PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)

FOLLOW-UP OBSERVATIONAL STUDY

PROTOCOL

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CHAPTER 1

BACKGROUND AND STUDY RATIONALE

1.1 OVERVIEW

The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) was designed as a Phase III, two-year study treatment, double-blind, randomized placebo-controlled trial including 200 children at 14 clinical centers. The final child was randomized into the BABY HUG Treatment Study in September 2007. At the end of their child's two years of study treatment, all parents/guardians will be offered the opportunity for their child to be treated with open-label hydroxyurea (HU) therapy, without regard to and without knowledge of their child's randomized treatment assignment. When originally consenting to participation in BABY HUG, parents/guardians were told explicitly of the investigators' intention to request permission to follow their child for many years, to evaluate possible long-term effects of treatment.

The purpose of the BABY HUG Follow-up Study is to provide structured follow-up of the children enrolled in the BABY HUG Treatment Study, in order to characterize the long-term toxicities and unexpected risks (if any) associated with treatment with hydroxyurea at an early age. Ideally this unique group of children should be intensively followed for growth, development, and clinical status at least through puberty or early adulthood to document any alterations in the natural history of sickle cell disease (SCD) associated with early HU therapy. This protocol is the initial installment in that effort.

All parents/guardians of BABY HUG participants will be offered enrollment of their child into the BABY HUG Follow-up Study, regardless of their original randomized treatment assignment. All children enrolled will be followed to a common termination date of December 31, 2011 or longer as funding permits. This plan will potentially provide approximately 2 to 6 years of follow-up after the

original randomized treatment on all children. Although all parents/guardians will be offered treatment with open-label hydroxyurea for their child after completing the BABY HUG randomized trial, participation in the follow-up study will not be contingent upon their subsequent treatment choice.

In the BABY HUG Follow-up Study, parents/guardians will be asked to consent to periodic reporting of clinically obtained information on their child including growth parameters, blood test results, transcranial Doppler (TCD) or other clinically obtained routine "screening" studies, and details of sickle cell disease related hospitalizations and health events. Collection and ongoing evaluation of growth and clinical data are essential to the determination of long-term effects of hydroxyurea. Blood cells, serum, and urine will be collected at follow-up study entry and on a second occasion four years later or at study exit (4 years/exit) whichever comes first. This will provide samples for surrogate markers of toxicity and clinical efficacy, such as measures of renal and spleen function and markers of DNA damage (see Appendix A). The stored blood sample will be separated, aliquots made and stored for future studies. An application for storage of biospecimens will be made to BioLINCC (www.biolincc.nhlbi.nih.gov). If storage of specimens is approved, they will be available for future studies with links to the clinical database of the BABY HUG Treatment Study. It is anticipated that essentially all BABY HUG families will agree to this "passive" follow-up plan. Personal identifying information will be removed from the database. Studies may be conducted by BABY HUG investigators and other researchers investigating sickle cell disease and related disorders.

In addition, parents/guardians will be invited to participate in one optional "active" reassessment two years after exit from the BABY HUG Treatment Study. At that time, age-appropriate neuropsychological testing, abdominal ultrasonography, and a radionuclide liver-spleen scan will be done. In addition, a blood sample for fetal hemoglobin, Howell Jolly Bodies (HJB), pitted cells, Cystatin C, and HPLC creatinine (see Appendix A) will be collected. Testing will be conducted at central laboratories. There will also be an additional neuropsychological test four

years after exit from the BABY HUG Treatment Study. These studies will allow simultaneous assessment of unexpected nephrotoxicity or splenic enlargement and possible prolonged protection from organ dysfunction.

Data collected in this follow-up study will be descriptively analyzed according to the original treatment assignment (HU versus placebo), as well as the subsequent independent decision by families concerning use of open-label HU in the follow-up period. Data collected in the passive follow-up portion will determine whether early hydroxyurea treatment is associated with long-term toxicities and provide limited data regarding long-term efficacy. Data collected in the active follow-up portion will identify long-term effects on organ dysfunction, and determine if duration of treatment and age of initiation (early vs. late) affect hydroxyurea's efficacy and toxicity. Information obtained from this follow-up study is vitally important to understanding the risks and benefits of early treatment, and ultimately for creation of an optimal paradigm for hydroxyurea therapy in young children with sickle cell anemia.

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CHAPTER 2

OBJECTIVES AND DESIGN OF THE STUDY

2.1 INTRODUCTION

The purpose of this observational study is to perform long-term clinical follow-up of children enrolled in the original Pediatric Hydroxyurea Phase III (BABY HUG) Clinical Trial, after they have completed their period of randomized study treatment. This long-term follow-up study was envisioned at the conception of the BABY HUG Treatment Study, and parents/guardians as well as local Institutional Review Boards (IRB) were made aware of this intent in the consent forms obtained at the BABY HUG Treatment Study enrollment. The overall goal of the BABY HUG Follow-up Study is to define more accurately the long-term risks and benefits of early HU treatment. All of this information is needed to determine the optimal use of HU in very young children with sickle cell anemia (SCA).

Long-term follow-up of BABY HUG participants treated with HU is critical for several reasons. First, certain HU toxicities identified during the BABY HUG Treatment Study may persist during the follow-up period, while other toxicities may decline in incidence or severity, and still other new toxicities may arise. The profile of these events may also be different in children starting HU treatment at 9 to 18 months of age (in the randomized treatment study) as opposed to children starting treatment at 33 months of age or later (by parent or guardian choice during the open-label follow-up study). Serious toxicities, particularly those concerning growth and development, could become evident only years after initial HU exposure and treatment. Children not treated with HU during the randomized treatment study, or at all represent an important comparison group and should be followed in the same manner.

