H-PRIME

Statistical Analysis Plan version 2.0 16 June 2025

Hydroxyurea - Pragmatic Reduction In Mortality and Economic burden

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H-PRIME Statistical Analysis Plan			
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Revision History

Version	Author	Date	Reason for Revision
Draft 0.1	Roisin Connon	6/9/2023	First draft based on protocol version 4.0
Draft 0.2	Roisin Connon	21/9/2023	Updated following comments from EG
Draft 0.3	Roisin Connon	9/10/2023	Updated following discussions with SW and EG.
Draft 0.4	Roisin Connon	26/1/2024	Added section on lab results.
Draft 0.5	Roisin Connon	29/1/2024	Minor wording edit in section 5.6
Draft 0.6	Roisin Connon	5/4/2024	Revised following comments from TW
Draft 0.7	Roisin Connon	15/4/2024	Further minor edits from TW
Draft 0.8	Roisin Connon	23/4/2024	Changes to analyses to be included in DMC interim report
Draft 0.9	Roisin Connon	3/5/2024	Clarifications regarding family randomisation after comments from DMC statistician
Draft 0.91	Roisin Connon	13/5/2024	Minor typographical corrections.
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Draft 1.1	Roisin Connon	2/6/2025	Updated to align with protocol v5.0.
Draft 1.2	Roisin Connon	10/6/2025	Updated following comments from EG
Draft 1.3	Roisin Connon	12/6/2025	Updated following comments from SW and TW
Draft 1.4	Roisin Connon	16/6/2025	Added details of HbF calculation
Draft 1.5	Roisin Connon	16/6/2025	Edit following comment from SW
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Contents

1. INTRODUCTION

1.1 Background and Rationale

The term sickle cell disease (SCD) describes a group of conditions in which the main form of haemoglobin produced is Haemoglobin S (HbS) and which are characterised by chronic anaemia, intermittent crises and progressive multi-organ deterioration. Without specific treatment, SCD results in high rates of both disability and early mortality.[1]

Most children born with SCD in high-income countries are diagnosed early and treated by specialists, and as a result, almost all now survive to adulthood.[2] There are a growing number of treatments increasingly being used to manage SCD patients in high-income countries. However, the majority of children with SCD are born in sub-Saharan Africa, and newborn screening has been rare in this region, although is now increasing. Until very recently, the majority of cases were never diagnosed and 50-90% of affected children died before their fifth birthday. Of the commonly available disease-modifying treatments, all are considered either too complex or too expensive to be realistic options for sub-Saharan Africa within the foreseeable future with the exception of hydroxyurea.

The standard approach to hydroxyurea therapy in high-income countries involves individually escalating the dose while closely monitoring haematological parameters to a point approaching toxicity - the maximum tolerated dose (MTD) - in the belief that this will bring the greatest benefits. The need for frequent visits to clinics that are staffed by clinicians with expertise in the use of hydroxyurea, access to multiple different formulations to provide very precise doses, and access to expensive laboratory monitoring mean that this individualised approach is unreachable for the majority of patients who live in sub-Saharan Africa. Where hydroxyurea is available in Africa, most often it is being used informally and with little laboratory monitoring as most clinics do not have the capacity to monitor hydroxyurea with the frequent clinical follow up and laboratory tests. Moreover, it is generally being reserved for children with multiple complications of SCD and is not widely being recommended as standard of care for all.

Malaria and bacterial infections are widely considered to be the commonest causes of early mortality among children with SCD in sub-Saharan Africa. Previous work from H-PRIME investigators suggests that although the absolute risk of malaria may not be increased, it more often results in catastrophic anaemia and subsequent death. Moreover, we have recently estimated that the risk of bacteraemia is exceptionally high, at approximately 5% per year.

Several drugs are commonly used for malaria prophylaxis in different parts of Africa, including chloroquine, proguanil and sulphadoxine-pyrimethamine (SP), which has recently been recommended as standard of care (SOC) in Uganda. However, given current rates of resistance, all these drugs are now sub-optimal. Similarly, although twice daily penicillin V (Pen V) prophylaxis and the introduction of newer vaccines will almost certainly reduce the rates of infections caused by both *S. pneumoniae* and *H. influenzae* type b, it is possible that greater benefits would accrue with protection against a broader range of infections.

1.2 Trial Design

H-PRIME is a randomised open-label factorial trial with a 2x2x2 design, conducted in four centres in Eastern Uganda.

The trial will have 3 intervention strategies aimed at reducing mortality and morbidity in children with SCD:

- **R1:** high-dose (daily) versus low-dose (thrice weekly) oral hydroxyurea dosing based on standard weight-bands and given with clinically driven (based on clinical signs/symptoms) rather than routine, scheduled laboratory monitoring
- **R2:** enhanced antimalarial prophylaxis with weekly dihydroartemisinin-piperaquine (DHA-PQP) vs standard of care (SOC) (monthly sulphadoxine-pyrimethamine, SP)*
- **R3:** antimicrobial prophylaxis with daily co-trimoxazole (CTX) throughout childhood/adolescence vs SOC (twice-daily penicillin V until the age of 5 years)

