

Description of Analyses

The analyses will initially be run separately for data from each study site. See section D of this document for a discussion of approaches to combining data across sites.

A. Objective: Feasibility

Outcomes:

1. Recruitment rate
2. Withdrawal
3. Adherence to monthly clinic visits
4. Completion of laboratory assessments: (i) by assessment; (ii) entire set of assessments.
5. Medication compliance: (i) pill counts and (ii) Modified Morisky Scale

Analyses:

All estimates of rates or proportions below will be accompanied by 95% confidence intervals based on binomial, Poisson or an overdispersed Poisson distribution of outcomes, as appropriate.

- a) Recruitment rate. We will download data on study recruitment from the REDCap system once a month and produce a graph of the cumulative number of children enrolled versus calendar time. We will add to this graph a separate line showing the target recruitment rates established at each site. These graphs will be updated monthly on the study web site and distributed at monthly steering committee meetings, so that sites have ongoing feedback on recruitment and can take measures to address slow recruitment or high withdrawal in the screening phase.
- b) Withdrawal. At each of the monthly updates, we will calculate the percentage of children who are recruited who are withdrawn from the study in the screening period. Over the treatment period, we will compute the monthly cumulative incidence of study withdrawal among those who complete the screening period and start hydroxyurea. By calculating these numbers as the study progresses, problems can be identified earlier and potential solutions proposed.
- c) Adherence to monthly clinic visits. At each month from the study baseline (hydroxyurea start, month 0), we will compute the percentage of children eligible for the visit who completed the visit. When it becomes feasible (after sufficient numbers of children have enough follow-up), we will plot cumulative incidence of withdrawal, accounting for competing risks of death and removal due to a serious adverse event. At any given time, we will summarize the percentages of visits completed across all study participants as the total number of completed visits divided by the total number of potential visits. This latter number will be calculated two ways, first assuming that all patients who start hydroxyurea should each have a monthly visit until their last potential visit in the study and secondly removing children from the potential visit count at the point of study withdrawal.
- d) Completion of laboratory assessments (e.g., fetal hemoglobin, hemoglobin, white blood cell count). We will examine these separately, in case there are problems with specific laboratory parameters being assessed, and in their entirety. To reflect potentially different study processes leading to missing values, we will calculate the percentage of children having complete assessments using three different denominators:
 - i. all children starting hydroxyurea, giving the crudest but perhaps most important measure from a feasibility standpoint;
 - ii. all children still being followed, giving a measure which reflects both non-attendance at a specific visit and non-completion of laboratory assessments within

- the visit; and
- iii. all children who attended the visit, giving a measure which reflects only procedural issues with completion of laboratory assessments within the visit.
- e) Medication compliance. Among children who are still being followed, monthly pill counts will be summarized as
 - i. the percentages with excellent, good, moderate and poor adherence (according to Protocol Section 7.1.2) and
 - ii. the median, IQR and range of the actual percentages of pills taken. Monthly adherence according to the Modified Morisky Scale (MMS) will be summarized by the percentages in each of the 4 categories of the MMS.

Trends in the percentages with excellent or good adherence over the duration of follow-up will be examined by use of generalized linear mixed models, with the binary variable for adherence as the outcome, time as a fixed predictor and child-specific random effects for the intercept and time. This model will also allow us to assess the importance of the child's characteristics (e.g., age, sex, distance from medical center) and hydroxyurea dose on adherence.

All feasibility outcomes will be assessed over the entire initial study period (out to 48 months), but the main focus for outcomes 2-5 will be

- i. the period of the first safety evaluation (zero to three months);
- ii. the period of fixed dosing (zero to 6 months); and (c) the period that include the dose escalation (six to 12 months).

Criteria for success.