Previous studies have provided limited assessment of late toxicities associated with HU therapy in a small number of young children with SCA. In the HUSOFT extension study (Wang et

al, 2001; Hankins et al, 2005), 17 infants (age 7 to 25 months) completed four years of treatment with HU and eleven completed six years. Hoppe (Hoppe et al, 2000) described eight children with a mean age of 3.7 years (range 2 to 5 years) treated with HU for an average of 2.6 years. While no unique adverse side effects and no apparent growth toxicities were reported in these two small series involving open-label HU treatment, longer follow-up on larger groups is required to support evidence-based decision making. Long-term follow-up of the 200 children enrolled in the BABY HUG Treatment Study will provide a critical and unparalleled opportunity to evaluate late toxicities associated with early HU exposure in a large number of well-characterized very young children with SCA and to specifically assess the potential spectrum and severity of growth, development, hematological and organ toxicities experienced. Ongoing concern about long-term effects on growth and development, as well as data regarding the possible mutagenicity/carcinogenicity of HU, can only be addressed in this long-term follow-up study.

Secondly, in conducting this follow-up study, it is important to document any long-term clinical and laboratory effects associated with HU therapy in this young age group. Clearly toxicities must be identified, but benefits identified during the BABY HUG Treatment Study may persist during the follow-up period, others may decline in magnitude, and still other new benefits may arise. Hematological effectiveness of HU therapy in terms of maintaining elevated levels of HbF was shown in HUSOFT, but confirmation of this benefit and its persistence with long-term treatment in a larger group of placebo-controlled children is needed. While it would be ideal to prospectively assess the beneficial effects of HU therapy in preventing or ameliorating chronic organ damage with "gold-standard" testing at several times over many years, simple less invasive measures of organ damage and function are likely to be validated as useful surrogates in the BABY HUG Treatment Study. Accordingly, we will collect blood and urine samples at study entry and 4 years/exit (which ever comes first) for all children enrolled in the BABY HUG Follow-up Study, for surrogate measure (pitted red blood cell [pit count], quantitative Howell Jolly Bodies [HJB], glomerular filtration rate [GFR] estimation from the Schwartz equations, Cystatin C, and urine microalbumin: creatinine ratio

[microalbuminuria]) assessment, VDJ recombination event assessment of genotoxicity, and storage. In addition, all children will be offered an optional active re-evaluation two years after the end of randomized treatment to include studies with more direct organ measurement (neuropsychological testing, abdominal ultrasonography, liver-spleen scan and GFR estimates) and one additional neuropsychological test at four years after the end of randomized treatment. Only with surrogate and direct measures of toxicity and organ function can we establish appropriate HU treatment paradigms for young children with SCA.

In addition, clinical benefits from long-term HU treatment, such as a decrease in vaso-occlusive pain crises or episodes of acute chest syndrome, may be observed during this follow-up study. The HUSOFT follow-up study (Hankins et al 2005) suggested that HU resulted in a decrease in the frequency of acute chest syndrome (ACS) events, but was limited by the small number of children. Follow-up of a group of 200 infants for 2 to 6 additional years, when clinical events are far more common than in early infancy, will allow determination of the role of HU in ameliorating the clinical complications of SCA in young children and provide insight into the risks and value of early initiation of such therapy.

Attempts to identify early clinical and laboratory characteristics that predict major complications and organ injury later in life have been only modestly successful. However, markers (early dactylitis, severe anemia, and leukocytosis) identified by the Cooperative Study of Sickle Cell Disease (CSSCD) to increase the risk of death, stroke, and recurrent pain and acute chest syndrome later in life are parameters likely to be impacted by the early use of hydroxyurea. Based on historical analyses, one might anticipate 20-30 deaths and 10 to 20 strokes in a cohort of 200 infants followed through the first two decades. (Miller et al, 2000; Quinn et al, 2004) At the end of this follow-up study we may begin to see a difference in natural history based on treatment with HU. Although long-term outcome may be somewhat obscured by the use of HU at the end of the blinded study, any discernible differences on modification of these late major sickle cell-related problems by

early, ever or never therapy with hydroxyurea would be of great significance to subsequently born subjects and their families.

The determination of the overall risk-to-benefit ratio for treatment with HU in this unique group of young children is likely to be complex. Regardless of the outcome of the BABY HUG Treatment Study, the safety and efficacy of HU treatment in SCA cannot be defined without collection of clinical and at least limited long-term laboratory follow-up data. Treatment recommendations for primary and secondary prevention based on observational data and expert opinion can be notoriously incorrect (Manson et al, 2003; Wasserthiel-Smoller et al, 2003). A modest treatment effect in the BABY HUG Treatment Study may become more apparent and compelling during long-term follow-up. Alternatively, the BABY HUG Treatment Study may provide evidence of benefit in preventing chronic organ damage in children with SCA, but long-term follow-up may demonstrate that the toxicity associated with HU treatment and/or time-limited organ protection (postponement rather than prevention of injury) make its use unwarranted. BABY HUG cannot clarify the role of HU in the treatment of very young children with SCA until the long-term risks and benefits are studied and better understood.

In summary, determination of the long-term risks and benefits of treatment with HU in infants and toddlers with SCA is absolutely essential to perform at this time. The participants in the BABY HUG Treatment Study represent a unique and “never-to-be-repeated” resource from which to address these issues. The BABY HUG Follow-up Study will allow more accurate characterization of the role and optimal timing of treatment with HU in young children with SCA. Follow-up of all participants for as long as possible is proposed. Initially, all children will be followed for at least two years to a common termination point of December 31, 2011 for clinical events and routine clinically obtained laboratory values. Ultimately, even longer-term follow-up will be proposed to allow a more complete assessment of the risks and benefits of early treatment with HU for children with SCA.

2.2 SPECIFIC AIMS

The specific aims of this follow-up study are:

1. To identify and define possible long-term toxicities in children who receive treatment with HU.
2. To determine if prolonged treatment with HU changes the risk and benefits of its use.
3. To investigate the optimal age for initiation of treatment with HU (early vs. late).

