1-STEP STATISTICAL ANALYSIS PLAN

Convenience and cost-aspects of a new 1-step reconstitution injectable artesunate compared to conventional 2-step injectable artesunate for the treatment of severe falciparum malaria: a multi-centre study

Protocol version: version 2.0 dated 18 August 2021

Trial registration number: NCT05140278

Version 0.4

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1. BACKGROUND

Parenteral artesunate is the first-line treatment for severe malaria.[1,2] The two largest ever treatment trials conducted in patients hospitalised with severe malaria, which compared parenteral artesunate (produced by Guilin/ Fosun) with quinine: SEAQUAMAT in Asia in 1,461 patients[3], and AQUAMAT in Africa in 5,425 children[4]. In both trials artesunate was associated with a significant and substantial reduction in mortality compared to quinine (a 34.7% relative reduction in deaths in SEAQUAMAT, 22.5% in AQUAMAT), which was not at the expense of an increase in severe sequelae. These findings resulted in a change of the WHO recommended first line antimalarial therapy for severe malaria in all endemic settings, and it has been estimated by the Medicines for Malaria Venture that since 2011 this has saved well over 500,000 young lives in Africa (https://www.mmv.org/our-impact/achievements/168-million-vials-artesun-delivered-treat-children-severe-malaria).

The conventional formulation of injectable artesunate requires a 2-step reconstitution and dilution of the artesunate hemisuccinate powder, including reconstitution in a sodium bicarbonate solution followed by further dilution in 5% dextrose or normal saline. The 2-step procedure takes time and is error prone, dissolving the artesunate powder in the bicarbonate solution is sometimes difficult, and the 2-step procedure requires additional consumables, like sterile syringes (https://www.severemalaria.org/toolkits-training/injectable-artesunate-tools-training). Preparation of conventional injectable artesunate (Artesun SmPC) requires shaking the vial for 3 to 5 minutes

to mix well until the powder is completely dissolved and the solution is clear. Another disadvantage of the conventional artesunate is the two steps needed to reconstitute artesunate powder by sodium bicarbonate first then to dilute the solution by sodium chloride should not be exchanged. Otherwise, artesunate will not be dissolved. Such malpractice can cause a waste of medicine and put critical ill severe malaria patient under risk. 1-step artesunate can avoid such mistakes.

2. GENERAL ANALYSIS APPROACH

The principle of intention to treat (ITT) will be the main strategy of analysis for the primary outcome. These analyses will be conducted on all patients assigned to the treatment groups as randomised. Any patients subsequently found not to be eligible during the trial or that did not receive a full course of study drug will be included in these analyses. Per Protocol (PP) analysis will be conducted to examine robustness of ITT results. This will be a form of sensitivity analysis to the intention to treatment analysis principle. In the PP analyses, all patients wrongly included in the study based on the inclusion/exclusion criteria will be excluded from analyses.

The cost analysis of 1-step parenteral AS will assess health facility-level costs, and health system costs to encompass all costs of a potential change from conventional to 1-step artesunate, including re-training, materials, drug replacement. Unit costs will be assigned to healthcare worker time spent on managing patients with severe malaria (derived from the time and motion surveys). Micro costing will be carried out in each of the sites to estimate the costs of equipment and consumables associated with provision of artesunate with each of the formulations. The resulting labour and consumable mean costs will be compared in patients treated with 1-step vs conventional artesunate formulation.

Time from opening box to completed preparation of syringe will be summarised using means and standard deviation for each each arm. Difference in means and the correponding 95% confidence intervals will be reported. We will provide overall comparisons by arm as well as for each site separately. The comparisons will be done for baseline times ans well as for all recorded times. Total cost of consumables in US dollars will be summarised in a similar way. A Student's independent t-test will be used for comparison of means between groups. The Central Limit Theorem justifies the use of the Student's t-test even if the times/costs may not be normally distributed because of the large sample size. However, if the data will be highly skewed, non-parametric tests such as Manny-Whitney (Wilcoxon) U test will be considered. Statistical significance will be declared at 5% significance level.

Data analysis will be performed using Stata 17 or higher, StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA

1.1 Data integrity

This study will be conducted in compliance with the protocol, relevant Standard Operating Procedures (SOPs), Good Clinical Practice (GCP) and the applicable regulatory requirement(s). All the analyses will be performed on clean data only.