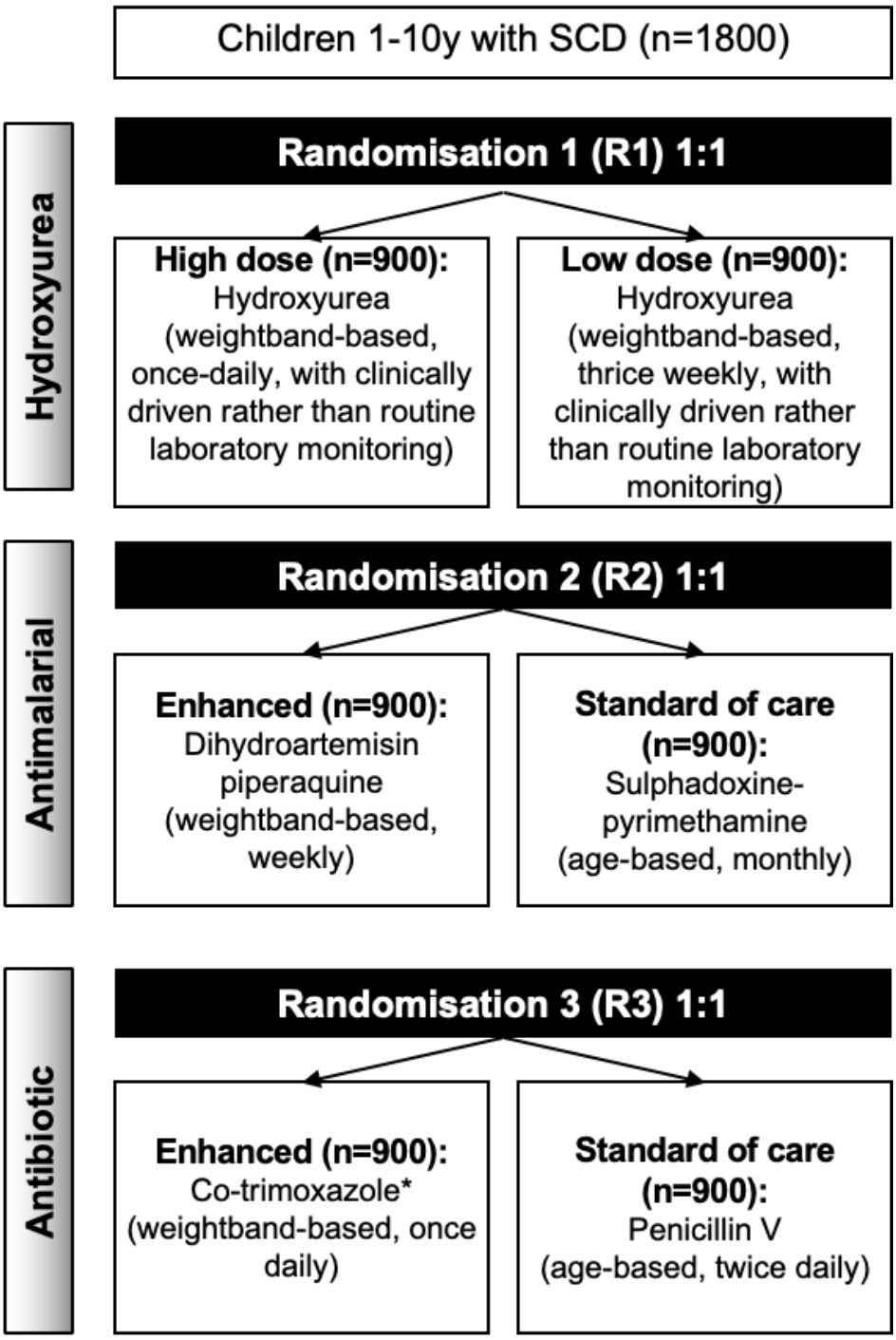
*Note: Because CTX and SP should provide similar anti-malarial cover and because both contain a sulphonamide component which, with its long half-life, increases the risk of overdosing if both are co-administered, children randomised to both CTX prophylaxis and SOC anti-malarials will receive CTX alone without additional SP.

All randomisations will be 1:1 using a factorial design, ie each randomisation will be balanced by design for allocation to other interventions or not. The primary analysis for each comparison will be based on a superiority framework. For the HDU comparison, if high dose is not shown to be superior to low dose, a secondary non-inferiority analysis will be performed to assess whether low dose is non-inferior to high dose (see section 5.7 for details).

Participants will be randomised over 2 years and will be followed until the common end of follow-up date 48 months after the first randomisation. The overall trial duration is therefore 4 years.

The trial design is summarised in the trial schema in Figure 1 below. Full details of the background to the trial and its design are presented in the protocol.

Figure 1



1.3 Inclusion criteria

- Aged 1-10 years inclusive
- Confirmed SCD (either by HPLC or IEF at a qualified laboratory)
- Have received conjugate pneumococcal vaccination against Hib and *S. pneumoniae*
- Carer willing/able to provide consent and to bring the child for follow-up visits, as demonstrated by either regular attendance at SCD clinics to date, or attending two visits (one of which may be the screening visit) before randomisation

1.4 Exclusion criteria

- Weighing <8kg
- Already meet criteria for starting hydroxyurea in national guidelines (frequent crises (>5/year), known abnormal transcranial Doppler ultrasound velocities, stroke or acute chest syndrome)
- Already receiving hydroxyurea
- Taking concomitant medications that are contraindicated with any of the trial medications (hydroxyurea, SP, DHA-PQP, penicillin V, cotrimoxazole) (including, but not limited to, nefazodone, verapamil, rifampicin, isoniazid, ethambutol)
- A positive pregnancy test at screening or enrolment visits
- Known cancer
- A clinical history of previous or existing liver or renal diseases unrelated to sickle cell disease
- Known cardiac ventricular dysfunction or failure or a previous history of cardiac arrhythmias
- Known HIV (these children should receive cotrimoxazole prophylaxis and many will be receiving antiretrovirals that are contraindicated with one or more trial medications (zidovudine, amprenavir, atazanavir, indinavir, nelfinavir, ritonavir))
- Current participation in any other clinical trial of an investigational medicinal product
- Presence of acute infection on the day of screening (e.g. symptomatic *P. falciparum* malaria, pneumonia, septicaemia, meningitis, newly identified tuberculosis)

1.5 Outcome measures

1.5.1 Primary outcome measure(s)

- R1: Mortality
- R2: Malaria-associated hospitalisations (diagnosed by either rapid diagnostic test (RDT) or microscopy or PCR)
- R3: Hospitalisations for any reason

All endpoints will be counted from enrolment to last assessment.

