42-day study period.
- ▶ Progression to severe malaria (modified WHO 2010 criteria<sup>21</sup>).
- ▶ Sensitivity and specificity of RDTs for the diagnosis of *P. knowlesi* malaria.
- ▶ Recurrence of any species of malaria during the 12-month follow-up period.

Overall cure rates at day 42 will be evaluated by survival analysis with cumulative incidences calculated by Kaplan-Meier method and also compared by the Mantel-Haensel log-rank test. No interim analysis will be performed.

### Other data included in final analyses

(1) A description of all patients screened and reasons for non-inclusion; (2) clinical, epidemiological and laboratory features of all patients included in the study; (3) the proportion of AEs and SAEs in all the patients included in the study; (4) the proportion of patients lost to follow-up or withdrawn, with reasons for withdrawal and (5) length of hospital inpatient stay and estimated cost to health sector.

### Withdrawal or censoring in analysis

Patients will be withdrawn from the analysis if PCR results are unclassifiable or shown not to be *P. knowlesi* mono-infection. Patient's results will be censored in the analysis if PCR genotyping indicates any treatment

failures are due to reinfection with *P. knowlesi* or new infection with another *Plasmodium* species, or if concomitant treatments with antimalarial activity are taken during the study period, including the initiation of rescue medication. Any patients withdrawn from the trial may still have data collected to that time point included in the analysis. The extent of missing data will be assessed and either consistent censoring of these patients results or multiple imputation methods will be considered.

## ETHICS AND DISSEMINATION

### Institutional Review Board approval

The study has been registered under <http://www.clinicaltrials.gov> (#NCT01708876). Any subsequent protocol amendments will be submitted to the ethics committees and made available to the study monitor.

### Informed consent

Patients will be included in the study only if they or parents or guardians of participants <18 years of age give written informed consent. Assent for those from ages 7 to 17 will be obtained in addition, as per Malaysian Medical Research Ethics Committee (MREC) guidelines. Informed consent will be obtained by trained study nurses in Bahasa Malaysia or English. If the patient is illiterate, then thumbprint will be obtained on the consent form in accordance with Malaysian GCP guidelines. A consent statement for pregnancy testing is also required for female participants of childbearing age who are sexually active. Informed consent may be withdrawn at any time during the study period and will have no effect on the patient's clinical management at the study site. Biospecimen storage for a period of up to 10 years at the participating institutions will be documented on the patient information sheet and consent form. Samples will only be used for MREC approved assays.

### Confidentiality

All information on patients will remain confidential and be shared only within the research team. Unique identifiers will be used for computer-based data entry and blood samples. Screening forms, the case report form and the completed identification code list will be kept in locked files.

### Healthcare services

Any person who decides not to participate or who cannot be enrolled into the study because he or she does not meet the criteria will be referred for standard Malaysian Ministry of Health management. It will be stated clearly that a decision not to participate in the trial will not affect subsequent care.

### Reimbursement of transport

Participants in the study will be reimbursed for their transport to attend all follow-up visits to the study sites.

Patients will be paid MYR30 for each follow-up visit. No other gifts or payments will be made.

### Trial-related injury

This is an investigator-initiated trial evaluating current first-line recommended standard therapies. As there is minimal additional risk from a normal standard of care, a trial Data Safety and Monitoring Board was not deemed necessary. Individuals will only agree to participate after being fully informed of inherent risks. There is no clinical trial insurance and patients will not receive financial compensation for any trial-related injury; however, patients will receive full access to standard Malaysian Ministry of Health hospital and outpatient care.

### Dissemination of results

At the end of the study, the principal investigator will finalise a report on the study and its main outcome. This report will be shared with the national malaria control programme and the Malaysian Ministry of Health. Results will be published in peer-reviewed journal articles, presented at academic meetings, and relayed to communities at Kudat, Kota Murudu and Pitas through public meetings. All investigators will have access to the full trial data set and be included in relevant publications.

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