REALIZING EFFECTIVENESS ACROSS CONTINENTS WITH HYDROXYUREA (REACH): A PHASE I/II PILOT STUDY OF HYDROXYUREA FOR CHILDREN WITH SICKLE CELL ANEMIA

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OBJECTIVES:

- To assess the feasibility of conducting a prospective research study using hydroxyurea therapy for SCA in sub-Saharan Africa (including adherence to monthly clinic visits and laboratory assessments, and medication compliance).
- To monitor the safety of hydroxyurea therapy, specifically documenting hematological toxicities (cytopenias) and serious infections (bacterial and malarial)
- To evaluate the benefits of hydroxyurea therapy, using both laboratory (e.g., fetal hemoglobin, hemoglobin, white blood cell count) and clinical parameters (e.g., pain, hospitalization, growth).
- To explore the pharmacokinetic and genetic basis for any observed inter-patient variability in the clinical or laboratory response to hydroxyurea.
- To evaluate the economic cost of providing hydroxyurea therapy in the REACH study sites.
- To investigate the effects of hydroxyurea dose escalation on laboratory and clinical parameters

DESIGN:	Prospective phase I/II open-label dose escalation pilot trial of oral hydroxyurea for pediatric patients with sickle cell anemia (SCA)
POPULATION:	Children with confirmed SCA between 1.0 and 10.0 years of age at the time of enrollment
SAMPLE SIZE:	Approximately 150 per study site 4 clinical sites
TREATMENT:	After patient enrollment, a two-month pre-hydroxyurea evaluation phase will be used to perform baseline evaluations including nutritional and infectious assessments, and to provide supplements or treatments as deemed necessary. After the pre-hydroxyurea evaluation and supplementation phase, hydroxyurea dosing will be administered as a single daily dose, using capsules provided as a monthly supply in 200mg, 300mg, 400mg, or 500mg sizes.
DURATION:	Six months of hydroxyurea treatment will be given at a fixed dose of 15-20 (17.5 ± 2.5) mg/kg/day, followed by another six months of treatment with dose escalation as tolerated to 20-30 mg/kg/day to establish a maximum tolerated dose (MTD). After twelve months of hydroxyurea treatment, children with an acceptable toxicity profile and favorable hematological responses will be given the opportunity to continue hydroxyurea therapy, through 18-years-old pending biennial review of BMS support.

1.0 INTRODUCTION

1.1 Background and Rationale

Sickle cell anemia (SCA) is among the world's most common forms of inherited hemolytic anemia, and results in significant morbidity and early mortality. SCA is most prevalent in Africa, with as many as 300,000 babies born annually, representing up to 2% of newborns in some sub-Saharan countries. [1,2] Assuming most of these babies die early in childhood, the World Health Organization (WHO) estimates that SCA causes 6-16% of under-five mortality for many African countries, [3] and this burden is projected to further increase substantially in the next 40 years. [4] This alarmingly high contribution of SCA to under-five mortality makes the recognition and management of SCA an important cornerstone of efforts by many African countries toward achieving Millennium Development Goal #4, the reduction of child mortality. [5] Since deaths due to SCA mostly occur in children under 5 years old, efforts to save lives must include early diagnosis and treatment. In addition to early death, SCA also causes profound adverse effects among surviving children on their education and future employment with resultant loss of productivity. As the overall mortality declines in the region due to demographic transition, along with the expansion of vaccine and malaria control programs, the economic and health burden of non-communicable diseases such as SCA will undoubtedly increase proportionately and consume significant medical and social resources in Africa [2,4].

In the US and Europe, each of which contributes only about 1% of the global annual sickle cell births, SCA is a medical condition with recognized impact upon both the quality and length of affected lives. However, due to effective early identification with newborn screening, aggressive early interventions with pneumococcal immunizations and prophylactic penicillin, and now with availability of hydroxyurea therapy, the morbidity and mortality associated with SCA in developed countries have dramatically improved with 95-99% survival to adulthood. [6] This is not the case in the global setting, however, where the demand for impactful intervention for individuals with SCA is long overdue. Without any identification by newborn screening or early interventions, the vast majority of these children will die from acute anemia or infection, especially bacterial sepsis or malaria. [1, 7-12]

WHO has recently recognized SCA as a significant health problem for Africa and recommends that sub-Saharan countries develop screening and treatment programs for SCA. [3] With the development and implementation of newborn screening and treatment programs, the early mortality of SCA is likely to be significantly reduced; however, these interventions will not treat or cure the underlying disease. Increased identification of SCA from public awareness and widespread screening efforts are likely to increase the perceived morbidity and burden of SCA in these countries, with many more children identified by screening, surviving early childhood, and suffering from its medical complications. Accordingly, the introduction of hydroxyurea, as a once-daily inexpensive oral medication with a well-established short-term safety profile, is an intriguing and potentially ideal option for affected patients with SCA in the developing world, since access and safety of other potential therapeutic options, primarily transfusions or stem cell transplantation, are currently not realistic options. [13]

1.2 Hydroxyurea Therapy for SCA

Hydroxyurea is a cytotoxic, antimetabolic, and antineoplastic agent used for several decades to treat a variety of medical disorders, most notably myeloproliferative neoplasms, [14] chronic myelogenous leukemia, [15] and HIV. [16] The efficacy of hydroxyurea for these varied medical conditions is due to its mechanism of action as a potent inhibitor of ribonucleotide reductase, [17] a ubiquitous intracellular enzyme that converts ribonucleotides to deoxyribonucleotides, which are required for DNA synthesis and repair.

The first clinical application of hydroxyurea for patients with SCA was reported in 1984, when Platt and colleagues demonstrated a rapid and dramatic increase in HbF-containing reticulocytes without significant bone marrow toxicity. [18] This and other 'proof-of-principle' experiments led to a critical phase I/II study of adults with SCA treated with hydroxyurea at maximum tolerated dose (MTD), which demonstrated significant dose-dependent increases in hemoglobin and HbF along with concurrent reduction in total white blood cell count, neutrophils, and reticulocytes. [19] Similar results were observed in phase I/II studies involving school-age children, toddlers, and infants, all documenting the safety, laboratory benefits, and clinical efficacy of hydroxyurea for young patients with SCA. [20,21] A pivotal Phase III trial of adults with SCA, using a randomized, double-blinded, placebo-controlled study design, proved the clinical efficacy of hydroxyurea for reduction of vaso-occlusive pain, acute chest syndrome, transfusions, and hospitalizations. [22]

The phase III study of hydroxyurea in infants (BABY HUG) has recently been completed; although equivocal benefits for organ protection were observed during the 2-year treatment period, significant laboratory efficacy and clear clinical benefits for reducing pain, acute chest syndrome, hospitalizations, and transfusions were demonstrated. [23] Three reports have documented the benefits of hydroxyurea on reducing mortality in SCA: two adult trials [24,25] and one pediatric study. [26] Taken together, accumulated evidence over 25-30 years documents the short-term and long-term safety and efficacy of hydroxyurea for children and adults with SCA.

1.3 Hydroxyurea in Developing Countries: The Knowledge Gap

Hydroxyurea is a once-daily oral medication with excellent bioavailability but variable pharmacokinetics and pharmacodynamics. [27] Hydroxyurea has a cytotoxic and myelosuppressive effect upon the bone marrow due to its mechanism of action as a potent inhibitor of the enzyme ribonucleotide reductase, [17] and this effect results in mild and predictable cytopenias. [28] These cytopenias are expected and can be used to determine the maximum tolerated dose (MTD). In developed countries, these mild reductions are observed primarily in the white blood cell (WBC) count, absolute neutrophil count (ANC), absolute reticulocyte count (ARC), and platelet count but have not resulted in any significant clinical toxicities. [28,29] However, hydroxyurea has not yet been used systematically as a disease-modifying therapy in Africa for the treatment of SCA, and it is unknown whether the significant infectious and nutritional comorbidities in sub-Saharan African children will exacerbate the marrow-toxic effects of hydroxyurea.

Currently there is limited and only sporadic use of hydroxyurea for SCA within Africa. No treatment consensus guidelines have been established, and no prospective clinical trials have been performed. It is critical to conduct properly designed clinical research on this topic, to prevent improper and potentially dangerous use. The REACH protocol was presented in concept at the 1st Global Congress on Sickle Cell Disease (Accra, Ghana in June 2010) and more specifically at REDAC consortium meetings (Dar es Salaam, Tanzania, June 2012 and Yaounde, Cameroon, June 2013); the response was extremely positive at all 3 meetings where attendees appreciated the importance of conducting clinical trials with hydroxyurea, before its use becomes more widespread without appropriate safety and efficacy data. Even if short-term benefits were observed, the safety profile with long-term use throughout childhood is critical to investigate and document.

The limited medical capacities of many countries in sub-Saharan Africa, coupled with the significant infectious and nutritional comorbidities of these populations will undoubtedly present substantial challenges for initiation and maintenance of hydroxyurea therapy. The prohibitive cost of hydroxyurea for most patients also must be managed. But with a carefully planned approach and local medical personnel willing to be trained and involved in the care of these children, hydroxyurea can be introduced into this highly burdened population via solid North-South research partnerships. [1,9] If proven to have safety and efficacy for children with SCA in developing countries, hydroxyurea could realistically emerge as the main disease-modifying therapeutic intervention to help reduce the global burden of SCA [30].

1.4 Potential Clinical Sites

The primary goal of this prospective research trial is to investigate the feasibility, safety, and benefits of hydroxyurea for children with SCA in sub-Saharan Africa, using a select group of clinical sites and investigators who will conduct high-quality research. Through the Global Sickle Cell Disease Network, clinical programs in sub-Saharan Africa were invited in January 2013 to complete a survey requesting detailed information about their site, which focused on the issue of readiness to conduct high-quality clinical research. The survey contained questions about the size and resources of the program, but also about research support, internet capability, investigational pharmacy, and many other research-related topics. The REACH Principal Investigator and selected co-Investigators reviewed these survey responses to determine if adequate clinical, laboratory, pharmacy, and data management facilities and operations are in place.

Based on 23 completed surveys, 6 sites across sub-Saharan Africa were identified with the best likelihood of conducting high-quality research that is required in the REACH protocol. These six sites included the following locations: Luanda, Angola; Dar es Salaam, Tanzania; Kampala, Uganda; Kinshasa, Democratic Republic of Congo; Accra, Ghana; and Kilifi, Kenya. In each setting, the country and region has a known substantial burden of SCA, an experienced local clinical investigator who could lead the trial, and adequate local resources to conduct the trial.

A 90-minute teleconference was then held with each site leader, to ask specific questions about their level of interest and ability to conduct the REACH trial. Subsequent communication further documented the ability of individual sites to conduct the REACH trial. Following this screening process, four clinical sites within sub-Saharan Africa (Luanda Angola, Kilifi Kenya, Kinshasa Democratic Republic of Congo, and Mbale Uganda) were identified as the initial clinical sites for the REACH study, based on investigator interest and site qualifications. Other sites may become eligible, but at most 4 sites will be activated for study treatment.

All four clinical sites were provided a draft version of the protocol and encouraged to make comments and edits. Over the period of several months and frequent email and teleconference communication, several key changes were made in the study design including eligibility criteria, frequency of monitoring visits, and support for the participants. This final protocol version represents a joint writing effort, and has been reviewed by all investigative teams and approved by all co-Investigators.

1.5 Study Design

REACH is a prospective, phase I/II open-label dose escalation pilot trial of hydroxyurea for children with confirmed SCA between 12 months and 10 years of age. The short-term goal is to obtain critical pilot data regarding the feasibility, safety, and benefits of hydroxyurea for children with SCA in multiple research settings in Africa. Based on that information, the longer-term goal is to develop a safety and benefits profile associated with extended hydroxyurea use throughout childhood, and to make hydroxyurea more widely available for children with SCA in Africa, particularly those identified with SCA through expanded newborn screening programs.

After enrollment, a two-month screening phase will be used to perform baseline evaluations including nutritional and infectious assessments, along with supplements and treatments as deemed necessary by local guidelines (e.g., penicillin, pneumococcal immunization, Vitamin A and albendazole/mebendazole treatment, malaria prophylaxis, and iron/folate supplementation). Hydroxyurea capsules will then be provided as a monthly supply in 200mg, 300mg, 400mg or 500mg sizes, to be administered as an open-label single daily dose. Six months of treatment will be given at the fixed dose of 15-20 mg/kg/day, followed by another six months with dose escalation (2.5-5.0 mg/kg increments every 8 weeks) as tolerated to 20-30 mg/kg/day or MTD. Interval history, physical examination, and blood counts will be conducted every month, until MTD.