1.5.2 Estimands

R1: Hydroxyurea

Treatments	Daily oral high dose hydroxyurea vs thrice weekly oral low dose hydroxyurea, with standard of care antimalarial and antibiotics
Population	The population is children aged 1-10 years inclusive, with confirmed SCD and meeting all other inclusion criteria and exclusion criteria as defined in sections 1.3 and 1.4.
Endpoint	Mortality
Population-level summary measure	Hazard ratio
Strategy for intercurrent events	
Any deviation from randomised strategy, including dose or duration of treatment	Treatment policy

R2: Antimalarials

Treatments	Weekly DHA-PQP vs monthly SP with penicillin V or CTX only (combined), with low dose hydroxyurea and standard of care antibiotics.
Population	The population is children aged 1-10 years inclusive, with confirmed SCD and meeting all other inclusion criteria and exclusion criteria as defined in sections 1.3 and 1.4.
Endpoint	Number of malaria-associated hospitalisations (diagnosed by either rapid diagnostic test (RDT) or microscopy or PCR).
Population-level summary measure	Incidence rate ratio
Strategy for intercurrent events	
Any deviation from randomised strategy, including dose or duration of treatment	Treatment policy
Death before end of follow up	While alive, with one event added if death occurred more than 14 days after the last hospitalisation, and either the child had a positive malaria test in the 7 days prior or malaria is listed as the primary or secondary cause of death by the clinician.

R3: Antibiotics

Treatments	Once daily CTX given throughout childhood, vs penicillin V given twice daily until 5 years of age, with low dose hydroxyurea and standard of care antimalarials.
Population	The population is children aged 1-10 years inclusive, with confirmed SCD and meeting all other inclusion criteria and exclusion criteria as defined in sections 1.3 and 1.4.
Endpoint	Number of hospitalisations for any reason.
Population-level summary measure	Incidence rate ratio
Strategy for intercurrent events	
Any deviation from randomised strategy, including dose or duration of treatment	Treatment policy
Death before end of follow up	While alive, with one event added if a death occurred without a prior hospitalisation (defined as at least 14 days from the last date of discharge).

1.5.3 Secondary outcome measures

- Mortality (for randomisations in which mortality is not the primary outcome)
- Malaria-associated hospitalisations (where not the primary outcome) (as defined above)
- All-cause hospitalisations (where not the primary outcome) (as defined above)
- Any of the following specific SCD-specific complications requiring medical intervention (Grade 2 or above):
    - Painful crisis
    - Hand-foot syndrome
    - Splenic sequestration
    - Acute chest syndrome
    - Stroke
- Number and volume of blood transfusions received
- Haemoglobin and fetal haemoglobin (HbF) values
- Grade 3 or 4 renal (creatinine) or liver function test (AST/ALT) results
- Febrile events that are treated with intravenous antibiotics
- Specific bacterial bloodstream infections confirmed by blood culture or molecular typing
- Serious adverse events (SAEs)

1.5.4 Other outcome measures

- Hospitalisations lasting 7 days or longer
- Total duration of hospitalisations

- Febrile events reported at follow up visits or detected during hospitalisation
- Grade 3 and 4 adverse events (AEs), further categorised according to whether or not they are potentially related to SCD
- Grade 3 and 4 adverse events (AEs) considered possibly, probably or definitely related to hydroxyurea, or malaria/bacterial prophylaxis (for each specific randomisation)
- Measures of nutritional status (including Z-scores for weight, height and mid upper-arm circumference (MUAC))
- Quality of life (QoL)
- Costs and cost-effectiveness

2. STUDY METHODS

2.1 Randomisation

Randomisation will use permuted blocks, stratified by site and initial hydroxyurea weight band (defined in Table 2 in the protocol). Each randomisation will also be stratified for the other randomisations in the factorial. Children randomised to both CTX prophylaxis and SOC anti-malarials will receive CTX alone without additional SP. Children from the same family will be randomised to the same allocations. To ensure an equal number of families are allocated to each arm, 'family randomisation' will be treated as a separate strata within the weight band stratification. Randomisation lists will be prepared by the statisticians at the MRC CTU.

Randomisation will be performed by the Mbale data centre using an online randomisation server.

2.2 Sample Size Calculations

H-PRIME aims to enrol 1800 children in total, giving 90% power to detect a 51% reduction in mortality associated with high dose hydroxyurea assuming a mortality rate of 2.5 per 100 person-years in the low dose group.

Further details regarding the sample size calculations are given in protocol Section 8.1.

2.3 Interim Analysis

During the trial an independent Data Monitoring Committee will meet to review unblinded data for all three randomised comparisons at least annually. They will review data on enrolment, safety, adherence to randomised strategies, efficacy and safety at regular intervals and in strict confidence. The DMC will meet within 6 months of the first recruitment, and will subsequently determine the frequency of their meetings, which could be more frequent than annually if they think necessary.

The significance level will not be adjusted to account for interim analyses.