2.3 DESIGN OF THE STUDY

After completion of active participation in the BABY HUG Treatment Study, each family will be asked to consent to have their child participate in this long-term follow-up study. If applicable, their child's retrospective data will be collected beginning with the exit visit from the BABY HUG Treatment Study. Follow-up of these children will continue to a common termination point on December 31, 2011, and beyond if further funding permits. Depending on the parents/guardians choice of "passive" or "active" follow-up, we will collect some or all of the following parameters that may not be part of routine clinical care: fetal hemoglobin (Hb F); pitted cell counts; HJB; liver-spleen scan, abdominal ultrasound; estimation of the GFR from the Schwartz equations and Cystatin C measurements; microalbuminuria; and neuropsychological testing. The plan for the performance of these tests is shown in Appendix A.

All children enrolled in the BABY HUG Treatment Study who participated for at least 18 months are eligible for the follow-up study regardless of their initial treatment assignment, their current treatment status, their prescribed dose and perceived compliance, or the follow-up group (passive versus active) selected. At the completion of the BABY HUG treatment study, all families will be offered the option to place their child on open-label HU, based on discussions with their local physicians and other health care providers. This decision will be made without knowledge of the primary study results or an individual child's treatment assignment, and will not influence the opportunity to participate in this follow-up observational study. Based on the initial 40 child pilot sub-study, approximately 2/3's of the children/families exiting the BABY HUG Treatment Study will choose open-label HU treatment.

Although the BABY HUG Follow-up Study is intended to monitor the longer-term risks of HU therapy, it will also test the hypothesis of an early versus late effect of starting HU. Experience from the MSH study (Steinberg et al. 2003) suggests that important covariates to be studied in the BABY HUG Follow-up Study include the unrandomized comparisons of ***ever*** used HU versus ***never*** used HU, and a time-dependent covariate indicating whether a child is "on" or "off" HU treatment during follow-up. The assessment of this last variable will require careful collection of HU treatment information over the course of the follow-up study and enumeration of significant clinical adverse events. We believe that these data can be collected by the current dedicated study personnel at each Clinical Center, using semi-annual review of the child's medical record and structured reporting forms. It is likely that children in the BABY HUG Treatment Study will continue to be medically followed at their Clinical Center by physicians and nurses knowledgeable about and dedicated to the goals of the treatment study, making the probability of long-term local data submission feasible Since continued HU treatment will not be randomized, it will not be possible to itemize all of the treatment and response trajectories that may occur in this study and statistically assess each possibility. Instead, the follow-up study statistical design will have a generalized and comprehensive analytical plan that will allow investigators to detect changes in these trajectories and associations as the follow-up study continues.

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CHAPTER 3

CHILD ELIGIBILITY, RECRUITMENT, ORIENTATION, AND INFORMED CONSENT

3.1 INTRODUCTION

All children who have completed at least 18 months of the BABY HUG Treatment Study will be eligible for this follow-up study. A new informed consent from parents or guardians will be required for participation. When families consented to enrollment in the BABY HUG Treatment Study, parents/guardians were made aware that the investigators wished to maintain contact with the child after the treatment study ended, and because most children were clinically followed in the same Clinical Center where they participated in the BABY HUG Treatment Study, this was felt to be realistic. Information about the concept of a follow-up study was repeated during the course of the study and consent will be requested at the child's 24-month (exit) visit from the treatment study. During the follow-up study, passive data collection with retrospective data abstraction (including information from other health providers when necessary) will be carried out for all children whose parents/guardians give consent to be in the follow-up study. In addition, blood cells, serum, and urine will be collected for measurement of important surrogate markers of efficacy and toxicity at follow-up study entry and 4 years/exit. Some of the optional "active" assessment studies are more than minimal risk, but will allow ongoing evaluation of organ function both for absence of new toxicities as well as potential for preservation of function that would usually be lost by children with sickle cell disease in early childhood. These additional studies will objectively assess the developmental safety of early HU use, and provide information about the duration of BABY HUG Treatment Study primary organ endpoints.

3.2 FAMILY ORIENTATION

At the end of the BABY HUG Treatment Study all children will be offered treatment with open-label HU by their child's physician. Parents/guardians will make this decision without knowing

their child's randomized BABY HUG treatment study assignment. Given the staggered exit of children from the treatment study, it is not practical to inform families of the treatment assignment, and to do so could result in potential unmasking of the primary study.

The rationale and the importance of the follow-up study will be presented and explained to the parents/guardians. They will be given a list of the passive follow-up data to be collected and the active assessment evaluations and tests that would be performed (see Appendix A). They will be advised that they are required to sign a new consent form in order to participate in the follow-up study. Open-label HU treatment will be managed according to local clinical care standards. Information about HU treatment will be collected on follow-up study forms. Parents/guardians will be advised that the follow-up study will not be paying for open-label HU treatment.

Families may continue to participate in the study even if they move out of the area of the Clinical Center. They will be encouraged to notify the coordinator in advance of the move so that plans for passive data collection in their new location can be developed.

3.3 INFORMED CONSENT

Individual Clinical Center consent forms will be prepared based on the model informed consent form presented below. The model consent form will be approved by the Observational Safety Monitoring Board (OSMB) prior to its release to the Clinical Centers for submission to the local IRBs. Each final consent form will be reviewed by Medical Coordinating Center (MCC) staff, NHLBI Program staff and a member of the OSMB, to ensure all required elements of the consent form have been addressed.

The Clinical Center Principal Investigator (PI) will attempt to obtain consent from each family in the BABY HUG Treatment Study by contacting the parent/guardians at the end of the treatment study. The family will be given adequate time and privacy to review the consent form. They will have the opportunity to have all of their questions and concerns addressed by the PI. An ombudsman, required for the BABY HUG Treatment Study consent process, may be present but is

not required. A copy of the signed consent form will be given to the parent/guardians and placed in the child's medical record. The original will be maintained in study files by the Principal Investigator.