After completion of the first 12-months of hydroxyurea treatment, children with an acceptable toxicity profile and favorable responses will be given the opportunity to continue hydroxyurea therapy until reaching the age of 18 years pending biennial review of BMS support. Data will be analyzed centrally using standardized data collection forms and a secure CTSA electronic database provided by Cincinnati Children's Hospital Medical Center in Cincinnati, Ohio.

REACH will be the first prospective study using hydroxyurea for children with SCA in Africa. The importance of gathering critical pilot data regarding the feasibility,

safety, and efficacy of hydroxyurea in this setting is enormous; these data could lead to transformation of treatment practices for SCA across Africa.

2.0 STUDY OBJECTIVES

2.1 To assess the feasibility of conducting a prospective research study using hydroxyurea therapy for SCA in sub-Saharan Africa (including adherence to monthly clinic visits and laboratory assessments, and medication compliance).

2.2 To monitor the safety of hydroxyurea therapy, specifically documenting significant hematological toxicities (cytopenias) and infections.

2.3 To evaluate the benefits of hydroxyurea therapy, using both laboratory (e.g., fetal hemoglobin, hemoglobin, white blood cell count) and clinical parameters (e.g., pain, hospitalization, growth parameters).

2.5 To explore the pharmacokinetic and genetic basis for any observed inter-patient variability in the clinical or laboratory response to hydroxyurea.

2.6 To evaluate the economic cost of providing hydroxyurea therapy in the REACH study sites.

2.4 To investigate the feasibility of hydroxyurea dose-escalation on laboratory and clinical parameters.

3.0 PATIENT SELECTION AND ENROLLMENT

3.1 Inclusion Criteria

3.1.1 Pediatric patients with documented sickle cell anemia (typically HbSS supported by hemoglobin electrophoresis, complete blood count, and peripheral blood smear)

3.1.2 Age range of 1.00-9.99 years, inclusive, at the time of enrollment

3.1.3 Weight at least 10.0 kg at the time of enrollment

3.1.4 Parent or guardian willing and able to provide written informed consent, with child's verbal assent as per local IRB/Ethics Board requirements

3.1.5 Willingness to comply with all study-related treatments, evaluations, and follow-up

3.2 Exclusion Criteria

3.2.1 Known medical condition making participation ill-advised, (e.g., acute or chronic infectious disease, HIV, or malignancy)

3.2.2 Acute or chronic severe malnutrition determined by impaired growth parameters as defined by WHO (weight for length/height or height for age >3 z-scores below the median WHO growth standards, as defined in Appendix I)

3.2.3 Pre-existing severe hematological toxicity (temporary exclusions)

a. Anemia: Hb <4.0 gm/dL
b. Anemia: Hb <6.0 gm/dL with ARC <100 x 10^9/L
c. Reticulocytopenia: ARC <80 x 10^9/L with Hb <7.0 gm/dL
d. Thrombocytopenia: Platelets <80 x 10^9/L
e. Neutropenia: ANC <1.0 x 10^9/L

3.2.4 Blood transfusion within 60 days before enrollment (temporary exclusion)

3.2.5 Hydroxyurea use within 6 months before enrollment (temporary exclusion)

3.3 Enrollment Procedures

3.3.1 Patients will be recruited from the existing sickle cell populations at each clinical site.

3.3.2 Clinical staff will identify eligible participants and discuss the study with the patient and family.

3.3.3 The parent or legal guardian will provide written informed consent at the time of patient enrollment.

3.3.4 Enrolled patients will begin a 2-month evaluation phase with screening studies, before commencing hydroxyurea treatment; treatment will have a fixed dose phase (6-months), an escalation phase (6-months), and maintenance phase (until the participant reaches 18-years-old pending biennial review of BMS support).

3.3.5 Participants who do not complete the initial screening phase will not begin hydroxyurea, and will be exited from the study and replaced with new patients.

3.3.6 Participants who start hydroxyurea but do not complete the initial 3-months of hydroxyurea treatment will be censored at the time of study exit, and then replaced with new patients, unless they already reached the primary safety endpoint. These censored data will still be used for the primary safety analysis.

3.3.7 Participants who complete at least 3-months of hydroxyurea treatment will not be replaced.

3.3.8 To ensure that at least 53 children have completed three months of hydroxyurea therapy for the first phase safety analysis, enrollment will be temporarily suspended after the first 60 participants are enrolled at each site, allowing for up to 12% participant drop-out or discontinuation of medication before restarting enrollment to the full 150 patients.

3.3.9 Patient enrollment is expected to be completed within 24 months after initiation at each clinical site. All participants will remain on study until reaching18-years-old pending biennial review of BMS support.

4.0 STUDY TREATMENT

4.1 Dosing Regimens

Overview of Dosing and Duration of Treatment. Eligibility Screening (ES) will occur at Month -2 and Month -1, followed by Baseline Evaluation (BE) and hydroxyurea initiation that occur together at Month 0. Hydroxyurea treatment will commence at 15-20 mg/kg PO daily and continue at this fixed dose for 6 months (24 weeks), with appropriate adjustments as needed for hematological toxicities as outlined below. Participants will have clinic visits and laboratory assessments (according to Appendix II) every month (4 ± 1 weeks). Six months of treatment will be given at the fixed dose of 15-20 mg/kg/day, followed by another six months with dose escalation (2.5-5.0 mg/kg increments every 8 weeks) as tolerated to 20-30 mg/kg/day or MTD. Interval history, physical examination, and blood counts will be conducted every month.

Study treatment with hydroxyurea will continue in a dose escalation phase through the 12-month (Week 48) evaluation, after which hydroxyurea will continue in a maintenance phase until the end of study participation. Whenever possible, an equivalent daily dose will be prescribed (i.e., the same mg of hydroxyurea will be given each day, rather than a variable daily dose throughout the week to give an average daily dose) using the hydroxyurea dosing calculator.

Dose Initiation Plan for Study Months 0-6. Using the participant's weight at Month 0, the daily dose will be calculated using available capsule sizes and a goal of 15-20 (17.5 ± 2.5) mg/kg/day using dosing calculator based on weight. At each 4-week interval visit, laboratory studies (Appendix II) will be used to assess treatment toxicity, typically anemia or neutropenia, but possibly also reticulocytopenia or thrombocytopenia. The daily dose will be held or lowered as per treatment toxicity guidelines outlined below. The daily dose will not be escalated until Month 6, thus representing 6 months of fixed dosage.

Dose Escalation Plan to MTD for Study Months 6-12. After 6 months of treatment, hydroxyurea will be titrated according to myelosuppression, and will be

increased to 20-30 mg/kg/day or the maximum tolerated dose (MTD) as defined by hematological toxicity, even if the participant has clinical improvement on lower doses. The target ANC on hydroxyurea therapy will be 2.0 - 4.0 x 10^9/L, which is similar to the goals of hydroxyurea in previous clinical trials, [23,24] but MTD may be based on the reticulocyte count. Hydroxyurea dose escalation will occur in 5.0 ± 2.5 mg/kg/day increments, adjusting every 2 months unless hematological toxicity occurs. Safety is paramount and so both toxicity and dose escalation thresholds have been established for REACH, based on previous clinical trials and experience using hydroxyurea.

Dose-limiting hematological toxicity and dose escalation criteria are summarized in Table 1. For on-study hematological toxicity criteria, any single parameter qualifies as a toxicity threshold; these criteria constitute the primary safety endpoint. For dose escalation criteria, all laboratory parameters should be within the ranges listed to allow hydroxyurea dose escalation. Minor adjustments to the dosing schedule may be recommended based on an individual participant's clinical course or laboratory trends.

Table 1. Dose-Limiting Laboratory Toxicity Thresholds

Toxicity	Parameter	Escalation Criteria	Toxicity Criteria
Neutropenia	ANC* (x 10^9/L)	>4.0	<1.0 x 10^9/L
Anemia	Hb* (gm/dL)	>6.5	Hb < 4.0 gm/dL OR Hb <6.0 gm/dL unless ARC >100 x 10^9/L
Reticulocytopenia	ARC* (x 10^9/L)	>150	ARC <80 x 10^9/L unless Hb >7.0 gm/dL
Thrombocytopenia	Platelets (x 10^9/L)	>150	<80 x 10^9/L

Table 1. Laboratory toxicity thresholds and hydroxyurea dose escalation criteria for hemoglobin, reticulocytes, neutrophils, and platelets. *ANC – absolute neutrophil count; Hb – Hemoglobin; ARC – Absolute reticulocyte count. Hydroxyurea dose escalation criteria are relevant only to Months 6-12.

Based on previous experience and published data from the US, [20,21,23,27-29,31] most pediatric patients require hydroxyurea doses of 20-30 mg/kg/day to reach the target myelosuppression. After reaching MTD, minor dose adjustments will be made as necessary based on weight changes and blood counts, to maintain the desired laboratory effects. Effects of hydroxyurea therapy (excessive myelosuppression; reticulocytopenia, severe anemia; thrombocytopenia; or drug noncompliance) will be collected at each visit. Medication adherence will be stressed at each visit and attempts will be made to collect treatment compliance data.

Dose Maintenance Plan for Study Months 12 through End of Study. After 12 months of treatment, hydroxyurea will be offered in a maintenance phase to compliant participants at MTD. Children will be escalated during Month 6-12 to establish the MTD, but may require further adjustments after Month 12, based on weight and blood counts, to maintain dosing at the MTD. Interval visits and laboratory monitoring will continue but at a less frequent interval, as outlined in the Schedule of Evaluations (Appendix II).

4.2 Drug Regimens, Formulation and Administration

Packaging, Administration and Storage of Hydroxyurea. Hydroxyurea will be provided by Bristol Myers Squibb (BMS) as capsules (200 mg, 300 mg, 400 mg, or 500 mg). The medication is stable at room temperature (20-30C) and does not require refrigeration. These capsules have been demonstrated to be stable with mixing or crushing, with dissolution in a wide array of liquids for immediate use. For example, for children incapable of swallowing capsules, the capsules can be opened daily and mixed with water or juice. Medication will be stored in local pharmacy space and a 30-day supply of capsules will be dispensed to study participants with each monthly clinic visit until reaching MTD, and a 90-day supply will be dispensed after that time. If a follow-up appointment needs to be scheduled more than 30 or 60 days from the previous visit (for example, due to clinic closings for a public holiday or other scheduling limitations), an adequate amount of hydroxyurea should be dispensed to last until the next scheduled visit. In these instances, up to an additional seven day supply can be dispensed. Study drug will be handled in accordance with guidelines set forth in the consent form and the REACH Manual of Operations, which will reflect language in the drug Package Insert and guidance from current US-based clinical trials that use hydroxyurea for children with SCA.

4.3 Toxicity Management and Dose Modification or Discontinuation

Dose Modification/Suspension Plan. Laboratory toxicities from hydroxyurea will manifest primarily as reversible and transient myelosuppression, especially of granulocytes. Myelosuppression with modest neutropenia and reticulocytopenia is a therapeutic goal of hydroxyurea therapy, however. Careful monitoring of CBC's will be performed every 4 weeks (± 1 week) since several studies have documented that monthly monitoring is acceptable. [23,29] If a hematological toxicity occurs during the dose escalation phase or the maintenance phase (e.g., the ANC falls $<1.0 \times 10^9$/L), hydroxyurea will be withheld and weekly blood counts performed. Upon recovery, the hydroxyurea dosing will depend on the recovery: if the toxicity has resolved within 1 week, then the hydroxyurea dose can resume at the same previous dose, but if it persists for > 1 week or occurs twice within a 3-month period, the hydroxyurea dose can be reduced by 2.5-5.0 mg/kg/day.

A similar algorithm will be used for platelet toxicity, with thrombocytopenia defined as $<80 \times 10^9$/L. For anemia, the thresholds will be any of the following: if the Hb concentration falls to <4.0 gm/dL; or Hb <6.0 gm/dL with the ARC $<100 \times 10^9$/L; or for reticulocytopenia (ARC $<80 \times 10^9$/L with Hb <7.0 gm/dL), hydroxyurea should be withheld until weekly counts document recovery. If the toxicity occurs during the dose

escalation phase, upon recovery the hydroxyurea dose will be restarted at a dose that has been reduced by 2.5-5 mg/kg/day. During the maintenance phase, if the toxicity resolves in 1 week, hydroxyurea should be resumed at the previous dose. If recovery takes longer than 1 week or occurs twice within a 3-month period, the dose can be reduced by 2.5 mg/kg/day.

These algorithms will serve as the general guidelines for the study. Individual toxicities will be evaluated by the MCC and recommendations may be made on an individual basis to ensure patient safety. Drug will be held during acute anemic events (e.g., malaria, parvovirus) and during acute renal toxicity (creatinine doubled from the baseline value and $\geq$1.0 mg/dL).