2.4 Stopping guidelines

The statistical stopping guideline for the trial is a Haybittle-Peto type rule based on the 99.9% confidence interval. At each review by the independent DMC, early stopping of the trial should be considered only if there is evidence beyond reasonable doubt (p-value < 0.001) of benefit on one or other of the co-primary endpoints. The independent DMC will also consider clinical criteria, other efficacy outcomes and safety outcomes in any decision about early stopping, as

well as wider implications for implementation where relevant. Reasons will be recorded for disregarding a statistical stopping guideline.

2.5 Final Analysis

The final analysis will take place after the final common follow up endpoint, 48 months after the first randomisation. All outcomes will be analysed at this time.

3. DATA

3.1 CRF Forms and variables

Full details of data collection and timings are described in the trial protocol. Details of the variables are presented as in the metadata which forms part of the Trial Master File. Details of the data management procedures are available in the Data Management Plan.

3.2 Management of datasets

- For an analysis for which a database lock is performed, the Trial Statistician will be responsible for defining when the data are clean and ready for database lock.
- For all analyses, datasets of all data stored in the database will be filed out from REDCap. This will act as the frozen dataset.
- For interim analyses, new data can continue to be entered onto the REDCap database. If a database lock is not performed and any outstanding data queries are resolved during the analysis that relate to data in the frozen dataset (e.g. problems that are found during analysis or amended CRFs that are data entered post-freeze), the data will be changed at the start of the set of analysis programs using an auditable statistical program, separate from all other programs (by the Trial/Delegated Statistician). The main REDCap database will be amended in parallel.

For the final analysis the Trial Statistician will be responsible for defining when the data are clean and ready for database lock.

3.3 Data verification

Data verification, consistency and range checks will have been performed at the data entry stage by the data manager, as well as checks for missing data (copies can be found in the Trial Master File). Additional range, consistency and missing data checks will be performed by the statistician on a regular basis, and prior to analysis. Variables will be examined for unusual, outlying, unlabelled or inconsistent values.

Any problems with trial data will be queried with the Trial Managers, Data Managers, or statisticians, as appropriate. If possible, data queries will be resolved, although it is accepted that due to administrative reasons and data availability a small number of problems will continue to exist. This will be minimised.

3.4 Derivation of data for analysis

Definition of baseline

For results of research haematology and liver and renal function tests, the baseline value will be defined as the average of the screening and day 0 values, unless the data item is missing at one of the timepoints, in which case the other value will be used as baseline.

For other data items, the baseline value will be those recorded on the baseline form.

Definition of follow up time

Time will be measured from the time of randomisation up to the date of the last study follow-up visit (including telephone visits), or the date of death for those that died.

Definition of visit schedule

Analyses of measurements at a given point in follow up will use the closest available measurement to that timepoint within evenly spaced windows. If a measurement was taken at a timepoint equidistant from 2 scheduled visits, it will be counted as within the window that has missing data for that measurement. If the data is missing for both visits it will be counted in the earlier window.

Definition of hospitalisations

New hospitalisations will be identified from the SAE form and dates of discharge recorded on follow up forms. If a death occurred more than 14 days after the last hospitalisation, one additional event will be added to the total number of hospitalisations.

Definition of malaria-associated hospitalisation

A hospitalisation will be defined as malaria-associated if there is a record of a positive malaria RDT or a positive malaria blood film or positive PCR result, from samples taken on any day from the date of admission until the date of discharge (inclusive).

Where a death without hospitalisation is being counted in the total number of hospitalisations (as described above), this will be considered malaria-associated if either the child had a positive malaria test in the 7 days prior to death, or malaria is listed as the primary or secondary cause of death by the clinician.

Counting of adverse events

SAEs will be analysed as episodes, with all components of the same clinical SAE presented as one episode. Analyses of grade 3 or 4 AEs will consider each component as separate events.

Death from an unknown cause will be analysed as a grade 4 event. Where cause of death is known and is itself a grade 4 event, the death will not be counted as a separate event to avoid double counting. If the cause of death is a grade 3 (or lower) event, it will be upgraded to a grade 4 event because it has led to death and counted as above.

Definition of censoring

For time-to-event analyses, participants not experiencing the event will be censored at their last trial assessment (including telephone visits). If participants are censored earlier due to loss to follow-up or withdrawal of consent, it will be assumed that such censoring is independent of the outcome.

Calculation of growth z-scores

Height-for-age and weight-for-age z-scores will be standardised to the WHO reference. MUAC-for-age z-scores will also be calculated based on the WHO reference for children up to 5 years old, and from reference data published in Mramba 2017 for children over 5 years old.[3]

Stratification factors

If any errors in the values of stratification variables used for randomisation (weight, site) are discovered after randomisation, analyses that are adjusted for stratification this use the corrected strata in the primary analysis.

Truncation of continuous values

All Hb and HbF values during the trial will be visually inspected. Outliers four standard deviations beyond the mean at each time point will be set to missing, after querying with site. Values larger than the 99th percentile or smaller than the 1st percentile across all time points will be compared to other values in the child's records. Any large deviations, greater than two standard deviations of values at that timepoint, from prior or subsequent measurements, where both exist, will be truncated to the 1st or 99th percentile.

Child randomised twice

If a child is inadvertently randomised twice, they will be analysed according to the randomisation at the site where they will be continuing follow up. Baseline data will be taken from the original randomisation as this is before receiving any HDU. If they are being followed up at the site where the second randomisation occurred, any data from follow up prior to the second randomisation will be included in analysis and may result in a period of non-compliance with the second randomisation.

Definition of site for participants who transfer

If following randomisation a child transfers to another site to continue follow up, for the purposes of analysis they will be considered to be from the site where they were randomised.