INFORMED CONSENT TEMPLATE

PURPOSE, PROCEDURES AND LENGTH OF STUDY

We are asking you to agree to your child's participation in the BABY HUG Follow-Up Study. This study in 14 Clinical Centers will follow the nearly 200 children enrolled in the BABY HUG Treatment Study to learn about the long-term effects of study treatment. This study is sponsored by the National Institutes of Health: National Heart, Lung, and Blood Institute (NIH-NHLBI). If you agree to allow your child to **enroll in the BABY HUG Follow-up Study, we will collect medical information on your child until at least December 31, 2011.**

The BABY HUG Treatment Study was designed to see if treatment with the drug hydroxyurea (also called HU) in children with sickle cell anemia could prevent organ damage, especially in the spleen and kidneys. There was also a chance that treatment could prevent painful crises, lung disease, stroke, and blood infection. **When your child exits (exited) BABY HUG, you and your child's doctor will decide (decided) whether or not to give HU from the pharmacy.** ***The main goal of the follow-up study is to study the long-term safety of HU.*** If treatment with HU provides a benefit or if a safety problem is discovered, it will be important to determine if the age that HU was started is important. Follow-up of your child will provide us important information about whether HU treatment should be given to infants and if so, the best age to begin treatment. **We would like your child to join the follow-up study whether or not you choose for him or her to take HU from the pharmacy now or ever.**

You may choose to let your child participate in the BABY HUG Follow-up **Study in one of two ways**. **The first is "passive" follow-up.** As your child is seen for clinical care of sickle cell disease, we will **collect information from routine tests ordered by your child's clinical sickle cell doctors**. These tests usually include a complete blood count and measurement of your child's height, weight and head circumference at each visit. At least twice a year we will also collect information about illnesses that your child has had since the last visit and how they were medically

treated. We will also record the results of TCD (transcranial Doppler or brain ultrasound) tests, imaging by CT or MRI and clinical consultations if ordered by your child's doctor.

We will collect **two teaspoons of blood and a urine sample from your child at the beginning and end (or after four years on study, whichever comes first) of this follow-up study**. These samples will be used to assess spleen and kidney function as well as changes in your child's genetic material or DNA. **The blood samples will be stored for use as new laboratory evaluations become available for future research on sickle cell disease and related disorders. We will store these blood samples <u>forever</u>.**

The second way you may choose to have your child participate is known as "**active" follow-up.** The same blood and urine samples will be collected as outlined in the above paragraph. Your child's health information will be collected and submitted to the BABY HUG coordinating center at least twice per year as with passive follow-up. **In addition, you agree to allow at one time in two years one more blood sample, imaging studies, and behavioral testing as were done at the beginning and end of the BABY HUG Treatment Study. You also agree to allow an additional behavioral test at your child's four-year visit.**

If you choose to participate in either group, we will collect information from your child's medical record since he/she exited the BABY HUG Treatment Study.

Family notification of their child's BABY HUG treatment will be sent to the family on or about December 2009. *We will inform families about the BABY HUG Treatment Study results as the data are analyzed.* At the end of the follow-up study, your family will be informed of the results and any new recommendations from the study doctors.

<u>STUDY TESTS TO BE PERFORMED</u>

Passive Follow-Up Group

We will collect **information from your child's medical record including height, weight, and the results of physical examinations and laboratory tests performed as part of routine**

clinical care. If your child has had any major health problems, imaging studies, or medical consultations between clinic visits, including the usual complications of sickle cell disease and especially those that require hospitalization, we will collect and submit that information. If your child has already completed the BABY HUG Treatment Study before the time that you consent to join this follow-up study, we will collect medical information back to the date he or she exited the treatment study. If TCD testing has been ordered clinically by your child's doctor, we will review the images and results of that testing. In the follow-up study, all data collection will end on December 31, 2011.

The information collected from testing your child's blood samples will be added to the BABY HUG Treatment Study data file for analysis. The laboratory tests performed as part of the follow-up study are not diagnostic tests for clinical disease. The results will be used as part of a large group analysis without identifying your child. **You will not receive the research results from your child's individual sample.** Knowledge of the test results will not change your child's current medical care.

If your family moves from this area, the study coordinator will contact you by telephone or in writing about every 6 months to obtain information about your child's growth and development and clinical events. We will request any information relevant to the follow-up study regarding your child's health from other health care providers. We will ask you to sign a release for us to obtain that information.

Active Follow-up Group

If you agree to have your child in the active follow-up group, **the same clinical information, blood and urine specimens as in the passive follow-up group will be collected, but there will be additional blood and urine tests, as well as tests to check the function of your child's brain, spleen and kidneys. These additional tests will be performed only once, approximately two years from now. Each of these tests may require a separate visit.**

There will also be a behavioral test, called the Vineland Adaptive Behavior Scale, performed at the four-year visit. A test requiring a small dose of radiation to evaluate the function of the spleen will be performed in exactly the same way it was done in the BABY HUG Treatment Study. For this test, small doses of radioactive material will be given through your child's vein. We will use a camera sensitive to radioactivity to take pictures of your child's spleen. The radioactive material will leave your child's body in urine or stool by the next day. In order to monitor your child's brain growth and development, neuropsychological testing will be performed. Your child will also have an ultrasound (sound wave) imaging test of spleen and liver size. These are the same tests that were performed twice in the BABY HUG Treatment Study. An extra blood sample will be collected to assess spleen and kidney function.

RISKS

The needle used to take blood may cause a sharp pain as it goes into the skin. Sometimes a bruise will form at the place the needle goes into the skin.

If your child is enrolled in the active follow-up group, he or she will have one medical imaging study. The **liver-spleen scan test requires that an IV be started in your child's vein and that a small amount of radiation** (like the amounts that people encounter naturally in daily life from space) is injected into your child's body. Like the blood draw, the risks of the needle stick for the IV are pain and bruising. Your child will receive about the same amount of radiation as he or she would get from living in our natural surroundings for about nine days. The radiation dose is what your child will receive from the follow-up study only and does not include any exposure he or she may have received or will receive from other tests.