If the statistical analysis of safety data for the initial cohort of children demonstrates that the starting dose of 15-20 mg/kg/day has too many dose-limiting hematological toxicities, then subsequent dosing will begin at 10-15 mg/kg/day with a similar dose escalation regimen and statistical analysis.

4.4 Procedures for Treatment Discontinuation

Participants can be discontinued from the study for any of the following reasons:

- Participants missing two consecutive monthly clinic visits or three clinic visits during any 12-month period;
- Severe allergic reaction or life-threatening drug-related toxicity;
- Request of the study sponsor, parent, legal guardian, or investigator.
- Should a female participant become pregnant, she will be required to stop study treatment.

Final decisions about study discontinuation remain with the clinical site, but the Medical Coordinating Center (MCC) should be contacted prior to any participant's permanent discontinuation of study treatment so that data can be gathered prior to the study exit. Reasons for withdrawal should be fully documented in the data collection forms.

4.5 Adverse Event Reporting Requirements

Following the family's written consent to participate in the study, all adverse events (AE) and serious adverse events (SAE) will be collected and reported to the MCC and Data Management Center (DMC) using the correct data collection forms. AE reporting will utilize the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, where all AE are categorized by organ system and graded by severity. A list of expected and potentially serious AE, as well as exceptions to the CTCAE, is included in Tables 2, 3, and 4 below.

Table 2. Sickle Cell Disease Symptoms and Associated Conditions

Acute chest syndrome	Empyema	Pain, long bone
Adenotonsillar disease	Hand-foot syndrome/dactylitis	Pain, severe abdominal
Albuminuria	Headache	Pain, sternal or rib
Amenorrhea	Hematuria	Priapism
Anemia (severe)	Hemiplegia	Proteinuria
Aplastic crisis	Hemolysis	Pneumonia
Arthralgia	Hepatic sequestration	Pulmonary embolism
Avascular necrosis of hip/shoulder	Hepatomegaly	Pulmonary hypertension
Bacteremia	Hospitalization >24 hours	Pulmonary infiltrate on chest x-ray
Bone infarction	Hyperbilirubinemia	Pyelonephritis
Cardiac arrythmia	Hypersplenism	Renal failure
Cardiomegaly	Hypertension	Renal insufficiency
Cerebrovascular accident	Hypocalcemia	Renal papillary necrosis
Cholecystitis	Hyposthenuria	Reticulocytopenia
Cholelithiasis	Hypotension	Reticulocytosis
Cognitive dysfunction	Hypoxemia (PO2 < 65mm Hg)	Retinopathy
Constipation	Ileus	Retinal hemorrhage
Cranial nerve palsy	Infection, bacterial	Rhabdomyolysis
Death	Infection, pneumococcal	Seizure
Decreased renal function	Infection, line	Septicemia
Decreased lung function	Infection, viral	Silent organ infarction
Delayed growth/puberty	Jaundice	Skin ulcer
Depression	Leukocytosis	Splenic sequestration
Dizziness	Meningitis	Splenomegaly
Electrolyte imbalance	Nephropathy	Stroke
Elevated urinary urobilinogen	Osteomyelitis	Transient Ischemic Attack (TIA)
Elevated serum transaminases	Pain, back	Tonsillar enlargement or infection
Elevated TCD velocities	Pain, chest	Transfusion, unanticipated
Fever	Pain, joint	Vaso-occlusive pain

Table 2. List of REACH expected and potentially serious adverse events associated with sickle cell anemia. Table 2 may not be all-inclusive and should not be considered the total universe of possibilities since some events may have been left off the list inadvertently. The Lead Investigator should use clinical judgment along with consensus knowledge about SCD in determining event expectedness.

Table 3. Hydroxyurea-Related Adverse Events

Allergic reaction	Increased creatinine	Reticulocytopenia
Anemia	Increased ALT	Skin ulcers/gangrene
Anorexia	Leukopenia	Splenomegaly
Constipation	Nail/skin hyperpigmentation	Thrombocytopenia
Diarrhea	Nausea	Vomiting
Gastritis	Neutropenia	Increase in the following laboratory parameters: MCV, MCH, MCHC, Nucleated RBC, %HbF)
Hair loss	Pancytopenia	Decrease in the following laboratory parameters: Reticulocytes, RBC, WBC, ANC, Platelets, Hematocrit, %HbS, Total Bilirubin, LDH, and Ferritin.
Hypersplenism	Rash	Skin ulcers

Table 3. List of REACH expected and potentially serious adverse events associated with hydroxyurea treatment of sickle cell anemia. Table 3 may not be all-inclusive and should not be considered the total universe of possibilities since some events may have been left off the list inadvertently. The Lead Investigator should use clinical judgment along with consensus knowledge about hydroxyurea in determining event expectedness.

Table 4. Laboratory Exceptions to the CTCAE List

Parameter	Grade 2	Grade 3	Grade 4
Hemoglobin (gm/dL)	5.0 – 6.0	4.0 – 4.9	< 4.0
Total WBC (x 10^9/L)	1.0 – 1.999	0.5 – 0.999	< 0.5
ANC (x 10^9/L)	0.5 – 0.999	0.2 – 0.499	< 0.2
Platelets (x 10^9/L)	50 – 79	20 – 49	< 20
Total Bilirubin (mg/dL)	5.0 – 10.0	10.1 – 20.0	> 20.0
AST (IU/L)	150 – 300	301 – 1000	> 1000
ALT (IU/L)	150 - 300	301 – 1000	> 1000
Creatinine (mg/dL)	doubling of baseline serum creatinine and value ≥1.0 mg/dL	1.6 – 2.0	> 2.0
ARC (x 10^9/L) and Hb < 7.0 gm/dL	50-80	10-49	< 10

Table 4. List of Laboratory Exceptions to the CTCAE, version 4.0, Guidelines

4.5 Serious Adverse Event Reporting Requirements

SAE reporting will use commonly accepted definitions (typically any life-threatening illness or condition) with the exception of hospitalization, which is common in children with SCA. Accordingly, all hospital stays more than 7 days must be scored as SAEs in REACH. Drug-related and unexpected SAE are of particular concern, however all SAEs including death require prompt reporting by the local site, using email communication or telephone to the MCC and Medical Monitor. BMS Worldwide Safety (worldwide.safety@bms.com) will be notified within 24 hours of the MCC becoming aware of the SAE, and then to the IRB of record within the required timeframe.

If an ongoing SAE changes in its intensity or relationship to the investigational product, a follow-up SAE report should be sent to the MCC and DMC. All SAEs should be followed until resolution or stabilization.

Pregnancy

If it is discovered that a study participant is pregnant or may have been pregnant at the time of exposure to the BMS product associated with this study, the pregnancy, AEs associated with maternal exposure and pregnancy outcomes must be recorded on the Pregnancy Form and reported following SAE guidelines. If only limited information is initially available, follow-up reports may be required. Follow-up

information should be obtained on pregnancy outcomes for one year following the birth of the offspring. The investigational product will be permanently discontinued in an appropriate manner. Protocol-required procedures for study discontinuation and follow-up must be performed on the participant. Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Form.

Any pregnancy that occurs in a female partner of a male study participant should also be reported. Information on this pregnancy will be collected on the Pregnancy Form. Any pregnancy that occurs in a female partner of a male study participant should be reported. Information on this pregnancy will be collected on the Pregnancy Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

4.6 Concomitant Medications

4.6.1 All concomitant medications should be recorded on the appropriate data collection form, including name and dose of the medication, along with the medication start and end dates. This includes malaria prophylaxis, if appropriate, as well as malaria treatment.

4.6.2 No known or suspected SCA disease-modifying therapy, whether registered or approved for therapeutic use, should be administered during the hydroxyurea treatment period. Herbs or other alternative medications that might be used to treat the signs and symptoms of SCA will be discouraged, and their concomitant use will be recorded.

4.6.3 Use of blood products (e.g., erythrocyte transfusion) is permitted but should be captured, first on paper and then entered into the electronic data capture system.

4.7 Investigational Agents

4.7.1 Hydroxyurea is the study treatment but may also be an investigational agent, depending on local national standards. Site investigators are responsible for ensuring compliance with local country regulatory for importation and use of hydroxyurea. BMS may provide guidance as feasible.

4.7.2 Supply, Distribution and Storage – all hydroxyurea capsules will be stored locally at room temperature in secure pharmacy storage space, under the supervision of local investigational pharmacy staff.

4.7.3 Study drug accountability - the local Study Coordinators will maintain a complete record of all study drugs, reagents and supplies. The Principal Investigators and their designees will work closely with local site co-Investigators to ensure that study drug will be dispensed only to patients enrolled on the study.

5.0 CLINICAL AND LABORATORY EVALUATIONS (SEE APPENDIX II - SCHEDULE OF EVALUATIONS)

Eligible patients will be enrolled in the study upon signing the informed consent document. Pre-treatment screening assessments can commence immediately, but should be completed within 8 weeks of initiation. All evaluations should occur on the study month indicated in Appendix II. Every effort should be made to ensure that all evaluations and supplementations occur around the time of the scheduled appointment.

5.1 Pre-treatment Evaluations (required)

5.1.1 Screening Month -2

- Medical history, physical exam, length or height (cm) and weight (kg), assessment of growth parameters to identify severe malnutrition
- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, HIV testing by local standards, malaria assessment by local standards, serum (stored at -20C or -80C for future studies), and blood for serum and genomic DNA stored at -20C or -80C.
- Measles and PCV-13 (if not yet completed); PPV-23 (if over age 2 years)
- Nutritional supplementation as needed, based on dietary history and growth parameters
- Folate and iron supplementation, based on local guidelines
- Malaria protection, either insecticide-treated bednets or chemoprophylaxis, as per local standards

5.1.2 Screening, Month -1

- Medical history, physical exam, length or height (cm) and weight (kg), growth assessments
- CBC with white blood cell differential, reticulocyte count
- If not collected during Screening Month -2, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, HIV testing by local standards, malaria assessment by local standards, serum (stored at -20C for future studies), and blood for genomic DNA stored at -20C.

- Treatment for possible helminth infection with either albendazole or mebendazole, as per local guidelines

- For children <5 years of age, vitamin A supplementation with 200,000 units orally, if not already received and documented within the past six months

5.2 Hydroxyurea Initiation, Month 0

- Medical history, physical exam, length or height (cm) and weight (kg), assessment of growth parameters

- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, malaria assessment

- Hydroxyurea (15-20 mg/kg/day) as a daily oral dose

5.3 Study Month 1 through End of Study

5.3.1 Study month 1

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment

- CBC with white blood cell differential, reticulocyte count

- Hydroxyurea (15-20 mg/kg/day)

5.3.2 Study month 2

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment

- CBC with white blood cell differential, reticulocyte count

- Hydroxyurea (15-20 mg/kg/day)

5.3.3 Study month 3

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment

- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, malaria assessment

- Hydroxyurea (15-20 mg/kg/day)

5.3.4 Study month 4

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment

- CBC with white blood cell differential, reticulocyte count

- Hydroxyurea (15-20 mg/kg/day)

5.3.5 Study month 5

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment

- CBC with white blood cell differential, reticulocyte count

- Hydroxyurea (15-20 mg/kg/day)

5.3.6 Study month 6

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment

- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, malaria assessment

- Hydroxyurea dose escalation by 5 ± 2.5 mg/kg/day every 2 months (8 weeks) as tolerated to MTD

- Treatment for helminth infection with either albendazole or mebendazole

5.3.7 Study month 7

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment

- CBC with white blood cell differential, reticulocyte count
- Hydroxyurea at 15-25 mg/kg/day

5.3.8 Study month 8

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment
- CBC with white blood cell differential, reticulocyte count
- Hydroxyurea dose escalation by 5 ± 2.5 mg/kg/day every 2 months (8 weeks) as tolerated or MTD

5.3.9 Study month 9

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment
- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, malaria assessment
- Hydroxyurea at 15-30 mg/kg/day or MTD

5.3.10 Study month 10

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment
- CBC with white blood cell differential, reticulocyte count
- Hydroxyurea dose escalation by 5 ± 2.5 mg/kg/day every 2 months (8 weeks) as tolerated or MTD

5.3.11 Study month 11

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment
- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), HbF level, malaria assessment.

- Hydroxyurea dose at 15-30 mg/kg/day or MTD

5.3.12 Study month 12

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment
- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, malaria assessment, saved serum and blood for DNA
- Hydroxyurea dose at 15-30 mg/kg/day or MTD
- Treatment for helminth infection with either albendazole or mebendazole
- PPV-23 booster at age 5 years and 12 years
- For children <5 years of age, vitamin A supplementation with 200,000 units orally, if not already received and documented within the past six months

5.3.13 Quarterly (Tri-Monthly) Visits after Reaching MTD until End of Study

- Medical history, physical exam, length or height (cm) and weight (kg), medication adherence assessment
- CBC with white blood cell differential, reticulocyte count
- Urine pregnancy test for girls who have reached menarche
- Tanner Staging for growth and development, menarche query
- Hydroxyurea dose at MTD
- PK Study Sample to be collected after MTD is officially established but only once after reaching MTD.