Calculation of fetal haemoglobin values

For analysis, the value of fetal haemoglobin will be recalculated from the CRF values as F/(F+S).

3.5 Data coding

Free text fields will be categorised based on self-evident corrections, e.g. spelling. Adverse events and hospitalisations will be coded consistently in consultation with the Chief Investigator.

4. STATISTICAL PRINCIPLES

All statistical analysis will be performed in Stata (updated and validated) unless otherwise specified.

4.1 Analysis populations and comparisons

The intention-to-treat (ITT) population will consist of all randomised participants excluding those demonstrably randomised in error; where randomisation in error will be judged by the participant meeting a major violation of the eligibility criteria, including for example a participant subsequently being discovered to be SCD negative, and will not depend on treatment allocation or post-randomisation follow-up.

H-PRIME will use the ITT population for the main analysis, with the groups defined according to the randomised allocation. For the anti-malarial randomisation, the main comparison will be between those randomised to DHA-PQP (with Pen V or CTX), and those randomised to either SP plus Pen V or CTX only (combined).

4.2 Statistical significance and p-values

All statistical tests will be two sided, with a p-value of 0.05 considered significant. Estimates will be presented with 95% confidence intervals. No adjustments will be made for multiple testing. Appropriate transformations for all variables may be applied prior to analysis after inspection of the data.

4.3 Analysis methods

Descriptive statistics will be reported overall and by randomised group, and percentages will be of non-missing values, with the number of non-missing values given if data is not complete.

Time to event analyses will consider time from randomisation until the earliest of the event date or censoring, presented as Kaplan Meier plots. Differences between groups will be tested using log rank tests and median survival time in each group will also be presented where this can be estimated (otherwise lower percentiles, eg 25^{th}). We will also present percentage with/without the event at 36 months. Cox proportional hazards models (stratified by site and adjusted for other randomisation stratification variables) will be used to obtain hazard ratios. We will test for non-proportional hazards using the Grambsch-Therneau test. If there is evidence of non-proportionality ($p<0.05$), we will estimate the restricted mean survival time (RMST) using a flexible parametric model. The estimated difference in RMST up to 36 months will then be presented. Depending on the actual duration of follow up, longer time periods may be considered.

Count outcomes such as hospitalisations will be analysed using Poisson regression if there is no evidence of over-dispersion, or negative binomial models if there is evidence of over-dispersion ($p<0.01$), with the time at risk under follow-up as the exposure. Zero-inflated models will be used if there is statistical evidence that more children than expected do not experience the outcome ($p<0.01$ for likelihood ratio test comparing the same Poisson/negative binomial model with vs without zero inflation). Children who died, withdrew or were lost to follow up before the end of the trial will be censored at the date last seen. The

difference between groups will be summarised by incidence rate ratios and estimated event rates per 100 person-years will be presented for each group.

Continuous outcome measures will be analysed using normal linear regression adjusted for baseline values. Generalised estimating equations (GEEs) with independent working correlation will be used to compare randomised groups across multiple timepoints. Appropriate transformations for all variables may be applied after inspection of the data.

The primary analysis of each outcome will be stratified (Cox) or adjusted (other models) by site and adjusted (all models) for the other factorial randomisations and initial hydroxyurea weight band. The reason for not stratifying by these other factors in Cox models is that small numbers of deaths may mean that entire strata get dropped – stratification in a Cox model allows the baseline hazard to differ non-proportionally across strata, whereas adjustment is a proportional effect.

Children from the same family will be analysed as individuals. Models will use cluster-robust variance to account for clustering of children from the same family, with family membership identified by their trial numbers.

Subgroup analyses will consist of tests for interactions within the models to assess whether there are differences in treatment effects in between subgroups. Forest plots will be presented to visually summarize the consistency of an effect over the subgroups.

4.4 Missing data

Outcomes will be analysed based on observed data only. Time to event analyses will censor any participants lost to follow up at the date last seen. For count outcomes it will be assumed that all relevant events have been recorded.

4.5 Protection from bias

All primary endpoints are as objective as possible, particularly death from all causes and laboratory test results at scheduled visits. Hospital admissions are clinically significant events, and it is reasonable to assume, therefore, that they will be approximately ascertained without bias. Any child lost to follow-up will be traced for vital status based on location and mobile phone data that will be verified at each trial visit.

5. ANALYSIS

The results of the analyses will be reported following the principle of the ICH E3 guidelines on the Structure and Content of Clinical Study Reports.

All analysis will be included in the final report, but only analysis in bold below will be included in the DMC report.

5.1 Recruitment and randomisation

- **Recruitment by centre**
- **Number of family randomisations, and total children as part of a family, by centre**

- **Screened, but not randomised: n overall, n (%) by category consent not given, at least one exclusion criteria met, tested negative for SCD**
- Number of stratification errors by treatment group and stratum

5.2 Protocol deviations

- **Protocol deviations: n (%) by protocol deviation type (critical, major)**

5.3 Baseline Characteristics

The following baseline characteristics will be summarised overall (i.e. not by randomised groups). Tabulations will also be presented by randomised group if there is a difference with p-value ≤ 0.05 (used as a flagging device for imbalance), with p-values from rank-sum tests for continuous variables, and from chi-square tests for categorical variables or Fisher's exact test if there are cell values <5.