1.2 Data cleaning and verification

All data will be cleaned and verified prior to statistical analysis. The study site will be monitored remotely at times agreed on with the Investigator. At the time of each monitoring session, the Monitor will review the completed CRFs to ascertain that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol. The Monitor will also check that the data in the CRF are consistent with the clinical records (Source Data Verification [SDV]) and that study results are recorded completely and correctly. The data manager will ensure that clean data is submitted to the statistician for analysis. The statistician will cross-check that the available data for analysis is clean. Any data cleaning queries will need to be resolved before statistical analyses.

1.3 Locking the dataset

After data cleaning and responding to all data queries, the clean data will be locked normally in the database that was used for data capturing. The data may also be locked and stored in other user-friendly formats such as MS Excel and Stata. The locked data will be stored at an identifiable secure place and should be available to the relevant researchers upon request following proper request procedures. The data will also be in other robust backup media.

1.4 Data format and Analysis logs

Prior to dispensing data to the trial statistician, the head of data management will make sure that the data to be sent to the trial statistician is clean. This will help the statistician to provide the analysis results in a timely manner as there will be a reduced amount of queries if clean data is provided to the trial statistician. Data will be given to the Trial Statistician by the head of Data Management (or designated person) in a format that is compatible with statistical software reading. Statistical analyses will be performed in Stata, version 17 or higher, or in R software. Statistical programs and output logs will be kept for all analysis and made available upon request.

1.5 Interim analyses

The interim reports will be upon request by the DSMB. No stopping rules both statistical as well as clinical will be specified. The need to stop the trial will be based on the perception of the accumulating data by the DSMB.

2. Study objectives and endpoints

2.1.1. Primary objective

Assessment of:

1. Convenience and rapidness of administration of 1-step vs. conventional 2-step parenteral artesunate formulations

and

2. Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations

2.1.2. Primary endpoint

1. Time to administration of treatment comparing 1-step vs. conventional 2-step parenteral artesunate formulations (by time-and-motion methods).

and

2. Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations at health facility, and at health system level.

2.1.3. Secondary objectives and endpoints

1. To assess and compare *P. falciparum* parasite clearance rates of 1-step vs. conventional 2-step parenteral artesunate formulations.
   Parasite clearance half-life and other parasite clearance parameters (PC50, PC90, 12-hour parasite reduction ratio) compared between 1-step vs. conventional 2-step parenteral artesunate formulations.

2. To assess and compare time from intravenous treatment to follow up treatment with oral drugs with 1-step vs. conventional 2-step parenteral artesunate formulations.
   Time from start parenteral treatment to follow up treatment with an oral ACT (recovery to per os treatment) of 1-step vs. conventional 2-step parenteral artesunate formulations.

3. To assess and compare disease outcome parameters between 1-step vs. conventional parenteral artesunate formulations.
   Fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5 ° C and remain there for at least 24 hours) of 1-step vs. conventional 2-step parenteral artesunate formulations.

4. To assess and compare adverse events between 1-step vs. conventional 2-step parenteral artesunate formulations
   Incidence of adverse events and serious adverse events by study arms within the first 28 days.

2.2. Study design

Brief Description

This is an Open-label Randomised Trial. The new 1-step artesunate parenteral formulation is thus expected to be equally effective, but likely more convenient and faster to use then conventional formulation. And to avoid reconstitution mistakes in field practice. Also, for the conventional formulation, the volumes of 0.9% sodium chloride used for IV route and IM route are different, for IV route: After reconstitution and dilution, one ml of solution for injection contains 10 mg artesunate. IM route: After reconstitution and dilution, one ml of solution for injection contains 10 mg artesunate. This can cause confusion among health workers and lead to mistakes. The 1-step formulation uses the same volume of solvent for IV and IM. In addition, the 1-step reconstitution is expected to be cheaper to administer, because it will use less of the health professional's time and will use less consumables. We propose a study to compare and quantify convenience and costs of the new 1-step artesunate parenteral formulation versus the conventional formulation in a randomised study. This study will also integrate social science studies to explore and compare acceptability of 1 step artesunate with the conventional formulation using SSIs and surveys among study staff, health staff and policymakers. In addition, efficacy data and adverse events will be captured.