5.3.14 Annual Visits after Reaching MTD until End of Study

- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, saved serum, blood cells, urine, and blood for DNA
- Treatment for helminth infection with either albendazole or mebendazole

- Urinalysis and urine for albuminuria

- Transcranial Doppler (TCD) Examination

- For children <5 years of age, vitamin A supplementation with 200,000 units orally, if not already received and documented within the past six months

- PPV-23 booster at age 5 years and age 12 years

5.4 Pharmacokinetics, Pharmacodynamics, and Pharmacogenomics

To explore the genetic basis for any observed inter-patient variability in the clinical and laboratory responses to hydroxyurea, a formal analysis of drug pharmacokinetics (PK) will be performed on children after achieving hydroxyurea MTD, followed by associations with drug pharmacodynamics (PD) and then pharmacogenomics (PG).

- Hydroxyurea PK studies will be performed as described, using a sparse sampling schedule of 4 time-points: a single collection before and 3 collections at about 15-20 minutes, 60 minutes, and 180 minutes after administering the hydroxyurea dose. [31] The whole blood specimens will be collected using Mitra® microsampling devices, with a total blood volume of <1mL for the PK studies.

- The samples will be stored at -20C or -80C until transport to Cincinnati Children's Hospital for quantitative analysis using either HPLC or LC-MS/MS analytic techniques. The PK analysis will be performed using a pharmacometrics strategy as described, [31] to calculate the hydroxyurea Tmax, Cmax, $t_{1/2}$, and other critical pharmacokinetic parameters

- PD variables will include Hb, MCV, HbF, ANC, hematological toxicities, and MTD dose, among others. Demographic variables and PK parameters will be correlated with these PD parameters, in an attempt to identify statistical associations and predictors of toxicities and response.

- Genomic DNA will be isolated from FTA cards or other storage devices and used to identify genetic variants that influence either the PK or PD parameters. Such PG analysis will require transport of the biological specimens to Cincinnati Children's Hospital for candidate gene analysis and unbiased whole exome/genome sequencing.

5.5 Discontinuation of Study Drug

- Medical history, physical exam, length or height (cm) and weight (kg), assessment of nutritional parameters, medication adherence assessment

- CBC with white blood cell differential, reticulocyte count, serum chemistries (creatinine, AST, ALT, bilirubin), %HbF level, malaria assessment, and serum, blood, urine, and DNA for future studies.

- Urinalysis and urine for albuminuria

- Tanner Staging for growth and development, menarche query

- Serum, blood and urine for future analysis as well as blood for genomic DNA purification and analysis, all stored at -20C or -80C.

- Transcranial Doppler (TCD) Examination

5.6 Lowering of Drug Dose due to Excess Toxicities

After the initial cohort of children is enrolled, toxicities will be closely monitored and analyzed. If the number of dose-limiting hematological toxicities is below the number allowed in the statistical analysis plan, enrollment will continue at the starting dose of 15-20 mg/kg/day. But if the number of dose-limiting toxicities is excessive, then the starting dose will be lowered by 5 mg/kg/day to 10-15 mg/kg/day and the first phase of statistical analysis will be repeated.

5.7 Data Management Issues

5.7.1 Research Electronic Data Capture (REDCap™) system

Study data will be collected and managed using REDCap electronic data capture tools. [33] REDCap is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

The REDCap system is available for use through institutions participating in the REDCap consortium. As of January 2013, this consortium is composed of 533 active partners in 49 countries. Cincinnati Children's Hospital Medical Center is serving as the home institution to allow the REDCap system for data collection in the

REACH study. The consortium supports a secure web application designed exclusively to support data capture for research studies, with a secure internet-based connection that uses low band-width, suitable for research in low-income countries.

5.7.2 Forms Management

Data will be entered into the electronic data record directly from each clinical site. Each enrolled patient will be given a study ID number, which will allow de-identified information to be collected without the child's name. All electronic data collection forms will be reviewed for accuracy and completion by the local Study Coordinator or Data Manager, as well as by the local study PI.

5.7.3 Database

An electronic database developed and managed by Cincinnati Children's Hospital Medical Center will accommodate data entry and management of the study's data.

A file for each study form will be created. The files will be templates of the data collection forms. A Data Entry Technician will access the database file templates to enter data from the study data collection forms.

5.7.4 Database Management

Programs will be created to run logical and missing value error checks on the data files. Edit reports will result from these programs and will be used to clean the data.

Quality control analyses are performed on the data at standard time intervals. Outliers are reviewed, resolved and the database is updated, as necessary.

Standard database reports will be generated monthly. These reports will include enrollment, withdrawal, endpoints, cumulative toxicities, and adverse events.

6.0 EVALUATION OF EFFECTS

6.1 Safety Endpoints

- The percent of participants and number of participants with pre-defined dose-limiting hematological laboratory toxicities; also the type and severity of the hematological toxicities.

- Number and type of serious infections, both bacterial and malarial

6.2 Feasibility Endpoints

- The percentage adherence to monthly clinic visits will be calculated between Month -2 and Month 12

- The percentage of laboratory assessments that were successfully obtained between Month -2 and Month 12

- The percentage of medication taken per pill counts (along with the Modified Morisky adherence score, described below) between Month 0 and 12

6.3 Efficacy Endpoints

- Change in laboratory measures of efficacy from baseline (e.g., fetal hemoglobin, hemoglobin, ARC, WBC, ANC, and bilirubin).

- Clinical complications will be captured, including pain, hospitalizations, and other sickle-related events.

- These on-study data will be compared to historical data for each patient gathered at study entry.

Correlate the PK parameters with PD variables, including hematological toxicities and treatment responses and identify genetic variants that affect both PK and PD parameters using a pharmacogenomics approach.

7.0 STATISTICAL CONSIDERATIONS

7.1 Sample Size

The enrollment goal for REACH is to recruit and treat a large cohort of children with SCA, up to 600 total participants at up to 4 different clinical sites. Because each local population of SCA will be different, based on a variety of criteria such as tribal ethnicity, genetics, income and nutritional status, each clinical site is considered independently for sample size calculations.

To achieve 90% power to detect significant differences in the primary safety outcomes described below, a sample size of 133 is required per site. Because of the possibility of excess drop-out during the initial 3 months of treatment, we will therefore aim for about 150 patients to be enrolled at each site (to ensure that 133 receive the entire initial 12-month treatment period), for a maximum total of 600 patients across 4 sites.

7.1.1 Safety endpoint – hematological toxicities

The specific safety hypotheses are as follows:

H_0: AE rate > Unacceptable value [unsafe]

H_a: AE rate < Unacceptable value [safe]

The investigators would like to reject H_0 and declare the medication safe. Low observed numbers of AEs will favor the alternative hypothesis. With this approach, early analysis will search for evidence that the drug is unsafe. Hematological toxicities and occasional dose adjustments or medication holds are expected with hydroxyurea therapy. Some of these adjustments will be due to drug-related toxicity while others will be sporadic. The recently completed BABY-HUG study reported toxicities of severe neutropenia (5%), severe anemia (1%), and thrombocytopenia (11%), but hematological toxicities were also noted in the placebo-treated arm. [19] The earlier HUSOFT study reported severe neutropenia (21% of patients) and severe anemia (25%), but that study had a much smaller cohort than BABY HUG, was open-label without a control arm, and had slightly different toxicity thresholds. [25] Based on these data, we estimate a dose-limiting hematological toxicity event rate of 20% for our sample size calculations, meaning 20% of the participants will have at least one hematological toxicity during the first 3-months of study treatment. However, given the potential for non-medication-related cytopenic events, an acceptable toxicity event rate of 30% will be the primary safety outcome measure.

Simon's two-stage procedure was used to calculate sample size and assess safety. An "expected" toxicity rate of 20% and "acceptable" toxicity rate of 30% were used for statistical calculations. Using this model, the first stage of analysis occurs after 53 participants at each site have completed 3 months of hydroxyurea therapy, but will also include participants who experience a dose-limiting hematological toxicity and withdraw from the study during the first 3 months of enrollment. To ensure that we have 53 children in this first phase, we will halt enrollment after 60 children have enrolled, allowing for up to 12% participant drop-out or discontinuation of medication. If ≤15 participants have hematologic toxicity as defined in Table 1, there is no early evidence against safety, and thus enrollment continues to the full 150 participants. If ≥15 of the initial participants experience hematologic toxicity, however, this is early evidence against safety. Future participants will begin at a lower dose of hydroxyurea (10 ± 2.5 mg/kg) and the study will in effect be restarted, with another 60 participants recruited for the same safety analysis at this lower dose. Upon final analysis of 133 participants at the same starting dose, safety for fixed-dose hydroxyurea can be concluded with 90% power if ≤33 participants experience hematologic toxicity. The main advantage of this two-stage design is the ability to identify that the drug is unsafe at an early part of the study, thus allowing dose modification to determine a safe hydroxyurea dose.

Serious infections, both bacterial and malarial, will be recorded descriptively and compared to pre-enrollment rates.

7.1.2 Feasibility endpoint – adherence to visits

Monthly clinic visits with laboratory testing are required in REACH, since this frequency of monitoring is the current standard or care in the United States, and also necessary to ensure safety while assessing efficacy. The feasibility of this monitoring schedule is relevant, since hydroxyurea cannot be safely administered with infrequent monitoring. Hydroxyurea treatment will be dispensed only 30 or 60 days at a time as outlined in Section 4.2, requiring a clinic visit every 4 ± 1 or 8 ± 2 weeks. Medication adherence and the ability for families to adhere to monthly clinic visits are important feasibility outcomes.

Families will be asked to bring their medication each month and pill counts will serve as one measure of medication compliance. Adherence will be scored categorically as follows: ≥80% adherence (Excellent), 60-79% adherence (Good), 40-59% (Moderate) and <40% (Poor).

Secondarily, the Modified Morisky Scale [34,35] will be administered verbally by nursing or medical staff. The adapted scale includes these four questions:

(1) Do you ever forget to give your child the hydroxyurea medication?

(2) Are you careless at times about giving your child the hydroxyurea medication?

(3) When your child feels better, do you sometimes stop giving your child the hydroxyurea?

(4) Sometimes, if your child feels worse when taking the hydroxyurea, do you stop giving them?

A simple yes/no will be used to assess adherence. Each "yes" answer is 1 point, and each "no" is 0 points. The answers are summed to give a score (range, 0-3). Adherence will be scored as follows: 0 (Excellent), 1 (Good); 2 (Moderate); 3 (Poor). Statistical analysis of the Feasibility Endpoint will be descriptive.

7.1.3 Efficacy endpoint – laboratory measures

The benefits of hydroxyurea will be primarily assessed through fetal hemoglobin (HbF), comparing treatment with baseline values. Additional measures of laboratory efficacy will include changes in Hb, MCV, WBC, ANC, ARC, and bilirubin. Clinical events such as vaso-occlusive pain will be captured as secondary outcomes.

7.2 Patient Recruitment and Retention

The clinics at the proposed clinical sites have large populations of children with SCA, with most seeing hundreds or even thousands of new patients annually. All patients will be recruited from these existing centers. Most patients live locally and travel to clinic by foot or local bus/taxi. Accordingly, physical distance to the clinic is not likely to affect recruitment and retention of our study population living in the urban centers where these clinics reside.

Recruited patients will be informed of study procedures and expected monthly follow-up schedule. Patients will be enrolled based upon the family's ability to maintain such a follow-up schedule. Patients will be enrolled after written, informed consent has been obtained from the parent or surrogate, according to the guidelines of the main IRB and the local Ethics Committees. Given the burden of traveling to the clinic each month, remuneration per visit will be provided for families, to help defray the costs of travel, meals and lost wages. The amount of this remuneration will be determined locally by the clinical site Investigator.

Given the early mortality of SCA in these countries, enrollment will primarily target young patients. With a goal of 150 participants per site, in each location we aim to enroll about 100 participants who are between twelve months and five years of age, about 50 participants between five and ten years of age. We have chosen twelve months of age as the minimum age, based on published experience from the Phase III infant hydroxyurea (BABY HUG) clinical trial. [19] We will also enroll some older children as available, because they will also receive clinical benefits from hydroxyurea therapy and data from these children are necessary to establish evidence-based guidelines for children with SCA of all ages. We will not address the treatment of adults.