BENEFITS

At this time no one knows whether HU will help your child. The results of this follow-up study, along with those from the BABY HUG Treatment Study, will help doctors decide in the future if and when to give HU to young children with sickle-cell anemia and how long to give it.

FREE CHOICE

Your child's participation in the follow-up study is up to you. You are free to take your child out of this study at any time. You are free to start or stop HU from the pharmacy, after talking with your child's doctor at any time. You are free to choose to have your child's information included in the data file, decide if your child's blood sample can be saved indefinitely, and to choose which active follow-up tests your child should have. **You are free to change your mind about the active follow-up tests you will allow your child to have up until the tests are performed.** If you take your child out of the study or do not take part in the study, we would still like to provide medical care for your child. That is, you may choose not to be part of the follow-up study, not allow us to collect information from that care, and just allow your child to receive standard care for sickle cell disease in this hospital and clinic. You and your child's doctor may plan to use or not use hydroxyurea. Other treatments may be possible for your child. These treatments include blood transfusions and stem cell or bone marrow transplantation. Your choice to continue in the follow-up study will not change the way your child is treated in this hospital or the treatments available to your child. In or outside of the study, we want to give the best care for your child.

COSTS

All costs that are considered part of routine clinical care will not be paid by the study. Routine care will be billed, as before, to you and/or your health insurance provider. **Costs related to the blood and urine samples obtained just for this study (at entry and exit), and procedures performed in the active follow-up group, will be paid for by the study. If you**

decide to have your child take hydroxyurea, the costs of the medication and the laboratory studies to monitor its effects will be billed to you and/or your health insurance provider.

PAYMENT

If you chose to allow your child to participate in the active follow-up group you will receive $100 for the extra clinic visits required. Payment will be made when you arrive at the clinic for the start of the active group tests. This is to cover your costs of parking, travel, meals and other expenses to get your child to the clinic for these extra tests. Payment will not be given for your child's regular clinic visits.

PRIVACY

Your child has a right to privacy. All information in this study that can single out your child or your family will remain private. A numbering system that does not allow anyone outside this Clinical Center to know your child's name will be used to identify your child. Your child will not be named in reports of results from this study. Your child's medical reports and family data will be kept private.

CONSENT FOR A DATA FILE

At the end of the follow-up study, a computer file of the study results will be made for future use by researchers studying sickle cell and related diseases. The information collected from your child's participation in the follow-up study, all testing done during the study, and the saved blood samples will be linked to the child's participant number in the follow-up study data file. This data file will not have your child's name, your name or any facts that could be linked to your child or family directly. The computer file may be used by other doctors to study sickle cell anemia or related disorders. Data may be given to the National Institutes of Health, the Food and Drug Administration (FDA) or other U.S. or state agency as required.

I ☐ agree ☐ do not agree _____ for the data file to include my child's information.
Initials

CONSENT FOR STORED BLOOD SAMPLES

These are the blood samples taken at study entry and exit.

I ☐ agree ☐ do not agree _____ Initials to the saving of my child's blood samples indefinitely.

I ☐ agree ☐ do not agree _____ Initials to the use of my child's blood samples for future research on sickle cell disease and related disorders.

CONSENT

I agree for my child to participate in the ☐ _____ Initials passive follow-up group.

OR I agree for my child to participate in the ☐ _____ Initials active follow-up group.

LIMITATIONS

The <<insert Clinical Center name>> will not provide compensation for children who may incur injuries as a result of being in this research except as is required by law. **This means that while all study doctors will do everything possible to provide careful medical care and safeguards in the conduct of this research, the medical center will not offer to pay for injury resulting solely from the research itself. The study sponsor, The National Heart, Lung, and Blood Institute, does not offer financial compensation or payment if you are injured as a result of participating in this research study.**

You can discuss the rights of children who participate in research with the Chairman of the Medical Center's Institutional Review Board, telephone number ( ). This board is composed of doctors and lay people who have reviewed and approved this study. Dr. _____, Principal Investigator of this study, is also willing to talk about any of your concerns about the study at telephone number ( ).

COPY OF CONSENT

If you agree to have your child take part in this research study, you will receive a signed copy of this consent form.

FOR SUBJECT FAMILIES WHO HAVE ALREADY SIGNED A CONSENT AND HAVE ENROLLED THEIR CHILD INTO THE ACTIVE GROUP BUT HAVE NOT HAD THE 24 MONTH VISIT YET

The only change to the BABY HUG Follow-up Study for your child is that the 24 month special studies visit will no longer include a DTPA/GFR test and an additional neuropsychology test, called the Vineland Adaptive Behavior Scale, will be done at the four year visit.

PARENT, INVESTIGATOR AND WITNESS SIGNATURES

I have read all of the consent form. I have been given a chance to ask questions and have received answers about areas I did not understand. I willingly give my consent for my child to join this study.

I understand that I may withdraw my child from the study at any time. In doing this, I will, in no way, change my child's ongoing medical care at this medical center or elsewhere.

_____	_____	_____	_____
Child's name	Signature of parent or legal guardian	(Date)	(Time)
	_____	_____	_____
	Signature of parent or legal guardian	(Date)	(Time)
_____	_____	_____	_____
Investigator's name	Signature of Investigator	(Date)	(Time)
_____	_____	_____	_____
Witness' name	Signature of Witness	(Date)	(Time)

PEDIATRIC PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 4

STUDY ENDPOINTS

4.1 INTRODUCTION

The primary objective of the follow-up study is to monitor the continued safety of HU treatment. Safety of HU will be assessed by ongoing clinical monitoring of growth and development, age-appropriate neuropsychological evaluation, serial hematologic parameters, and the frequency of expected and unexpected clinical events related to sickle cell disease. For this reason follow-up should continue as long as practically possible. Disease and treatment-related effects on the spleen will be measured by a liver-spleen scan, pitted cell counts and HJB enumeration. With the deletion of the DTPA/GFR, kidney function will now be measured by GFR estimation from the Schwartz formulae or Cystatin C measurements and the urine microalbumin:creatinine ratio. These evaluations will also allow us to monitor the effectiveness of ongoing HU therapy. We will also seek to study how early treatment with HU changes the child's disease trajectory as open-label HU becomes available after the child's participation in the BABY HUG Treatment Study.