Figure 1. Study sites: Kinshasa, DRC, and Korogwe, Tanzania

There are 2 centres of recruitment in 2 different countries (DRC and Tanzania). 100 patients will be recruited from each of two centres: Kinshasha DRC, and Korogwe Tanzania).

2..2.1 Determination of sample size

Sample size calculations for non-inferiority study is based on dissolution test data from Guilin Pharmaceutical Co. Ltd. ("Study report on the solubility of artesunate for injection (60mg), Doc. RR-QC-ZSYQHHZ (60mg)RJX-2021 30th April 2021).[23] This is supported by unpublished in-house experience (from a bioequivalence study in healthy volunteers, personal communication, Dr Podjanee Jittmala, Mahidol University) that the conventional formulation takes about 4 minutes with a standard deviation of 2 minutes to administer injectable artesunate. Our non-inferiority margin is 1 minute of drug re-constitution for the new simpler artesunate formulation i.e. if participants in the 1-step artesunate formulation will have a mean of the time of 5 minutes or less of drug re-constitution, with a standard deviation of 2 minutes, then the new formulation will be considered to be non-inferior to the old formulation. With this non-inferiority margin, detecting non-inferiority with 90% power and with a one-sided alpha of 0.025, we will need to recruit 85 participants in the new formulation and 85 in the old formulation arm giving a total of 170 participants. In order to compensate for a 15% loss to follow-up or withdrawal, we will need 100 participants in each arm giving a total of 200 participants in the two arms combined. These calculations were performed in Stata 16 (StataCorp LLC Software).

The sample size calculation dovetails with pragmatic needs for the other study activities the 200 participants provides sufficient variability of participant characteristics to allow staff to gain experience of using 1-step artesunate and be able to provide an informed opinion of its pros and cons. Recruitment of 100 patients per site is feasible over 12 months based on the expected numbers of cases of severe malaria (Dr Fanello and Dr Gesase, personal communication April 2021). The sample size calculation is based only on the time-to-treatment of participants and not on the costing analysis.

3. Data Analysis

3.1 Trial Profile

The number of patients who will be screened, reasons for non-enrolment, number of patients randomized, number of patients lost to follow up and the number of patients assessed for 28-day endpoints will be summarised in a CONSORT flow diagram, figure 2, below.