Participants of reproductive age should not become pregnant, and should either practice abstinence or use medically-approved contraceptive measures since hydroxyurea has teratogenic potential. There are no adequate and well-controlled studies in pregnant women, so precautions will be taken in the REACH trial to recommend against pregnancy and to regularly monitor menstruating female participants for pregnancy. Male participants also will be counseled about the need to avoid fathering a child while taking hydroxyurea treatment. Should a female participant become pregnant, she will be asked to stop study treatment.

7.3 Expected Drop-Out Rate

Based on data from the US in clinical trials and discussion with the local site investigators, we estimate ≤ 25% dropout rate over the 12-month treatment period. Participants who drop-out during the screening phase, before receiving hydroxyurea treatment, will be replaced by new recruitments. Data from participants who dropout during the first 3-months of the treatment period will be censored but still included in the analysis, using data available from

successfully attended clinic visits. These participants will be replaced to provide sufficient data for analysis of safety. For the possibility that the drop-out rate exceeds 25%, some additional patients will be enrolled (150 per clinical site).

7.4 Planned Statistical Analyses

Formal statistical analyses will be developed after additional support and funding are secured. Initial goals will be descriptive summaries of enrollment, toxicities, and effects based on participant age, gender, and clinical location.

8.0 HUMAN SUBJECTS

8.1 Institutional Review Board (IRB) and Informed Consent

This protocol, the informed consent document, patient recruitment brochures, and any subsequent modifications will be reviewed and approved by the IRB at Cincinnati Children's Hospital (MCC and DMC) and the IRB or Ethics Committees at each participating institution. Compliance with GCP guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements presented in ICH E6, Good Clinical Practice: Consolidated Guideline. The Investigators will comply with all clinical trial disclosure and registration regulations. Approval of the local IRB or Ethics Committee will be received prior to initiation of the study, along with external IRB approval at the institutions of record for both the MCC and DMC. Written informed consent will be obtained from the parent or legal guardian of patients, all of whom are below age 10 years so cannot consent for themselves (e.g., those below the local legal age). If required by the local IRB or Ethics Committee, the patient's assent will be obtained regarding the nature, significance and risks associated with the study. A notation that written informed consent was obtained will be made on the participant's case report form. The signed informed consent forms will be retained at the study site; a copy will be put in the participant's records. A copy of the consent form will also be provided to the parent or legal guardian (and to the patient when applicable).

8.2 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified by a coded number only to maintain patient confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be completed with coded numbers only. Clinical information will not be released by the REACH MCC or DMC without written permission of the patient/parent/guardian, except as necessary for

monitoring. Clinical information can be shared by the site investigators and staff for patient care reasons, e.g., local consultations.

8.3 Study Discontinuation

Data from this study will be reviewed in real-time by the Principal Investigator and Co-Investigator team members. Data will be reviewed periodically with an external DSMB assembled by the MCC team. The study will be discontinued if at any time the study team feels that it is in the best interests of study participants to do so.

9.0 DATA AND SAFETY MONITORING BOARD (DSMB)

9.1 Rationale

Since REACH is a Phase I/II pilot trial in a potentially vulnerable patient population, it is important that an independent group have access to the feasibility, safety, and efficacy data. Periodic review by the DSMB will be critical to ensure that the participants are protected from harm, while also ensuring that the study integrity is not compromised.

9.2 Composition

A group of international investigators will be invited to join the REACH trial and serve as an independent DSMB. Among these projected 5-7 persons, there will be experience and expertise in international clinical trials, pediatric hematology, malaria, and the use of hydroxyurea in sickle cell disease, as well as representation from biostatistics, ethics, and patient advocacy.

10.0 ORGANIZATIONAL STRUCTURE

The REACH trial will be guided by both a Medical Coordinating Center (MCC) located at Cincinnati Children's Hospital Medical Center and a Data Management Center (DMC) through the CTSA located at Cincinnati Children's Hospital Medical Center. The MCC will be led by Dr. Russell Ware, who will serve as overall Principal Investigator (PI) for the study, and Dr. Patrick McGann (co-Investigator) who will be the primary Clinical Coordinator to assist the sites with clinical questions, adverse events, and other questions related to interpretation of the protocol. The DMC is led by Dr. Russell Ware and assisted by a team of experienced CTSA personnel who have substantial REDCap expertise in multicenter clinical trials. The DSMB and a special African Advisory Board will provide input to the Steering Committee.

Each REACH clinical site (Angola, DRC, Kenya, and Uganda) will be led by a co-Investigator who will serve as site Lead Investigator, along with a team of local co-investigators. The MCC will be assisted by the REACH Hydroxyurea Consultant who will provide specific expertise for hydroxyurea dosing and toxicities, and adverse events that might be drug-related. The REACH Investigational Pharmacist will assist the MCC and clinical sites, to ensure the hydroxyurea is stored, managed, and dispensed properly.

A monthly REACH Steering Committee call will include representatives of the MCC, DMC, and Lead Investigators from all local sites. Operations Committee calls will occur every 2-4 weeks throughout the study. An illustration of the organizational structure for the REACH trial is shown on the following page.

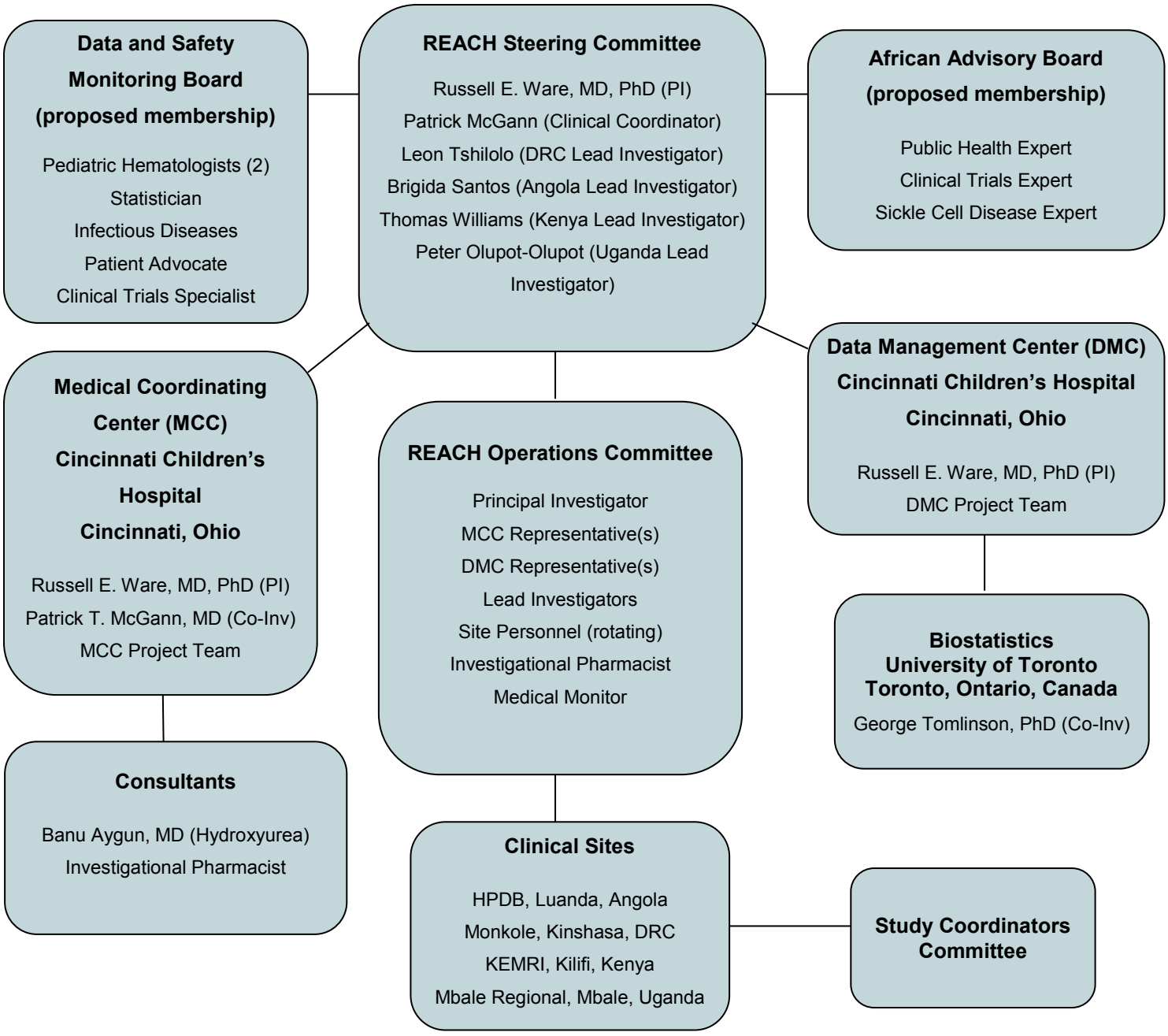

Figure 1. Organizational Structure for the REACH clinical trial

11.0 PUBLICATION OF RESEARCH FINDINGS

The results from this clinical trial have the potential for immediate public health applicability for the sickle cell community around the world. The target audience will be reached through publications, oral presentations, and seminars. Data analysis and manuscript preparation will occur during the last 6-12 months of this proposed trial. At the end of the trial, the main outcome paper will be submitted to a prestigious journal, such as the New England Journal of Medicine, Journal of the American Medical Association, or Blood. The trial results will be presented at the annual meetings of the American Society of Hematology (ASH) and regional meetings in Africa (REDAC). Any presentation, abstract, or manuscript will be made available to all study team members for review prior to submission.

Other results generated from the trial that are not published in the main outcome manuscript will be submitted for publication in prestigious medical journals and presented at scientific meetings listed above. All plans for dissemination of study results will be discussed with the investigators and the DSMB before implementation. BMS will have the ability to review draft publications approximately 30 days prior to submission, but will not have access to the primary data, authority to edit the results, or influence any presentations or publications.