Demographics

- **Sex: n (%) male, female**
- **Age at last birthday: median (IQR), n (%) in categories 1-2, 3-4, 5-10 (depending on numbers, the last category may be split).**

Child's family

- Number of siblings: n (%) 0, 1-2, 3-4, 5+
- Any siblings with sickle cell disease: n (% of those with siblings) yes, no, don't know

Past medical history specific to sickle cell disease

- Ever admitted to hospital, suffered a painful crisis, received a blood transfusion, suffered from dactylitis, suffered from splenic sequestration, suffered from malaria: n (%) 0, 1, 2+ times in total; 0, 1, 2+ times in last 12m
- Ever suffered from leg ulcers, had an operation to remove their spleen: n (%) yes, no
- Are leg ulcers present now: n (% of those with leg ulcers ever) yes, no
- Ever suffered priapism: n (% of males) yes, no
- **Taken hydroxyurea in the past: n (%) yes, no, don't know**

Past medical history – general

- Diagnosed with asthma, diabetes: n (%)
- Other diagnosed medical conditions: n (%)

Symptoms in the last 4 weeks

- Fever, weight loss, weakness/tiredness, pallor, jaundice/yellow eyes, rash, new bruises/masses/bumps, muscle aching/pain, abdominal aching/pain, poor appetite, difficulty feeding, sore mouth or throat/ulcers/thrush, vomiting/nausea, diarrhoea, moderate-severe dehydration, cough, difficulty/fast breathing, ear discharge/pain, difficulty walking, delayed milestones, new visual problems, poor sleep/bad dreams, numbness/pain in hand or feet, depression/withdrawn, headache, skin infections(s): n (%) yes, no
- Other symptoms: n (%)

Physical examination

- **Weight (kg, Z-score, categorised based on dosing for hydroxyurea), height/length (cm, Z-score), MUAC (cm, Z-score), axillary temperature (°C), pulse (beats/minute), spleen size (cm), head circumference (cm) if < 2 years: median (IQR)**
- **Scleral icterus: n (%) yes, no**

Lab results

- **Haemoglobin (g/dL): median (IQR), histogram of distribution**
- **Absolute reticulocyte count (x10^9/L): median (IQR)**
- **White blood cells (10^9/L),** lymphocytes (%), monocytes (%), neutrophils (%), basophils (%), **red blood cells (10^{12}/L), haematocrit (%), MCV (fL), MCH (pg), MCHC (g/dL), platelets (10^9/L):** median (IQR)
- **Haemoglobin type: n (%) HbAA, HbAS, HbSS, missing/not done, other**
- **HPLC results: A_0, A_2, F and S-windows (%): median (IQR), n (% with results)**
- **Creatinine (µmol/L), AST (IU/L), ALT (IU/L), bilirubin (mg/dL): median (IQR)**

5.4 Follow up

- **Distribution of time from randomisation to latest follow-up visit in months**

The following will be tabulated overall. The denominator in each case will be those who have been enrolled long enough ago for that visit to have occurred, including those who have been lost to follow up. Tabulations will also be presented by randomised group if there is a difference with p-value ≤ 0.05 (used as a flagging device for imbalance), with p-values from chi-square tests for categorical variables.

- **Visits considered complete, defined as attended visit, or had a home or telephone visit, or died before the visit took place, at 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48 months and overall: n (%)**
- **Child status at 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48 months and overall: n (%) attended in clinic, telephone visit, home visit, died, lost to follow up, withdrawn, missed visit.**

5.5 Adherence to treatment

Hydroxyurea

The following will be tabulated for the high dose vs low dose HDU randomisation.

- **Prescribed correct dose at randomisation: n (%) yes, no**
- **Average daily dose prescribed at randomisation (mg/kg): median (IQR)**
- **Time-averaged area under the curve of prescribed daily dose (mg/kg): median (IQR)**
- **Stacked bar chart of percentage of prescribed daily dose, out of total child time at risk at 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27, 27-30, 30-33, 33-36, 36-39, 39-42, 42-45, 45-48 months**
- **Kaplan Meier plot of time until stopping HDU, censoring at death.**
- Summary of reasons for stopping
- Number with temporary reductions of HDU: n (%)
- Number with at least one self-reported missed dose: n (%)

Antimalarial prophylaxis

The following will be tabulated for the anti-malarial randomisation, excluding those not receiving SP due to being randomised to CTX unless otherwise stated.

- **Antimalarial prescribed at randomisation [including those randomised to CTX]: n (%) DHA-PQP, SP, none**
- **Time-averaged area under the curve of prescribed daily dose (mg/kg): median (IQR)**
- **Stacked bar chart of percentage of prescribed daily dose, out of total child time at risk at 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27, 27-30, 30-33, 33-36, 36-39, 39-42, 42-45, 45-48 months**
- Number with at least one self-reported missed dose: n (%)

Antimicrobial prophylaxis

The following will be tabulated for the antibiotic randomisation.

- **Number randomised to Pen V aged ≥5 years: n (%) [Pen V group only]**
- **Antimicrobial prescribed at randomisation: n (%) CTX, Pen V**
- **Number who stopped Pen V due to turning 5: n (% of those starting Pen V)**
- **Time-averaged area under the curve of prescribed daily dose (mg/kg): median (IQR)**
- **Stacked bar chart of percentage of prescribed daily dose, out of total child time at risk at 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27, 27-30, 30-33, 33-36, 36-39, 39-42, 42-45, 45-48 months**
- Number with at least one missed dose: n (%)

5.6 Laboratory results

The following will be tabulated by randomised group for the hydroxyurea randomisation.

- **Proportion of research samples requested by site**

The number and proportion of scheduled research samples (FBC, renal and liver function tests) where the site requested the results to be returned or ran the test locally.

- **Local tests completed**

The number of additional haematology, liver and renal function that were run locally at timepoints when a research sample was not taken.

- **Proportion of requested or locally run tests with a grade 4 adverse event**

The number and proportion of tests where results were requested by the site or run locally (at any timepoint) where the results showed at least one grade 4 adverse event.