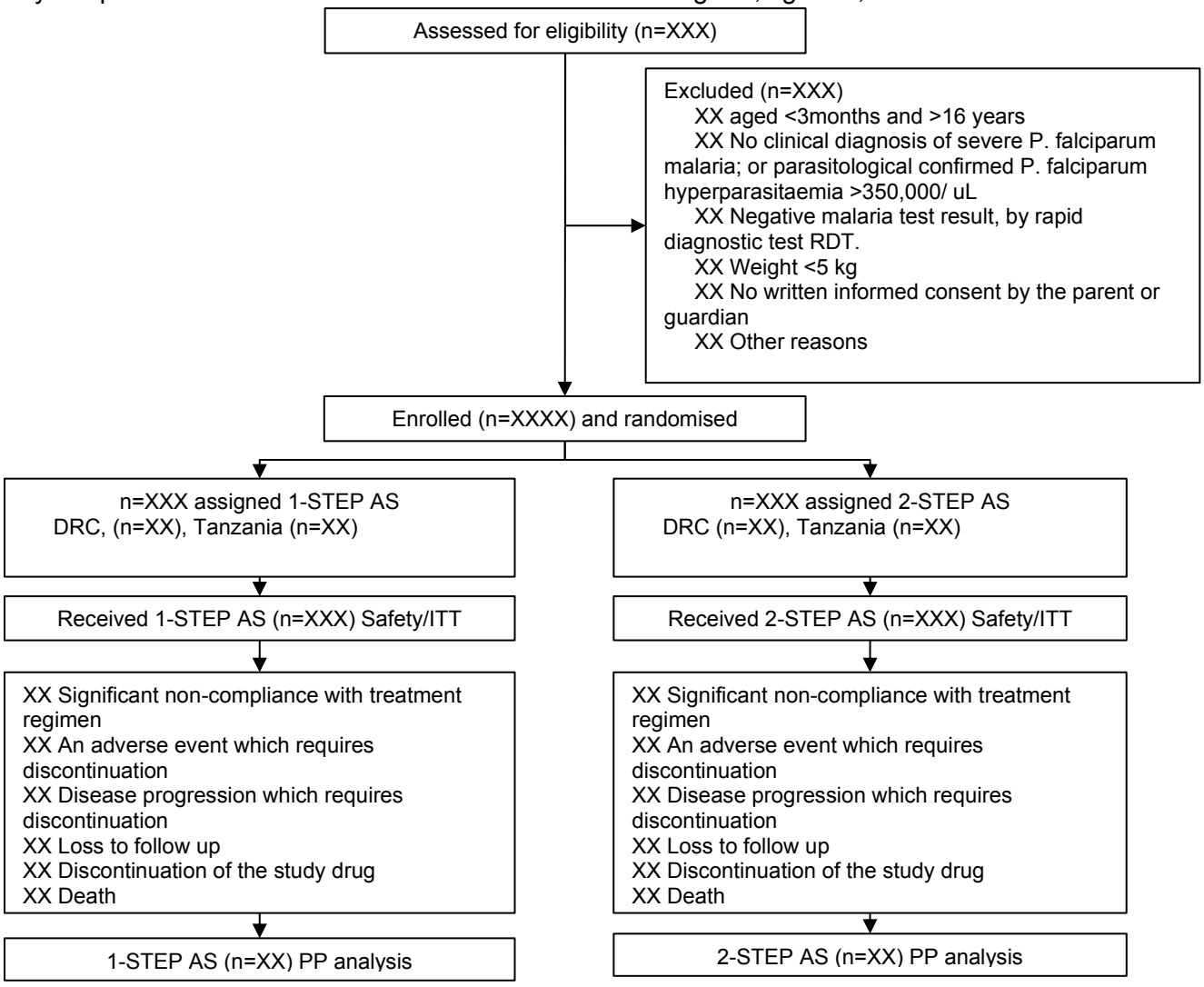

Figure 2 Consort Trial Profile by Arms

3.2 **Demographics and other baseline characteristics**

The following baseline characteristics will be described by study arm in table 1 (below). Variables such as age, heart rate, respiratory rate will be summarized using median and interquartile rage. Continuous variables such as weight, height, systolic and diastolic blood pressure, , haemoglobin will be summarized using mean ± standard deviation. Parasitaemia at baseline will be described as geometric mean and range. Categorical variables such as sex, presence of fever and gametocytaemia at baseline will be summarized using frequencies and percentages.

Table 1. Baseline Characteristics of the Patients

Characteristics	1-STEP AS (N=XXX)	2-STEP AS (N=XXX)	Total (N=XXX)
Age (years), med (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Male sex, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Weight (kg), mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Height (cm) mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Hb (g/dL), mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Parasites/μL, geometric mean (range)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Impaired consciousness or unrousable coma, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Prostration, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Failure to feed and drink without assistance, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Multiple convulsions, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Deep breathing, respiratory distress, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Circulatory collapse or shock, systolic BP < 70 mmHg, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Clinical jaundice plus evidence of other vital organ dysfunction, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Haemoglobinuria, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Suspected pulmonary oedema, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)

Renal failure (< 20 ml urine per hour) , n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Spontaneous bleeding/disseminated intravascular coagulation, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Several episodes of vomiting in the preceding 24 hrs, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Severe pallor with respiratory distress OR HCT<15% OR hb<5.0g/dl, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)

3.3 Convenience and rapidness of administration of 1-step vs. conventional 2-step parenteral artesunate formulations and Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations

Primary objective

To assess:

1. Convenience and rapidness of administration of 1-step vs. conventional 2-step parenteral artesunate formulations

and

2. Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations

Primary endpoint

1. Time to administration of treatment comparing 1-step vs. conventional 2-step parenteral artesunate formulations (by time-and-motion methods).

and

2. Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations at health facility, and at health system level.

Table 2 Drug preparation times

Time from opening box to completed preparation of syringe, at baseline only				
	Minutes and seconds, mean (SD)		Difference in means, (95% CI)	p-value
	1-STEP AS (N=XXX)	2-STEP AS (N=XXX)		
Overall	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
By site				
DRC	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
Tanzania	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
Time from opening box to completed preparation of syringe, at all recorded times.				
Overall	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
By site				
DRC	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
Tanzania	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
Total cost of consumables in US dollars, mean, SD.				
Overall	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
By site				
DRC	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX
Tanzania	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X - XX.X)	X.XXX

USD calculated from average cost of consumables used, and wastage of drug.