1. Recruitment rate: this is not part of the feasibility outcome, but may be useful in designing future trials at these same or similar sites.
2. Withdrawal: similarly, withdrawal by itself will not be used to evaluate feasibility but will be useful in designing a future study. The effect of withdrawal will be seen in the feasibility outcomes adherence to monthly visits, completion of laboratory assessments and medication compliance.
3. Adherence to monthly visits: feasibility of a future study will require that we can attain >80% adherence at each of the study visits over the first 12 months of treatment and >60% visit completion overall among those starting hydroxyurea.
4. Completion of laboratory assessments: we will consider it feasible to collecting these values on patients who are still on study if at least 80% of visits have key assessments (hemoglobin, fetal hemoglobin, WBC, ANC, ARC) completed.
5. Medication compliance will be considered satisfactory if at least 80% of patients starting hydroxyurea take at least 80% of the required pills over the first 12 months of the study.

B. Objective: safety over initial fixed dosing period

Outcomes:

1. Hematological toxicities, as outlined in Table 1 of the protocol.
2. Serious infections

Analyses:

1. Hematological toxicities. We will download data on toxicities from REDCap on a regular basis. The analyses will follow the rules for the same Simon two-stage design independently at each site. The first 53 children to begin hydroxyurea will comprise the first stage cohort at a site, with the exception described in Protocol Section 3.3.6 – a child who withdraws with no toxicity

before completion of three months of hydroxyurea treatment will not be considered ‘exposed’ enough to allow a full assessment of safety, so will be replaced by a later recruit. Without this potential wrinkle, we could halt enrolment at exactly 53 children and then wait a further 3 months for the evaluation of the safety outcome on the last enrolled child. However, to increase the probability that we have 53 children in this first phase, we will halt enrolment after we have started 60 children on hydroxyurea, allowing for up to 12% to halt the medication before the three-month period ends. The phase one cohort that we evaluate at three months will comprise the children with the 53 earliest recruitment dates and will include two groups: children who completed three months of hydroxyurea without a toxicity and children who had a toxicity before completing three months of hydroxyurea. If this second group has 15 or fewer children, then we will start recruitment with the same dosing scheme and continue until 150 children have started hydroxyurea, to give a high probability of having a total of 133 children complete three months of therapy. Then the stage 2 cohort will comprise 133 children in all and as above, the final cohort we evaluate will be the 133 with the earliest recruitment dates. If 33 or fewer have a toxicity, then we will declare hydroxyurea treatment safe at that site.

If in the first stage, we observe more than 15 toxicities at three months or before three months of follow-up are complete on the first 53 children at any particular site, then we will begin a new period of stage one recruitment at a lower initial dose as described in Protocol Section 4.3. If this dose also proves to be unsafe, then the study will end at that site.

In the case that we go through two stage one phases and find the drug unsafe, feasibility of treatment and follow-up is relatively unimportant, but we will report feasibility outcomes on both stage one cohorts. If the initial dosing regimen leads to a high proportion of toxicities and we restart the first stage with a lower dose, and this second dose proves safe, we will separately report the feasibility outcomes from the initial dose cohort of 53 and the full cohort of 133 using the revised dosing scheme.

In addition to the rule-based decision on safety, we will also estimate the proportion of patients at each site having a hematological toxicity and its 95% confidence interval, using the methods described in Koyama and Chen (Statistics in Medicine, 2007) that account for the two-stage nature of the sampling.

We have exclusions for study entry based on acute infectious disease and severe hematological toxicity, so we will not see these at visit -2. They could occur at visit -1 or 0 before hydroxyurea starts, so we will compute the rates of these severe hematological toxicity over the screening period to establish a baseline rate of events per month of follow-up while not on hydroxyurea. The statistical comparison of baseline rates and follow-up rates will use generalized estimating equations with a Poisson (or negative binomial if necessary) distribution for the outcome of the number of events in each time period, the logarithm of follow-up time as the offset, and treatment period as the primary predictor. We will estimate the rate ratio (and its 95% confidence interval) comparing rates of these hematological toxicities on and off hydroxyurea.