12. REFERENCES

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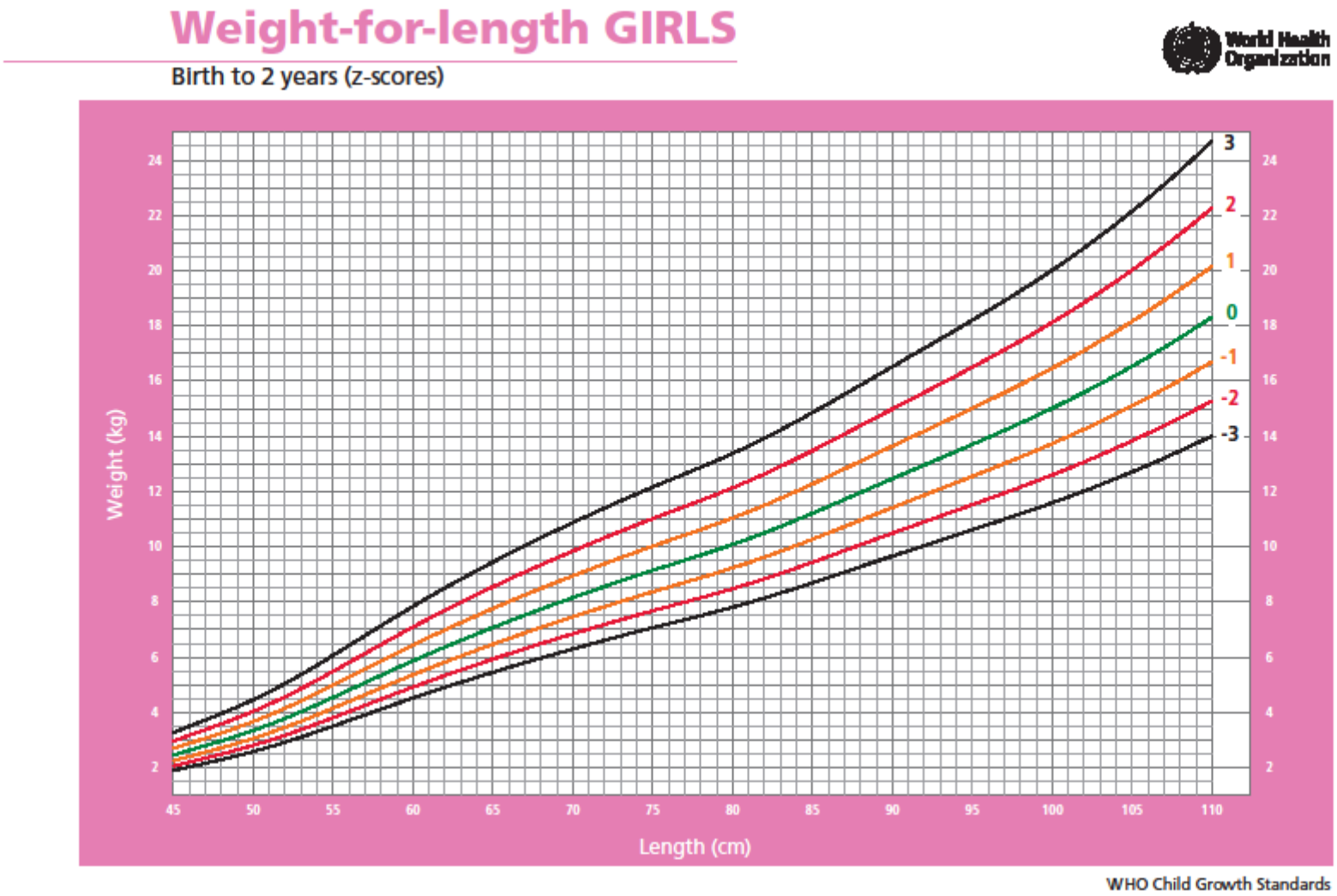
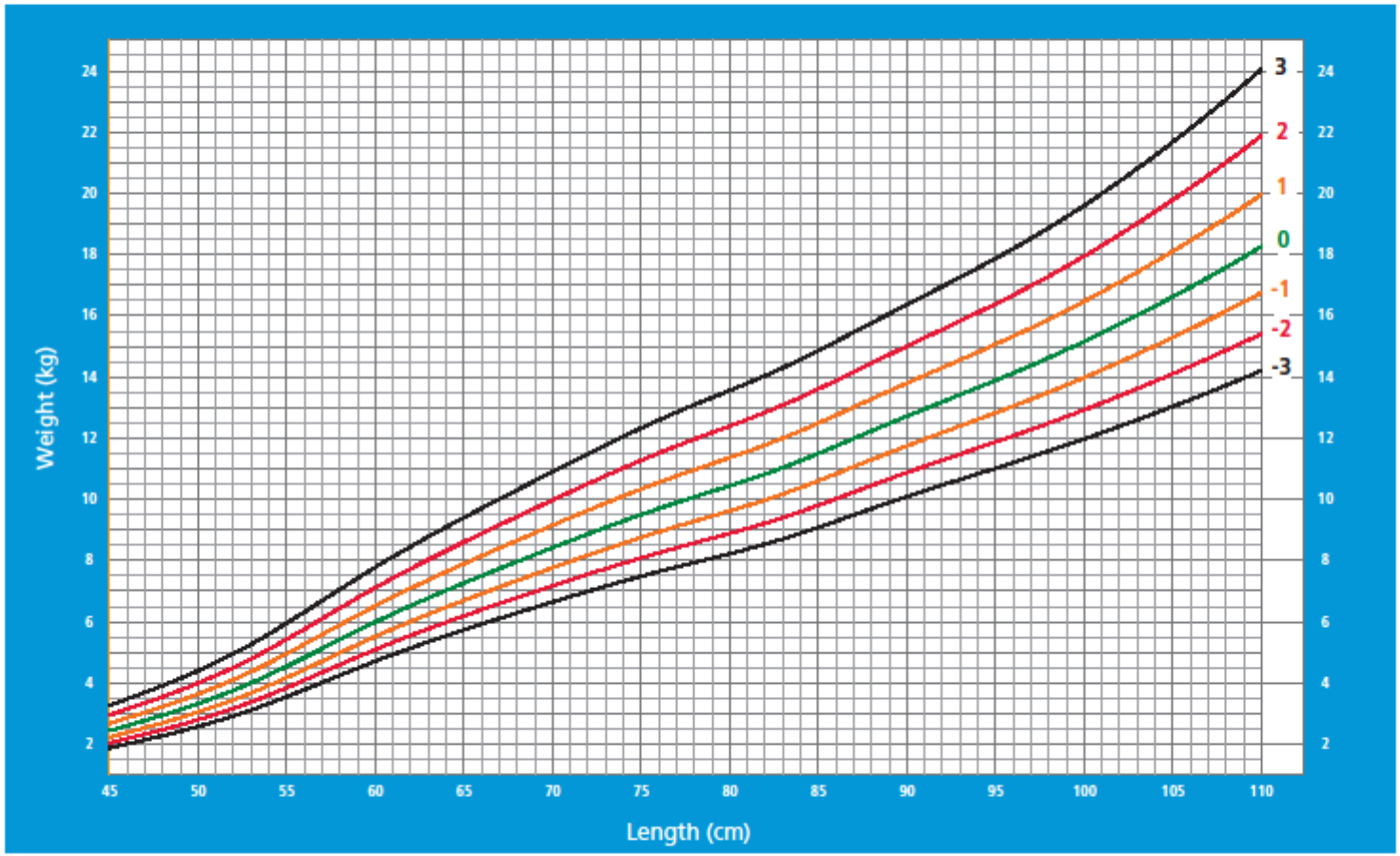
APPENDIX I: WHO WEIGHT-FOR-LENGTH, WEIGHT-FOR-HEIGHT, AND LENGTH/HEIGHT-FOR-AGE GROWTH CURVES



Weight-for-length BOYS

Birth to 2 years (z-scores)

World Health Organization

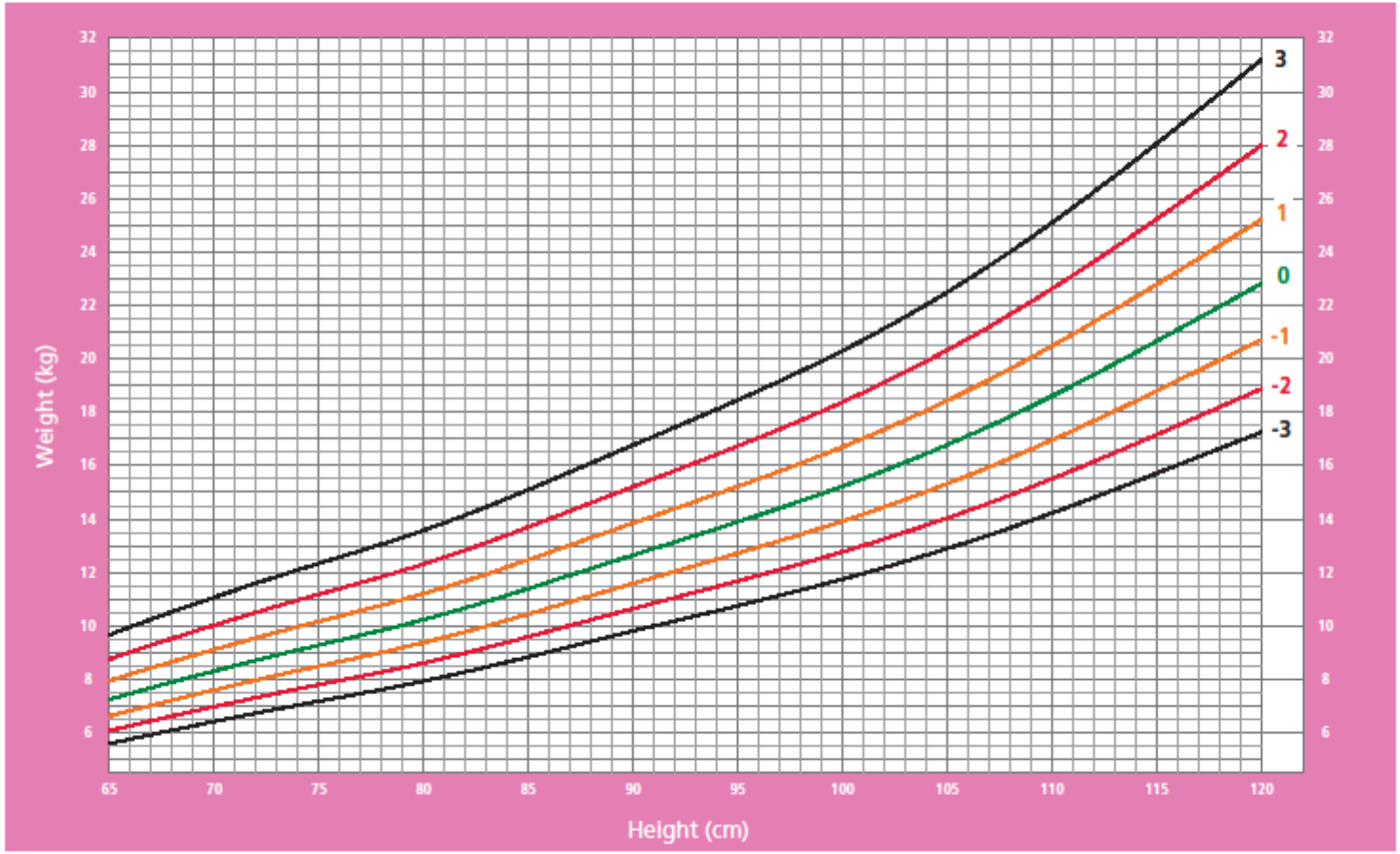


WHO Child Growth Standards

Weight-for-Height GIRLS

2 to 5 years (z-scores)

World Health Organization

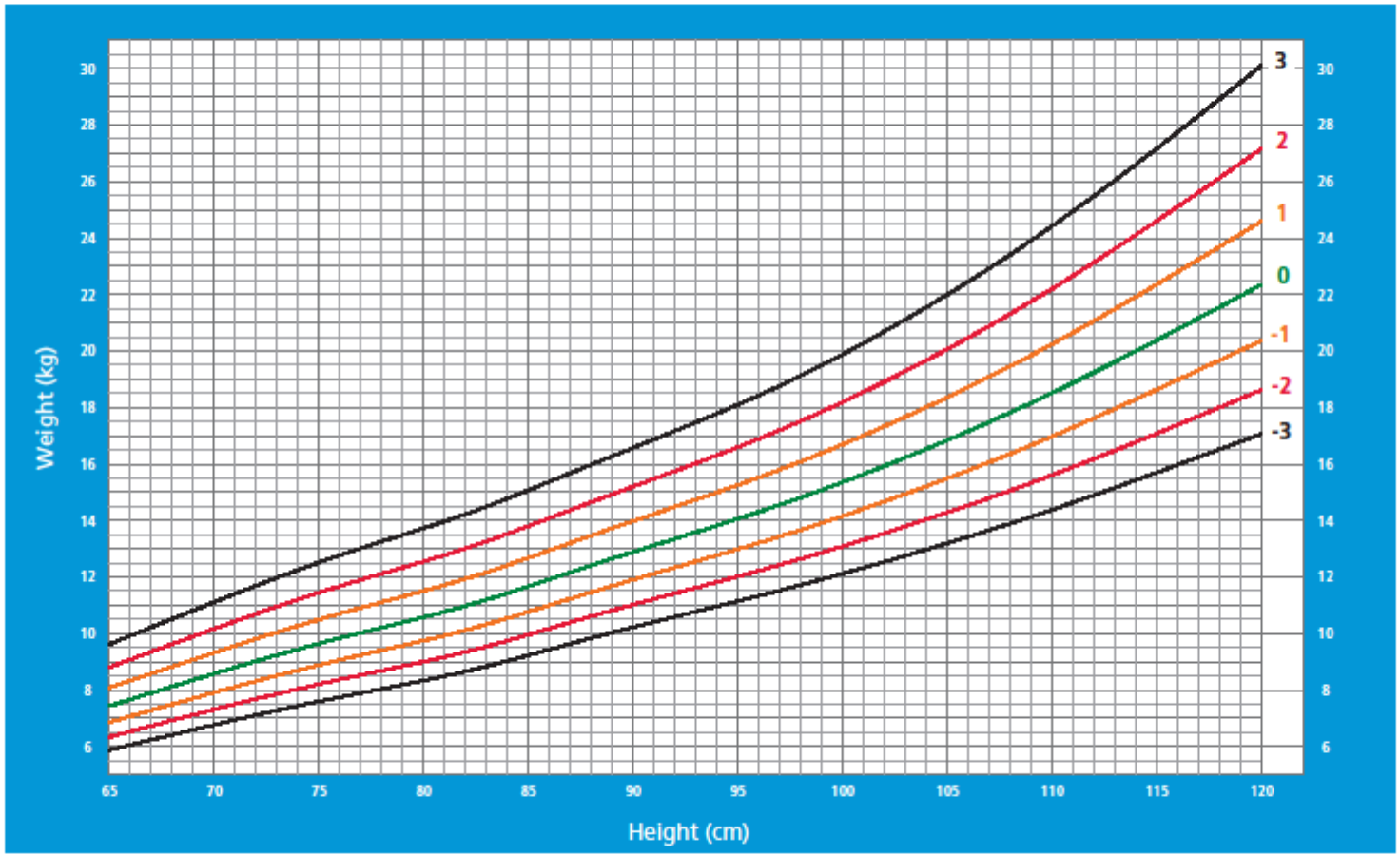


WHO Child Growth Standards

Weight-for-height BOYS

2 to 5 years (z-scores)