3.4 **Fever clearance and parasite clearance during treatment, by treatment allocation**

Table 4 Clinical recovery*, fever clearance, and parasite clearance by study arm**

	1-STEP AS N=XXX	2-STEP AS N=XXX	Both groups N=XXX	P-value (2-sided)
Hours to fever clearance, mean (SD) (baseline to start of first 24h period <37.5C; temperature was recorded every 4 hours, until day 2, then 6 hourly)				
DRC	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
Tanzania	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
All study sites	**XX.X (X.X)**	**XX.X (X.X)**	**XX.X (X.X)**	**X.XXX**
Day 3 blood smear still positive, n/N (%)** (taking prior clearance or discharge as negative)				
DRC	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Tanzania	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
All study sites	**XX/XXX (XX)**	**XX/XXX (XX)**	**XX/XXX (XX)**	**X.XXX**
Hours to tolerate oral medication, mean (SD)* (baseline to start of oral ACT)				
DRC	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Tanzania	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
All study sites	**XX/XXX (XX)**	**XX/XXX (XX)**	**XX/XXX (XX)**	**X.XXX**
Parasite clearance half-life in hours, mean (SD)** (excluding patients with initial count insufficient to estimate half-life)				
DRC	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
Tanzania	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
All study sites	**X.X (X.X)**	**X.X (X.X)**	**X.X (X.X)**	**X.XXX**

*Protocol recommends patients receive a minimum of 3 doses of injectable artesunate before switch to oral ACT. Patients may vary if discharged before 24 hours, died, or referred.
** Parasite dynamics will be affected by patients who are transfused.

3.5 Safety assessments and tolerability (Incidence of adverse events and serious adverse events by study arms within the first 28 days

Safety analyses will be based on the whole population that get administered the study drug. That is, the safety and tolerability data will be pooled from all the sites that received the same antimalarial treatment.

The safety and tolerability of 1-STEP AS versus 2-STEP AS will be assessed by comparing the frequency (%) of adverse events and serious adverse events, with particular attention to the variables listed below, which pertain to severe malaria.

Safety data will be presented in tabular and/or graphical format and summarized descriptively. Any clinically relevant abnormalities or values of potential clinically concern will be described. Patients will be analysed following the intention to treat analysis. All adverse event summaries will refer to treatment emergent adverse events, i.e. adverse events that newly started or increased in intensity after the study drug administration. Adverse events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE). The safety and tolerability summaries will be presented in table 5.

Table 5 Incidence of adverse events within first 28 days by study arm

	1-STEP AS		2-STEP AS		p-value
Number of subjects	XX		XX		
Serious adverse events (SAEs) , n/N, (%)	XX		XX		X.XXX
Possible, probable or definite drug related SAEs, n/N, (%)	X/XX (X.X)		X/XX (X.X)		X.XXX
Neurological sequelae at discharge	X/XX (X.X)		X/XX (X.X)		X.XXX
Neourological sequelae at 28	X/XX (X.X)		X/XX (X.X)		X.XXX
Neurological sequelae persisting at end of follow up	X/XX (X.X)		X/XX (X.X)		X.XXX
Grading of adverse events, n/N, (%)	1-2	3-4	1-2	3-4	
Symptoms					
Impaired consciousness or unrousable coma, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Prostration, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Failure to feed and drink without assistance, n (%)					
Multiple convulsions, n (%)					
Deep breathing, respiratory distress, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Circulatory collapse or shock, systolic BP < 70 mmHg, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Clinical jaundice plus evidence of other vital organ dysfunction, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Haemoglobinuria, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Suspected pulmonary oedema, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX

Renal failure (< 20 ml urine per hour) , n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Spontaneous bleeding/disseminated intravascular coagulation, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Several episodes of vomiting in the preceding 24 hrs, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Severe pallor with respiratory distress OR HCT<15% OR hb<5.0g/dl, n (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Total	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Laboratory abnormalities					
Creatinine	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Total bilirubin	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Direct bilirubin	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Alanyl transfarase (ALT)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Aspartate transfarase (AST)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Alkaline phosphatase	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Hematocrit decrease	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX

Adverse event grading: grade1 to grade 5 (CTCAE)