2. Serious infections. We do not have a formal rule for deciding safety based on the absolute rates of serious infections, as background rates of infection can be variable over time and across sites. Instead, we will record all occurrences of serious infections and compute prevalence at each study visit, both before (at visits -1 and 0) and after initiation of treatment. Over each interval, we will classify a child as either having or not having had a serious infection. A generalized estimating equation log-link binomial regression model will be fitted to

these 5 repeated binary outcomes to estimate the relative risk of infection between the post-treatment and pre-treatment periods. Although this comparison (as with the comparison of hematological toxicities) in section is confounded by the passage of time, it is hoped that variation in background infection rates and timing of enrolment will behave in such a way that some children will be in the pretreatment phase when rates are higher and some will be in the treatment phase when background rates are higher. The absolute rate of infections, the point estimate of the relative risk and the upper limit of the 95% confidence interval for the relative risk will all be taken into consideration by the study steering committee in the judgment of safety of treating with hydroxyurea with respect to serious infections. A wide confidence interval for the relative risk having a high upper bound, for example, could arise if we have few serious complications at a site, so would not itself be ground for concern; the study is not designed to rule out high rates of infection. Similarly, a moderate point estimate of the relative risk may or may not be of concern depending on the absolute rate of infections.

C. Objective: Benefits of therapy

Outcomes:

1. Hematological toxicities (dose-limiting toxicities as outlined in Protocol Table 1)
2. Serious infections (primarily malaria, bacterial, other sepsis)
3. Laboratory parameters (e.g., hemoglobin, fetal hemoglobin, WBC, ANC, ARC)
4. Clinical outcomes (e.g., pain, acute chest syndrome, transfusions, hospitalization, growth)
5. Dose escalation

Analyses:

1. Laboratory parameters (hemoglobin, fetal hemoglobin, WBC, ANC, ARC and others). We will generate summary estimates of these outcomes at each study visit and also estimate differences in laboratory measures between the pre-treatment phase (visits -2, 1 and 0) and the post-treatment phase (visits 1, 2 and 3) using a linear mixed effects model with factors for time, and treatment status and allowing for repeated measurements within a subject by use of a correlation structure on the residuals. Laboratory measures will be log-transformed where appropriate (e.g., bilirubin, ANC)
2. Clinical outcomes (pain, hospitalization, growth). These clinical outcomes are secondary measures of treatment effect. As with the laboratory measures, we will generate summary measures at each study visit and use longitudinal modeling to compare outcomes within individuals between the pre-treatment and post-treatment phases. The effects of treatment on pain scores and growth will be assessed with linear mixed effects models. Hospitalization will be recorded as the number of hospitalizations in the previous month (since the last study visit, for all except visit -2) and analyzed with a generalized linear mixed model using the Poisson distribution.
3. Dose escalation to maximum tolerated dose (MTD). Dosing and toxicities will be compared to results from other NHLBI-funded studies led by Dr. Ware, specifically HUSTLE, SWITCH, SCATE, and TWITCH.

D. Combining initial safety and benefit results across study sites.

Once the final results are available for all four study sites, we will make both qualitative and

quantitative comparisons of the findings. For the first safety outcome, hematological toxicities, there are five possible outcomes at any one site:

- (1) Find that hydroxyurea is safe in all 133 children at the original dosing scheme;
- (2) Find that hydroxyurea is safe in all 133 children at the revised dosing scheme, after restarting stage 1 at a lower initial dose;
- (3) Find that hydroxyurea is unsafe in all 133 children at the original dosing scheme;
- (4) Find that hydroxyurea is unsafe in all 133 children at the revised dosing scheme, after restarting stage 1 at a lower initial dose;
- (5) Find that hydroxyurea is unsafe in 53 children, after restarting stage 1 at a lower initial dose (i.e., unsafe in two sets of 53 children).