World Health Organization

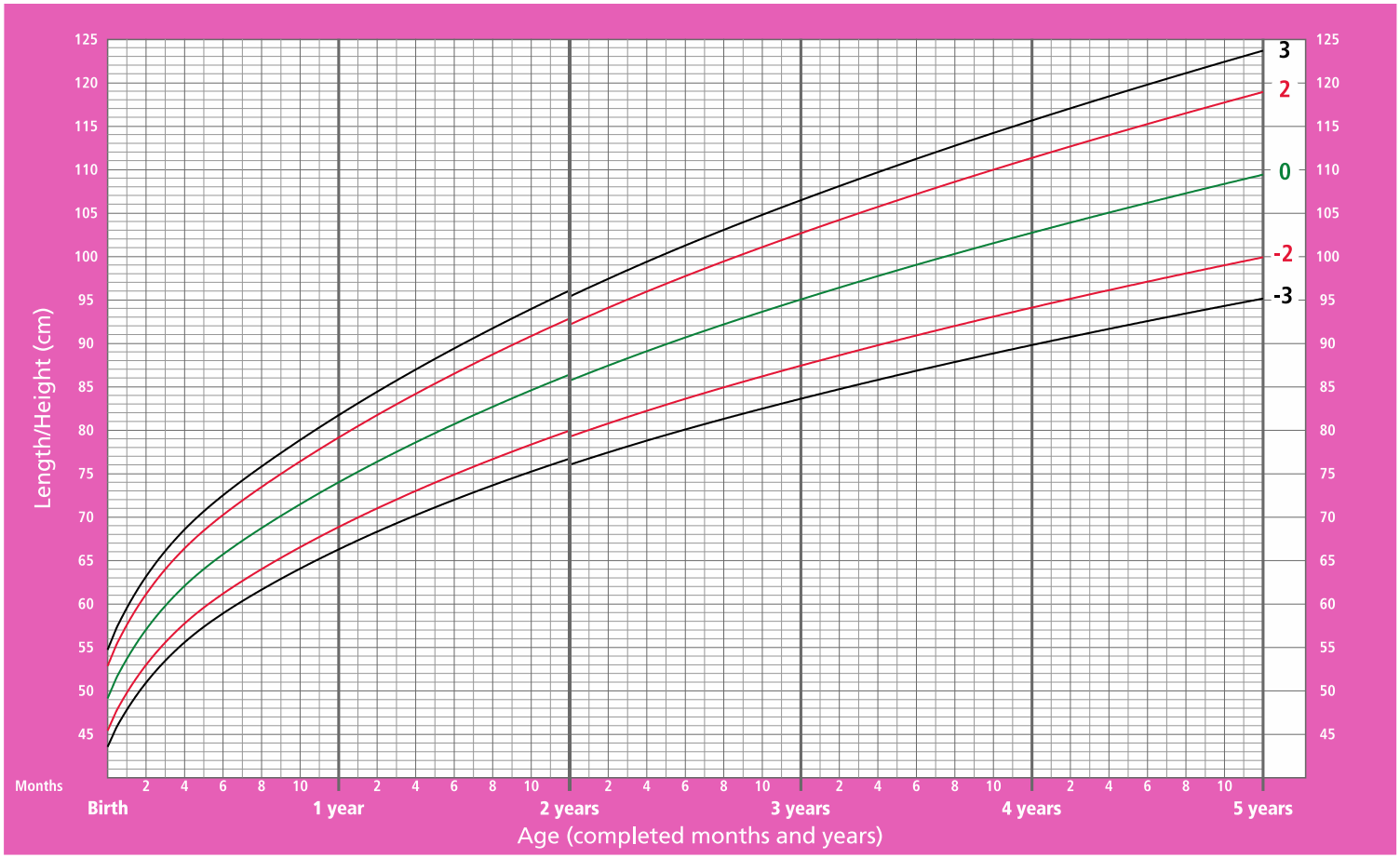


WHO Child Growth Standards

Length/height-for-age GIRLS

Birth to 5 years (z-scores)

World Health Organization

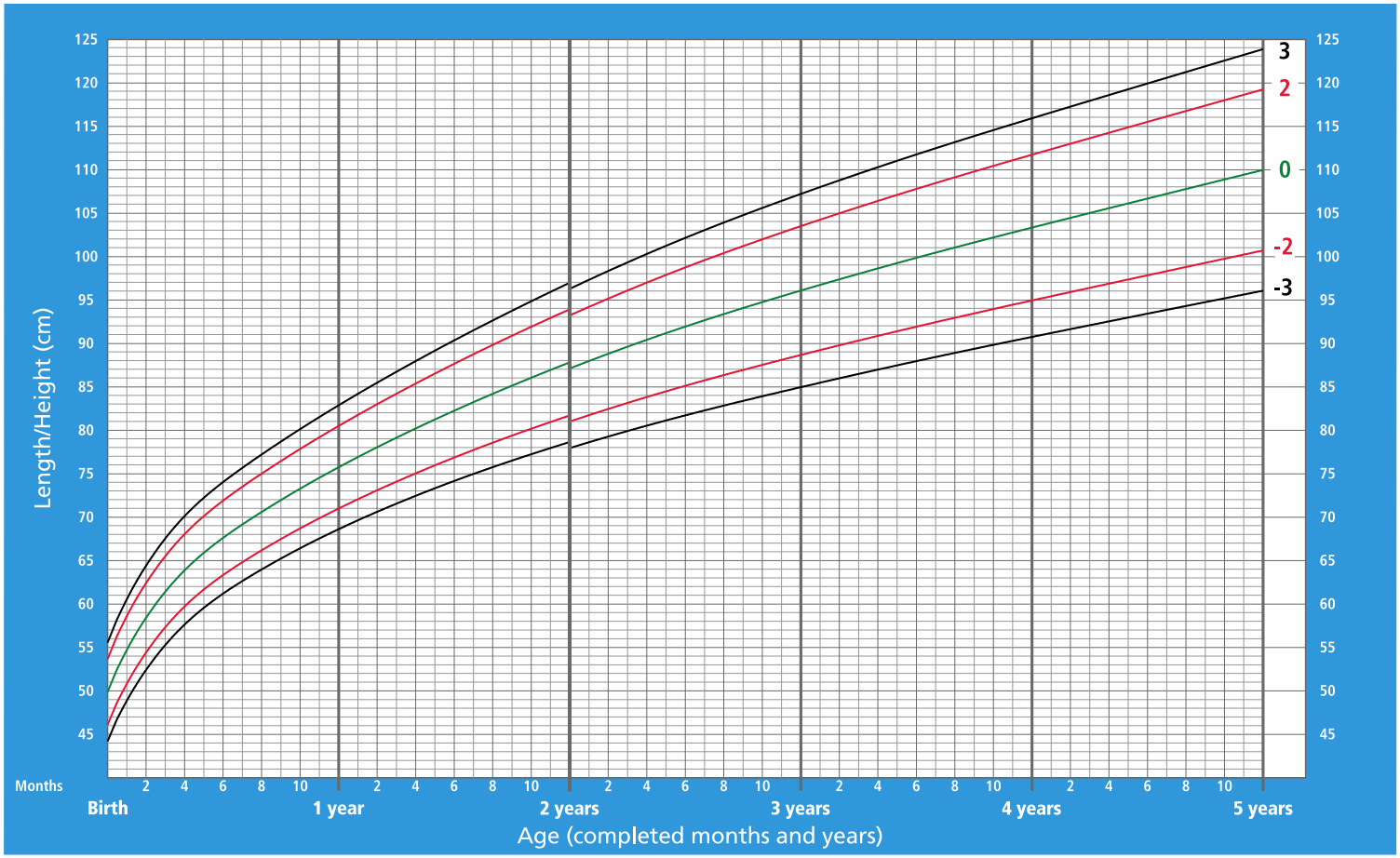


WHO Child Growth Standards

Length/height-for-age BOYS

Birth to 5 years (z-scores)

World Health Organization

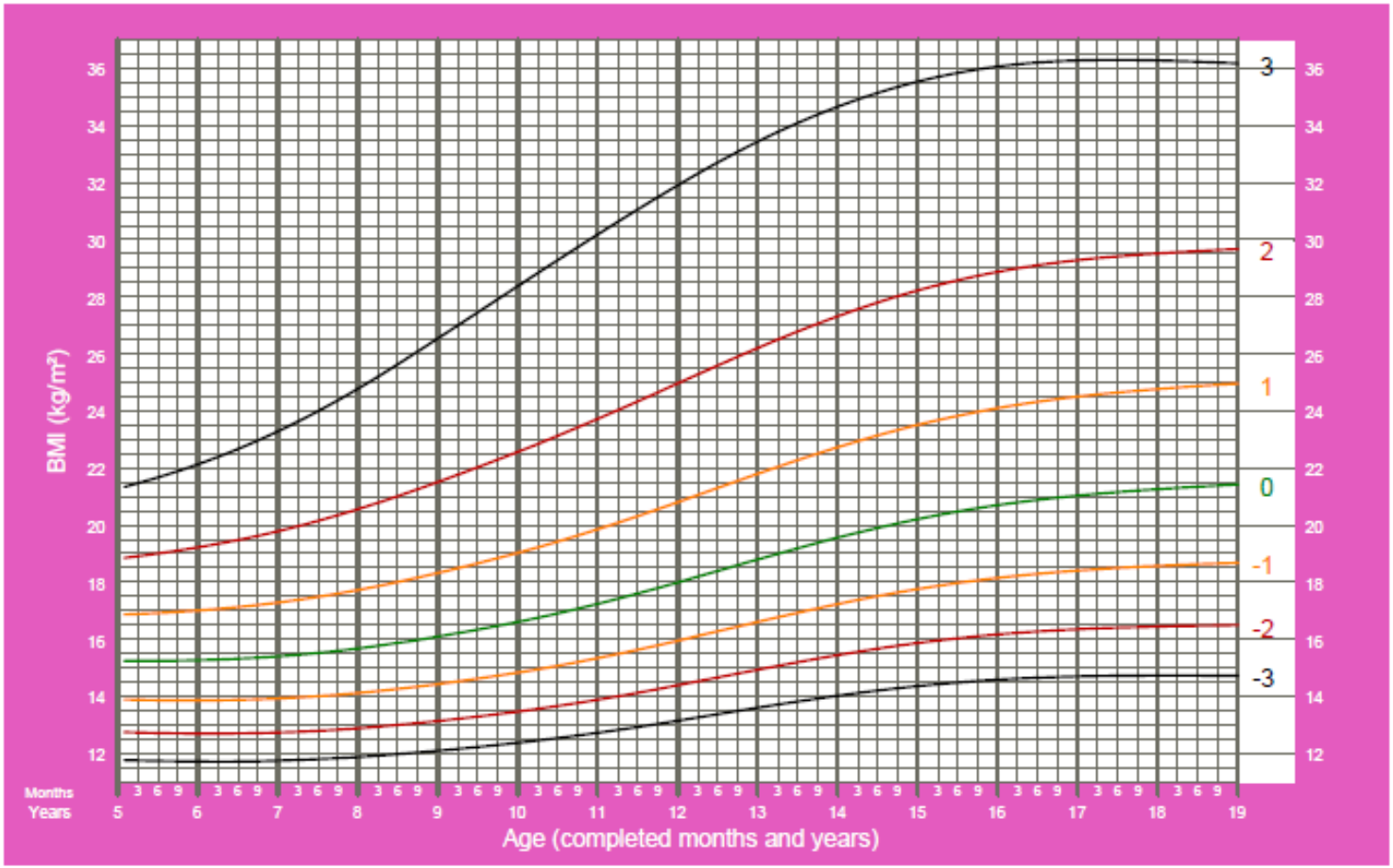


WHO Child Growth Standards

BMI-for-age GIRLS

5 to 19 years (z-scores)