4.2 FOLLOW-UP ENDPOINTS

4.2.1 Growth, Development, and Education

Height, weight, physical examination parameters (for example the presence of a palpable spleen or liver) are routinely assessed at clinical care visits which usually occur every 3-4 months through the first five years of life and every 6-12 months at older ages in children with sickle cell anemia. Children will be carefully monitored for height or weight percentile and compared to standardized growth curves. In particular, the growth curves of children ever exposed will be further divided into those who have taken HU both in the treatment study and follow-up study versus those treated with HU only in the follow-up study and compared to those never on HU. (HU children in the

trial whose parents elect not to have their child take HU in the follow-up study will be excluded from this analysis.)

Careful evaluation of the BABY HUG participants through young adulthood for growth, school performance, development of pubertal characteristics (menarche and Tanner Score), and fertility is beyond the scope of resources currently available to this follow-up study, but is highly desirable. During the period demarcated for the follow-up study, the first children may complete the second grade, although the majority will not yet have entered formal education. We will collect self-reported data about school placement (special education placement and/or repeated grades vs. those continuing without such assistance) and the presence or absence of parental concern about language development and behavior.

4.2.2 Hematologic and Clinical Events

Complete blood counts (CBC) will be collected for all children at their regular clinic visits in accordance with standard clinical practice. Creatinine and hepatic transaminase values will be routinely recorded for children on hydroxyurea. The investigators will continue to compare children on and off of treatment with HU to assess the effect of HU treatment on the absolute neutrophil count, white blood cell count, mean cell volume, reticulocyte count and platelet count.

For any children having serious clinical events, these events will be recorded including, but not limited to, stroke, splenic sequestration events (and presence of a palpable spleen), transfusions, and hospitalizations. This information will be collected by structured, retrospective review of the medical record at the semi- and annual reporting periods.

4.2.3 Central Nervous System

Neuropsychological evaluation with the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and the Vineland Adaptive Behavior Scale tests will be performed once each, with the other active assessments, at two years and four years (respectively) after enrollment in the BABY HUG Follow-Up Study. Results will be analyzed with the previously collected Bayley and

Vineland tests in the BABY HUG Treatment Study to carryout an analysis of covariance. Longitudinal profiles will not be analyzed because the neuropsychological scales must be changed as the child ages, which will prevent the creation of a neuropsychological trajectory based on a common measurement.

4.2.4 Spleen

The liver-spleen scan will be performed on subjects in the active follow-up group two years after completion of the treatment study. The results of this scan will be compared to the two scans performed at initial screening and exit from the BABY HUG Treatment Study. Thus, pre-treatment, post-treatment and follow-up scans performed at time zero, two and four years respectively from entry in the treatment study will be available for review and comparison. The follow-up scan will be categorized by the same panel of nuclear medicine specialists who read the previous scans. Once again these reviewers will be masked to all treatments received by the subject and will not be involved in the acquisition of the images.

Children will be compared between groups for the continued presence of a spleen with sufficient function to take up the radionuclide. A functional spleen can be seen as both having potential for toxicity (the child remains at risk for splenic sequestration, a potentially fatal complication that children with sickle cell anemia usually do not develop after 3-5 years of age) as well as demonstration of HU's ability to preserve organ function.

Pit counts and HJB quantitation will be performed once for all subjects, four years after exit, which is approximately six years after enrollment in the BABY HUG Treatment Study. Children who agree to active follow-up will have an additional pit count and HJB measurement at the time of the other active organ assessments. The results of the pit counts will be compared with those serially performed during the BABY HUG Treatment Study. This will provide an assessment of changes that have occurred over the entire period of study. The results for the HJB will be compared with those performed at screening and completion of the BABY HUG Treatment Study. This will provide an

assessment of serial changes in surrogate measures of spleen function. The non-invasive markers of spleen function will be compared to the results of the liver-spleen scans (when available) to continue to assess their reliability in providing the same information about ongoing risk from a functional spleen as the radionuclide scan.

4.2.5 Kidney

Central laboratory measurement of serum creatinine will be performed in order to estimate the GFR according to the Schwartz formula, GFR=kL/Pcr, where L = the body length in centimeters, Pcr = the serum creatinine concentration in mg/dl, and k = 0.55 mg creatinine/100 min x cm x 1.73 m^2. Blood Urea Nitrogen (BUN) and Cystatin C will also be collected to obtain a recently reported Schwartz GFR. The newly developed Schwartz formula is: GFR(ml/min per 1.73 m(2))=39.1[height (m)/Scr (mg/dl)](0.516) x [1.8/cystatin C (mg/L)](0.294)[30/BUN (mg/dl)](0.169)[1.099](male)[height (m)/1.4](0.188).[1]

Measurement of serum Cystatin C will also be used to estimate the GFR. Cystatin C measurement was added to the BABY HUG Treatment Study in October 2006 due to its apparent superiority over creatinine-based estimates of GFR (Alvarez et al, 2006). This may be especially true in individuals with sickle cell anemia due to their substantial tubular secretion of creatinine. This measurement is being obtained, to the extent possible, on residual samples of serum stored from creatinine measurement done with entry and exit DTPA scans early in the treatment study. It was performed on all children entering or exiting the treatment study after this change in protocol. Measurement of Cystatin C (by blood sample) will continue to be performed by the Clinical Chemistry Laboratory at St. Jude Children's Research Hospital and will be performed at entry into this study (if not done at exit from the treatment study) and four years after exit from the treatment study. Subjects in the active follow-up group will have an additional determination, two years after follow-up study entry, coincident with other organ function assessments. For most of the children who will enter the follow-up study immediately after exiting the treatment study, this measurement

will be available for comparison to the Cystatin C value collected in the follow-up study as well as the serum creatinine measurement. These two methods will be compared for their prediction of GFR for HU treatment groups, based on early vs. later or ever use of HU. As discussed in the BABY HUG Treatment Study protocol, the development of hyperfiltration is one of the earliest lesions in sickle cell nephropathy. Evaluation of renal function, particularly raw creatinine and/or Cystatin C values, will also allow assessment of any unexpected detrimental effects of HU therapy.