- **Grade 4 adverse events with no clinical request**

The number and proportion of tests which showed at least one grade 4 adverse event, where these results were not requested by the site or run locally.

5.7 Analysis of the Primary Outcome(s)

- **R1: Mortality**

The number and percentage of participants who have died will be tabulated by randomised group (high vs low dose of hydroxyurea).

Mortality will be analysed using time-to-event methods, with censoring as defined in section 3.4. A Kaplan Meier curve will be plotted by randomised group, and a site stratified log-rank test used to test for differences between the groups. The hazard ratio and 95% confidence interval will be calculated from a Cox model as described in section 4.3, comparing the hydroxyurea randomisation. The proportional hazards assumption will be tested and, if there is evidence of non-proportional hazards, restricted mean survival time (RMST) methods will be used as the primary analysis.

If the primary superiority hypothesis is not met (ie high dose is not shown to be superior to low dose in terms of mortality), a secondary exploratory non-inferiority analyses will be performed to assess whether low dose is non-inferior to high dose. This will be conducted at a one-sided significance level (as low dose is not expected to be superior to high dose in terms of mortality). The planned non-inferiority margin will be a 5% absolute difference in cumulative mortality at 3 years. Assuming no true difference between the arms, the expected mortality in the high dose group is 11% at 3 years; 1800 children will provide 91% power to demonstrate non-inferiority assuming 5% loss to follow up (one-sided alpha=0.025). If the high dose 3 year mortality is less than 7.5% (approximately 2.5/100 CY) then the non-inferiority margin will be modified to 3.5% absolute difference (without reference to the accumulating comparative data) to reflect the equivalent relative differences between arms.

- **R2: Malaria-associated hospitalisations (diagnosed by rapid diagnostic test (RDT) and confirmed by microscopy and/or PCR)**

Analysis of malaria-associated hospitalisations (defined in section 3.4) will use Poisson or negative binomial models as described in section 4.3, comparing the antimalarial randomisation. Incidence rates for each randomised group will be calculated and the incidence rate ratio with 95% CIs will be presented.

- **R3: Hospitalisations for any reason**

Analysis of hospitalisations (defined in section 3.4) will use Poisson or negative binomial models as described in section 4.3, comparing CTX to Pen V. Incidence rates for each randomised group will be calculated and the incidence rate ratio with 95% CIs will be presented.

5.7.1 Subgroup Analyses

For each of the primary outcomes, heterogeneity of treatment effects according to the other randomisations will be explored by including interaction terms in the outcome models. The estimated interactions will be presented with 95% confidence intervals and p-values to assess the assumption of no interaction.

We will also investigate a priori whether there was any evidence for a different impact of the interventions according to age, haematological indices, and time in the trial (to assess for attenuation of effects over time). These will be considered both as categorical variables, splitting at terciles (if not otherwise specified), and as natural cubic splines. The categories for age will be 1-2, 3-4, 5-10.

5.7.2 Sensitivity Analyses

- Multi-arm analysis

Each of the three primary outcomes will be analysed using a multiarm approach (ie with the 8 possible treatment combinations included separately in the model) to evaluate to what extent results from the primary analyses may be affected by departures from the underlying assumption of no interaction.

- Adjustment for stratification using randomisation strata

If there are errors identified in the stratification variables used for randomisation, the primary analysis will adjust based on the corrected strata. As a sensitivity analysis we will also analyse the three primary outcomes adjusted for the randomisation strata.

- Definition of malaria-associated hospitalisation

A sensitivity analysis for the malaria-associated hospitalisation outcome will consider admissions as malaria-associated as defined by each test (RDT, microscopy and PCR) separately.

An additional sensitivity analysis will also count deaths as malaria-associated where malaria is listed as a cause of death, in addition to those defined by a positive malaria test.

5.8 Analysis of the Secondary Outcomes

Secondary outcomes will be compared for each of the three randomisations unless otherwise specified.

- **Mortality (where not the primary outcome)**

Mortality will be analysed using time-to-event methods, with participants not known to have died censored at the date last seen. A Kaplan Meier curve will be plotted by randomised group, and a stratified log-rank test used to test for differences between the groups. The hazard ratio and 95% confidence interval will be calculated from a Cox model as described in section 4.3.

- **Malaria-associated hospitalisations (where not the primary outcome)**

Analysis of malaria-associated hospitalisations will use Poisson or negative binomial models as described in section 4.3.

- **Hospitalisations for any reason (where not the primary outcome)**

Analysis of hospitalisations will use Poisson or negative binomial models as described in section 4.3.

The frequency of hospital readmissions and adverse events will be tabulated by body systems and by randomised groups, and the number of events experienced by each participant will be compared across randomised groups using Fisher's exact test.

- **Any of the following specific SCD-specific complications requiring medical intervention (Grade 2 or above):**
  - **Painful crisis**
  - **Hand-foot syndrome**
  - **Splenic sequestration**
  - **Acute chest syndrome**
  - **Stroke**

For each of the above complications, the number and percentage of participants experiencing at least one event, and the total number of events, will be tabulated. Each complication will also be analysed as count data using Poisson or negative binomial models as described in section 4.3.

- **Number and volume of blood transfusions received**

The number and percentage of participants receiving a blood transfusion, and the number of transfusions will be tabulated. The number of blood transfusions will be analysed as count data using Poisson or negative binomial models as described in section 4.3. The mean total volume of blood transfusions will be analysed using normal linear regression.