World Health Organization

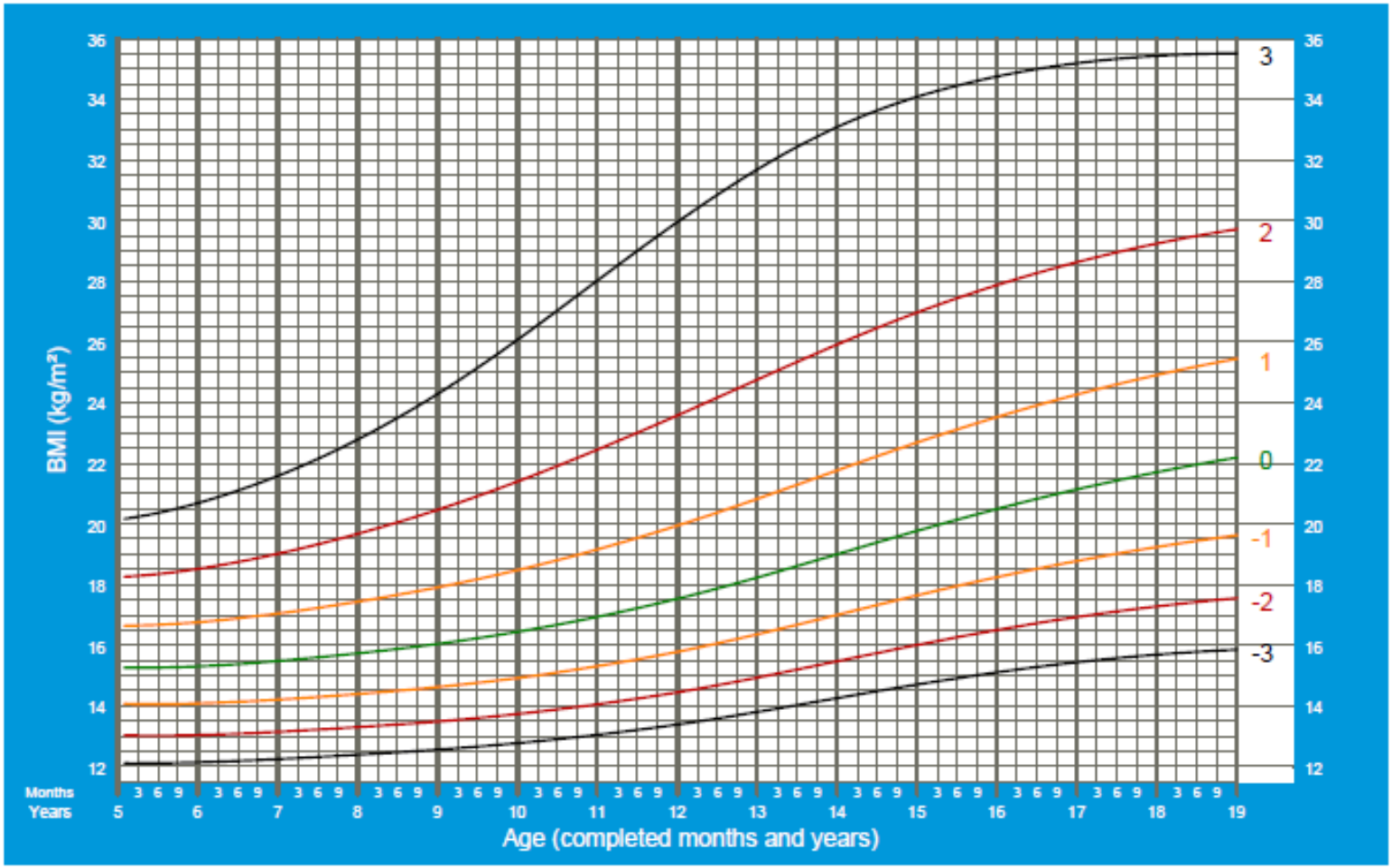


2007 WHO Reference

BMI-for-age BOYS

5 to 19 years (z-scores)

World Health Organization

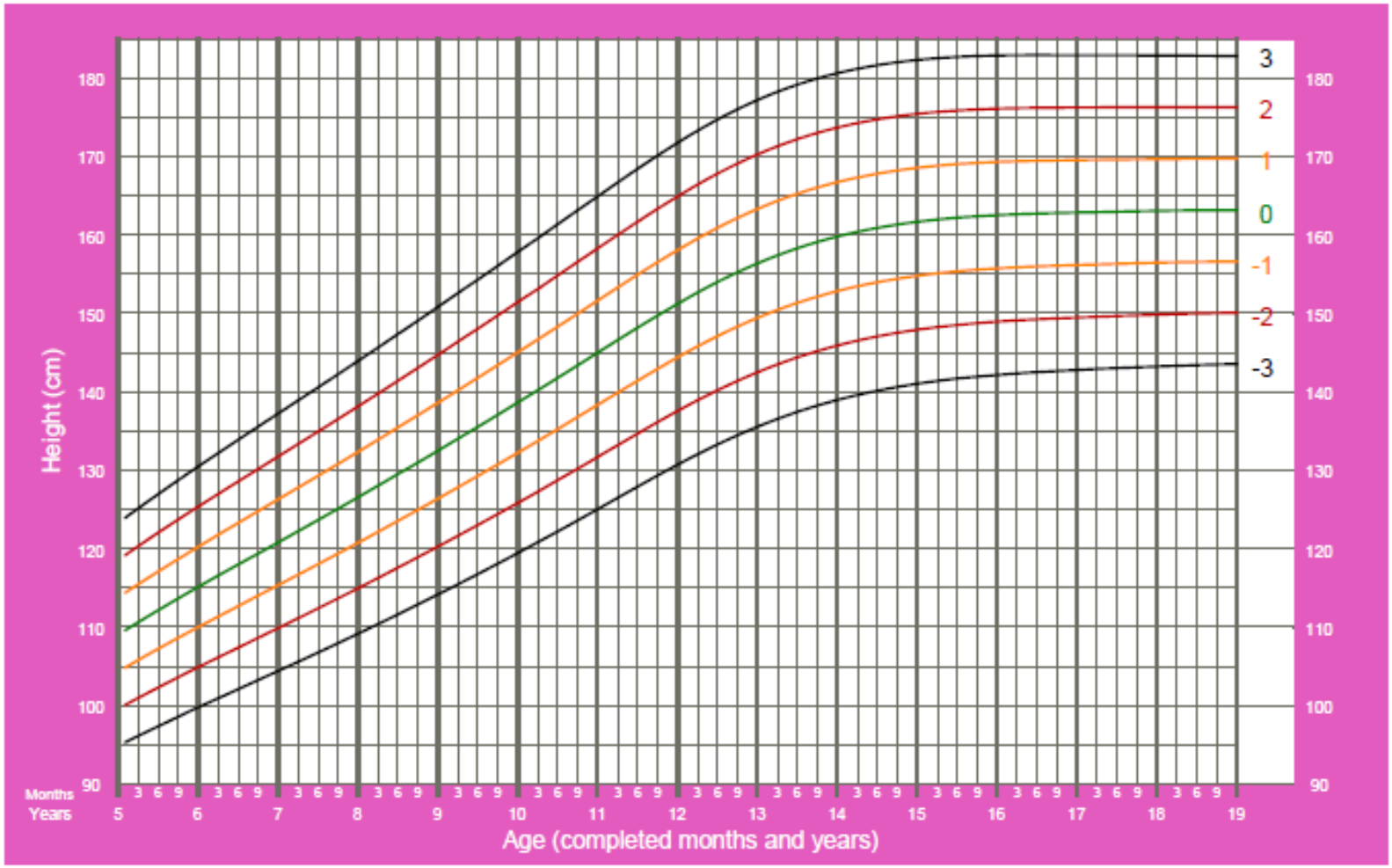


2007 WHO Reference

Height-for-age GIRLS

5 to 19 years (z-scores)

World Health Organization



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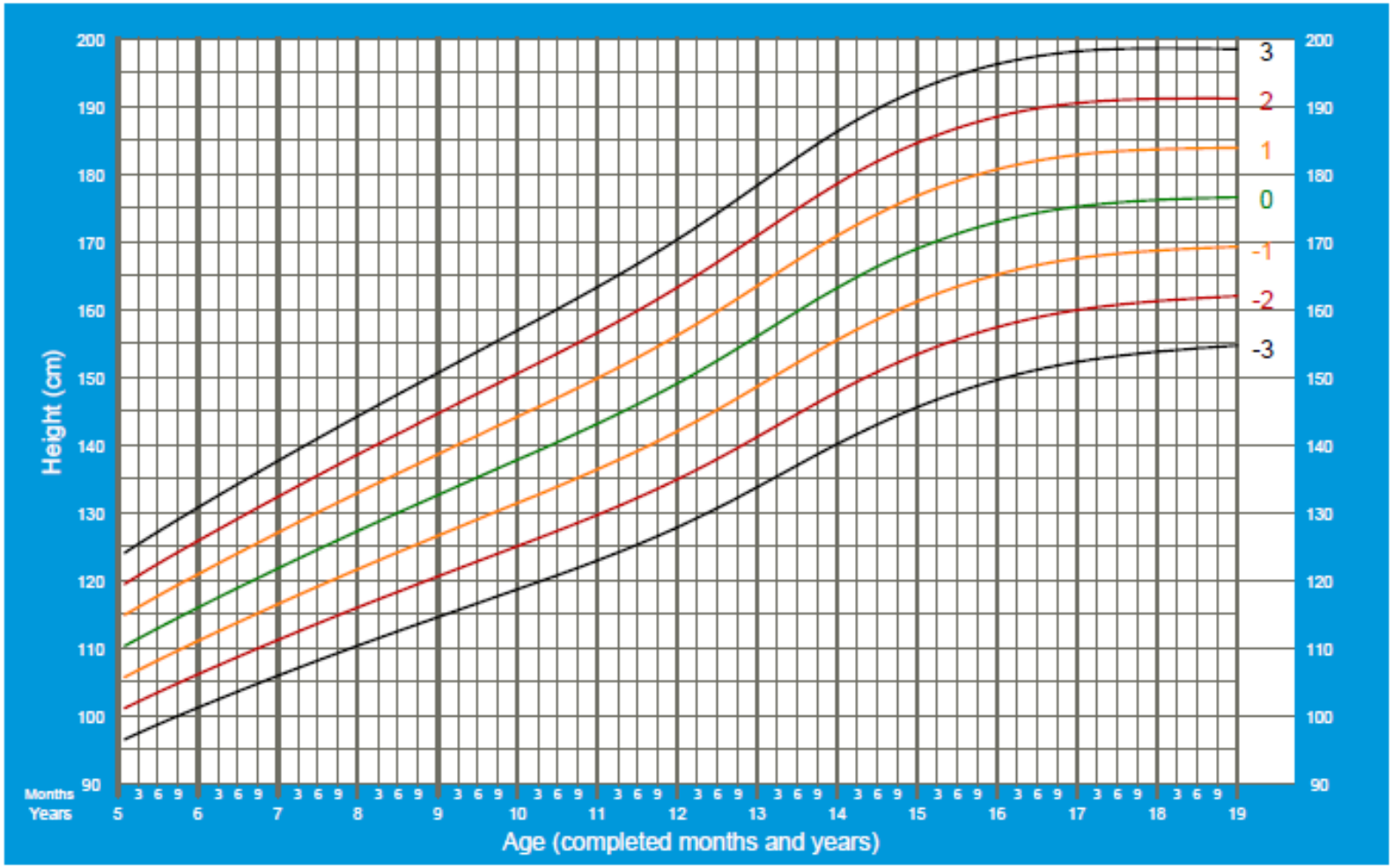
Height-for-age BOYS

5 to 19 years (z-scores)

World Health Organization



2007 WHO Reference

APPENDIX II: SCHEDULE OF EVALUATIONS

Evaluations	Screening		Initiation	Study Month [1]														
	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	Q3	Annual	EOS
Clinical Assessments																		
Medical history	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height/Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tanner Staging															X	X	X	X
Medication adherence				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TCD Examination																	X	X
Pregnancy Testing																X	X	
Laboratory Assessments																		
CBC with WBC differential	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reticulocyte count	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistries [2]	X		X			X			X			X			X		X	X
HbF level	X		X			X			X			X			X		X	X
HIV serology	X																	X
Malaria assessment	X		X			X			X			X			X		X	X
Urine for albuminuria															X		X	X
Serum and DNA collection [3]	X														X		X	X
Hydroxyurea PK Study																At a visit after MTD is established		
Treatment																		
Hydroxyurea (15-20 mg/kg/d)			X	X	X	X	X	X										
Dose escalation (15-30)									X	X	X	X	X	X				
MTD maintenance															X	X	X	
Treatment for helminths		X							X						X		X	
Vitamin A		X													X		X	
Measles, PCV-13, PPV-23 [4]	X																X	
Nutritional supplementation	As clinically indicated																	

[1] Study evaluations should be performed ± one week of the designated study month for the first 24 weeks\s, then ± 2 weeks of the designated study month thereafter
[2] Creatinine, AST, ALT, bilirubin
[3] Saved for future testing such as serum folate, B12, ferritin, retinol; urine for albumin and cystatin C; blood for micronuclei; and genomic DNA analysis
[4] Measles and PCV-13 immunizations if not previously provided; PPV-23 if participant is >2 years of age and not previously provided, then booster at age 5 years and 12 years