Microalbuminuria, assessed by the urinary microalbumin to creatinine ratio, may also be regarded as an early sign of sickle nephropathy (Darnidharka 1998). This ratio will be measured in all children at entry to this study and at exit from this study. Assessment of this parameter will provide additional information about the toxic or beneficial effects of HU on kidney function in young children with sickle cell anemia. For example, even if HU therapy prevents the rise in GFR of hyperfiltration, it may make other aspects of renal function, such as microalbuminuria, worse. Again this assessment will allow determination of the true spectrum of toxicity or benefit conveyed with early use of HU.

4.2.6 Fetal Hemoglobin

Maintenance of an elevated fetal hemoglobin with hydroxyurea therapy is one of the secondary endpoints of the BABY HUG Treatment Study. Fetal hemoglobin will be measured once in conjunction with the active follow-up assessment two years after entry into the follow-up study. The levels of fetal hemoglobin observed will be compared to the serial values available from the BABY HUG Treatment Study data to evaluate the persistence of a beneficial effect from hydroxyurea.

4.2.7 Stored Blood Samples

We will collect, separate and store components from a five milliliter blood sample from all follow-up study participants at entry into the study and at four years or exit. These samples will be linked to the treatment study and follow-up study datasets and will be available to researchers for

future studies of sickle cell and related diseases. It is likely that in the future, as with the addition of the Cystatin C and urinary microalbumin:creatinine assessments, novel investigations for toxicities or organ function effects of HU will be proposed. These samples will also be used and stored indefinitely. Storage of samples with known periods of exposure to HU will be an invaluable resource for these investigations. To this end, Investigators have agreed that saved serum samples will be shipped to a central repository at the NHLBI.

4.3 STATISTICAL ANALYSES OVERVIEW

The BABY HUG MCC staff will continue to assess the number of non-functioning spleens, according to assigned treatment, four years after the children were enrolled in the treatment study. The alpha level for the spleen test will be set at 0.05.

A review of the other endpoints and treatment variables to be analyzed shows three different treatment-variable constructs, and four classes of endpoint analyses, to be performed in the BABY HUG Follow-up Study. As mentioned in Chapter 2, the primary independent variables to be studied in the BABY HUG Follow-up Study are: 1) the BABY HUG Treatment Study treatment assignment, 2) a variable indicating whether a child has ever received HU (either as study treatment during the treatment study and/or open-label follow-up), and 3) a time-dependent covariate indicating HU treatment or no HU treatment over the BABY HUG follow-up interval. The last variable is not a variable per se but a treatment trajectory that will be assessed for its association with SCA-related symptoms and adverse events. The endpoint analyses to be considered in the follow-up study, consist of longitudinal data analyses of continuous endpoints (e.g., CBC results, fetal hemoglobin values, and quantitative spleen and renal evaluations), longitudinal analyses of qualitative evaluations (e.g., spleen activity as evaluated by two masked nuclear medicine physicians), time to event analyses (e.g., the time to the occurrence of adverse events), an analysis of covariance (the analysis of the four-year WPPSI score), and multivariate analyses e.g., (Vineland Adaptive Behavior Scale).

Because there is no hypothesized pattern of interest to be seen in the BABY HUG Follow-up Study, a generalized approach will be formulated that will allow the investigators to describe the changes that occur. This means that a large number of analyses will be performed in this study. The investigators will limit the number of spurious associations by setting the alpha level for determining statistical significance to 0.01 rather than the traditional 0.05 alpha level. This procedure is consistent with the statistical approach used to identify up-regulated genes in micro-array studies, where one seeks to make it "highly likely" that statistically significant results are real by adjusting alpha levels and powers (Benjamini et al., 1995). As an example, among a large group of independent statistical assessments for which 25% of the alternative hypotheses are true and 75% of the null hypotheses are true, setting alpha at 0.01 for each comparison and power to 0.9 for each comparison would mean that on average, 96% of the statistically significant hypotheses declared in the study would come from the 25% of tests where the alternative hypothesis is true. Setting alpha at 0.05 rather than 0.01 would reduce the number of positive predictions to 86%.

4.4 STATISTICAL CONSIDERATIONS IN DESIGN AND STUDY SIZE

4.4.1 Primary Treatment Comparisons

This follow-up study will have one primary endpoint analysis: comparison of the proportion of infants with decreased or absent spleen function.

The primary comparison of this outcome will be done according to the BABY HUG Treatment Study treatment assignment. In effect, this comparison will be a test of an early (BABY HUG subjects randomized to Hydroxyurea) versus late (BABY HUG patients randomized to placebo) onset of HU treatment since after the subject exits the BABY HUG Treatment Study, open-label HU treatment will be offered to all families of BABY HUG participants. We have presented graphical figures of power as a function of study size for the primary endpoint. There will be no interim monitoring plan proposed for the primary endpoint. The goal is to estimate treatment effects.

An interim monitoring plan will be constructed to monitor the appearance of adverse events and potential toxicities.

4.4.2 Spleen Endpoint

Study size calculations for the analysis involving the spleen endpoint (a proportion) are functions of the overall study size, the proportion of infants in each of the comparison groups, and the difference between the expected proportions of events in the different groups.