- **Haemoglobin and fetal haemoglobin (HbF) values**

Haemoglobin and fetal haemoglobin values at 3, 6, 12, 18, 24, 30, 36, 42 and 48 months will be analysed using normal linear regression adjusted for baseline values. Means and 95% CIs will be presented for each group, and the mean difference will be calculated. GEEs will be used for a global test of difference between randomised groups across all time points as described in section 4.3. Box plots of haemoglobin and HbF will be presented by randomised group and follow up visit.

Sensitivity analyses for HbF will include either a binary indicator for values taken within 3 months of a blood transfusion, or exclude those values completely, to assess whether blood transfusions impact the results.

The number and percentage of participants with an episode of severe anaemia (defined as Hb<5g/dL), and the number of episodes, will be tabulated. The total number of episodes will be analysed as count data using Poisson or negative binomial models as described in section 4.3.

- **Grade 3 or 4 renal (creatinine) or liver function test (AST/ALT) results**

The total number of grade 3 or 4 renal or liver function test results, and the number of grade 3 or 4 results in creatinine, AST and ALT separately, will be analysed as count data using Poisson or negative binomial models as described in section 4.3. The number and proportion of children having a grade 3 or 4 result, and the total number of grade 3 or 4 results will be presented for each test.

- **Febrile events that are treated with intravenous antibiotics**

The number of febrile events recorded as being treated with IV antibiotics will be analysed as count data using Poisson or negative binomial models as described in section 4.3. The number and proportion of children having an event, and the total number of events will be tabulated.

- **Specific bacterial bloodstream infections confirmed by blood culture or molecular typing**

The number of specific bacterial bloodstream infections will be analysed as count data using Poisson or negative binomial models as described in section 4.3. The number and proportion of children having an infection will be tabulated. The numbers with each specific pathogen will also be presented.

- **Serious adverse events (SAEs)**

The number and proportion of children ever having an SAE will be tabulated and compared across randomised groups with a chi-squared test. Relationship of SAEs to hydroxyurea and the other trial drugs (DHA-PQP, SP, CTX, Pen V) will be tabulated. The number of children having SAEs and number of events per child will also be tabulated by SAE criteria (fatal, life threatening, cause or prolonged hospitalisation, persistent or significant disability, other) and randomisation group.

All causes of death will be tabulated by each randomisation.

5.9 Other Analyses

- **Values of red blood cell indices (RBC, haematocrit, MCV, MCH, MCHC)**

RBC, haematocrit, MCV, MCH, and MCHC values at 3, 6, 12, 18, 24, 30, 36, 42 and 48 months will be analysed using normal linear regression adjusted for baseline values. Means and 95% CIs will be presented for each group, and the mean difference will be calculated. GEEs will be used for a global test of difference between randomised groups across all time points as described in section 4.3. Box plots of each measurement will be presented by randomised group and follow up visit.

- Hospitalisations lasting 7 days or longer

The number and proportion of children having a hospitalisation lasting 7 days or longer will be tabulated by group. The total number of hospitalisations lasting at least 7 days will be analysed as count data using Poisson or negative binomial models as described in section 4.3.

- Total duration of hospitalisations

The total duration of hospitalisations for each child will be calculated, and the median and IQR presented by randomisation group. The total durations will be analysed using normal linear regression. If there is a gross departure from normality ($p<0.0001$ by the Shapiro-Wilks test) the data will be transformed using a Box-Cox transformation before analysis.

- Febrile events reported at follow up visits or detected during hospitalisation

The number of febrile events will be analysed as count data using Poisson or negative binomial models as described in section 4.3. The number and proportion of children having an event, and the total number of events will be tabulated by randomised group.

- Grade 3 and 4 adverse events (AEs), further categorised according to whether or not they are potentially related to SCD

- Grade 3 and 4 adverse events (AEs) considered possibly, probably or definitely related to hydroxyurea, or malaria/bacterial prophylaxis (for each specific randomisation)

The number and proportion of children ever having an adverse event will be tabulated for each of the categories above and compared across randomised groups with a chi-squared test. The number of events will also be tabulated.

- Measures of nutritional status (including Z-scores for weight, height and mid upper-arm circumference (MUAC))

Mean weight-for-age, height-for-age and MUAC-for-age z-scores will be analysed at 1, 3, 6, 12, 18, 24, 30, 36, 42 and 48 months using normal linear regression adjusted for baseline values, and the mean difference between randomised groups will be presented, as described in section 4.3. GEEs will be used for a global test of difference.

- Quality of life analyses

- Total PedsQL score at 12, 24, 36 and 48 months
- EQ-5D health state (visual scale) across all scheduled follow-up time points

The means for each group and the mean differences between randomised groups will be estimated using normal linear regression adjusted for baseline values, as described in section 4.3. GEEs will be used as a global test of difference between groups across all time points.

- Health Economics

Health economics (HE) analyses will be performed by collaborators outside of MRC CTU and are not included in this analysis plan.

6. REFERENCES

6.1 References

1. Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. Lancet 2017; 390(10091): 311-23.
2. Telfer P, Coen P, Chakravorty S, et al. Clinical outcomes in children with sickle cell disease living in England: a neonatal cohort in East London. Haematologica 2007; 92(7): 905-12.
3. Mramba L, Ngari M, Mwangome M, et al. A growth reference for mid upper arm circumference for age among school age children and adolescents, and validation for mortality: growth curve construction and longitudinal cohort study. BMJ. 2017;358:j3423.

6.2 References to trial related documents (DMP, TMF, SMF)

The H-PRIME trial master file (TMF) is held at KEMRI-Wellcome Trust Research Programme Clinical Trial Facility. The Data Management Plan forms part of the TMF. The Statistical Master File (SMF) is held at MRC CTU.