This means that there are a total of $5^4 = 625$ possible combinations of study finding across the four sites. We may find that all sites return the same finding, or that two or three of four sites return the same finding, in which case we will consider pooling the shared results to obtain more precise estimates for this safety outcome. The two-stage-adjusted estimates from section B.2 of this document will be combined using the generalized-inverse variance weighting method of meta-analysis, with variances estimated from the 95% CI (assuming they are approximately 3.92 standard errors wide). We are not aware of any existing method for estimating a pooled proportion from multiple independent Simon two-stage designs.

Each of the efficacy results can be compared across sites by extending the analyses currently planned at each site to include site and a site by treatment interaction. Where the results are not statistically significantly different across sites and where the findings are judged to be clinically similar, we will generate a pooled effect estimate. Feasibility outcomes will not be pooled.

Note: The analyses comparing the screening and treatment phases, and the analyses comparing and combining data from the four sites are not currently set up to take into account the two-stage nature of the treatment phase, as we are currently not aware of an approach that takes the full design into account in these analyses.

E. Long-term treatment and organ function

Subsequent to the drafting of the analysis plan described to this point, support from NIH and from Bristol-Myers-Squibb made it possible to plan longer-term follow-up of the cohort. This will lead to many opportunities to assess the long-term benefits and potential harms of hydroxyurea treatment in the current participants in sub-studies that will need planning and approval and their own detailed analysis plans. Here, we outline only the continuation of the original REACH analyses past the initial planned four-year time period.

Outcomes:

1. Ability to continue to treat and monitor
 - a. Study retention
 - b. Adherence to scheduled clinic visits and completion of laboratory assessments.
 - c. Medication compliance: (i) pill counts and (ii) Modified Morisky Scale
2. Clinical and laboratory outcomes
 - a. Survival
 - b. Hematological (laboratory) adverse events

- c. Serious infections (malaria, bacterial, other sepsis)
- d. Laboratory parameters (e.g., hemoglobin, fetal hemoglobin, WBC, ANC, ARC)
- e. Clinical outcomes (e.g., pain, acute chest syndrome, transfusions, hospitalization, growth)

Analyses:

1. Ability to continue to treat and monitor
 - a. Study retention: Since feasibility of follow-up is not an issue in the longer-term, there is no need to treat death as a competing event, so it will be rolled into a composite outcome. This analysis will count an event as a withdrawal, death, movement away from the study location, or anything that means a child is no longer in active follow-up within REACH. A child who is alive will be censored at the date of the analysis or at the date he or she turns 18, whichever is earlier. We will produce a Kaplan-Meier estimate of the percentage of children still retained by the study at each time point from hydroxyurea initiation.
 - b. Adherence to scheduled clinic visits and completion of laboratory assessments. These analyses will calculate the percentages of patients still in follow-up who (i) complete a scheduled clinic visit and (ii) complete planned laboratory assessments at a scheduled clinic visit.
 - c. Medication compliance: (i) pill counts and (ii) Modified Morisky Scale. The analyses of pill counts will present per-patient percentages of pills not returned (and therefore assumed to have been taken) by time point. The analyses of the Modified Morisky scale will present the percentages of patients with scores of 0 or 1 (excellent or good) by time point.
2. Clinical and laboratory outcomes:
 - a. Survival
 - b. Hematological (laboratory) adverse events
 - c. Serious infections (malaria, bacterial, other sepsis)
 - d. Laboratory parameters (e.g., hemoglobin, fetal hemoglobin, WBC, ANC, ARC)
 - e. Clinical outcomes (e.g., pain, acute chest syndrome, transfusions, hospitalization, growth)

Analyses: These will initially be summarized and analyzed with similar methods to those used in the initial phase of the study. In addition, comparisons of long-term outcomes will be made between the four study sites. For outcomes with comparably long-term data from the US, comparisons will be made in aggregate or, if individual data are available, will be made by pooling the REACH data with the US data and adjusting for known potential patient-level confounders.