Let p_1 be the probability that the spleen is not functional four years after treatment initiation for children assigned to receive HU during the BABY HUG Treatment Study (early HU treatment) and p_2 be the probability that the spleen is not functional four years after treatment initiation for children assigned to receive placebo. The overall alpha level of this comparison will be alpha = 0.05 (two-tailed). We will test the hypothesis:

H_O: $p_2 - p_1 = 0$

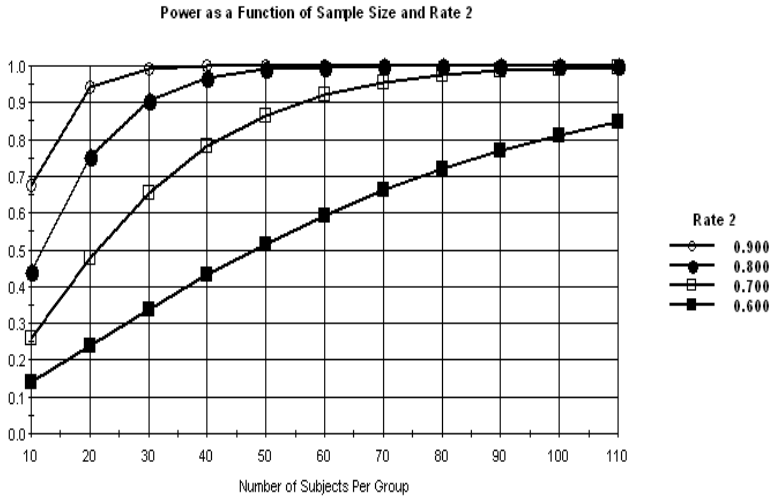
versus the alternative:

H_A: $p_2 - p_1 \neq 0$.

Figure 4.1 shows that the spleen failure rate in the late treatment (BABY HUG placebo [PBO]) group will have to be large (greater than 0.6) while the early treatment group event rate (BABY HUG HU treatment) is low (0.4) for the proposed study design to show with adequate power that early HU treatment is a superior organ-saving treatment. This is consistent with the BABY HUG Treatment Study goal of determining that HU cuts organ failure in half.

Figure 4.1

Power as a Function of Study Size and the Late (PBO) Event Rate (Rate 2)

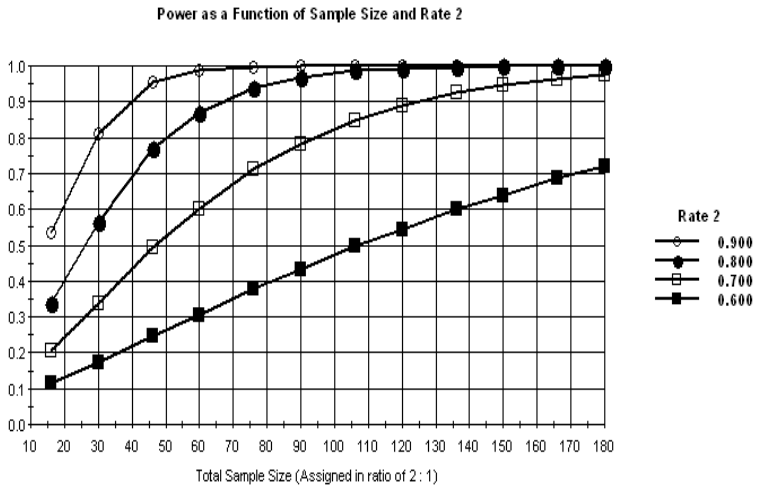


1:1 Allocation

Alpha = 0.050, Tails = 2, Rate 1 (Early HU) = 0.400

If the number of children enrolled in the follow-up study is unbalanced with respect to the BABY HUG treatment assignments for this hypothesis, Figure 4.2 can be used to address the hypothesis for the circumstance in which twice as many parents whose infants were treated with HU continue in the study as compared to placebo-treated children. The operating characteristic of the primary comparison with unequal allocation is similar to the equal allocation scenario so long as the imbalance does not exceed more than a 3 to 1 ratio.

Figure 4.2
Power as a Function of Study Size and the Late (PBO) Event Rate (Rate 2)



2:1 Allocation
Alpha = 0.050, Tails = 2, Rate 1 (Early HU) = 0.400

4.4.3 Data Analysis

4.4.3.1 Introduction

Primary analysis for the BABY HUG Follow-up Study will focus on estimating treatment effects on the designated endpoint: loss of spleen function. Assessment of a treatment difference will be based on pooling data across all participating Clinical Centers using all children entered. Secondary analyses will develop statistical models to determine associations and relationships between dependent variables, risk factors and the three treatment variables described earlier in this chapter.

Analysis of binary endpoints will be accomplished using contingency table analysis. Significance of results will be assessed with the Chi-square test uncorrected for continuity or Fisher's exact test. If necessary, contingency table analyses will be adjusted for confounding variables using logistic regression.

For continuous variables, comparisons of groups will be accomplished using Student's t test or the Wilcoxon rank sum test depending on the distributional properties of the data. Stratified designs will be analyzed using regression methods with the strata represented as randomized blocks for the analysis.

Analysis of continuous and categorical endpoints that are measured repeatedly over time, such as weight, height, and CBC measurements will use longitudinal data analysis models (Laird and Ware, 1982; Schlucter, 1992; Liang and Zeger, 1986). Estimation in these models can be in terms of point and interval estimates or trends (i.e., slopes of growth curves) over time.

The comparison between two treatments (or exposures) of time-to-event endpoints will be evaluated using the log-rank statistic. We will use the LIFETEST procedure in SAS to perform the test. The cumulative distributions of this outcome will be estimated using the methods of Kaplan and Meier (Kaplan and Meier, 1958). Multivariate adjustments to this comparison will also be made using the PHREG procedure for SAS to accomplish Cox proportional hazards models analyses (Cox, 1972).

Non-proportionality of the hazards will be investigated by plotting log[-log(S(t))], in which S(t) is the survival function, for important stratifying variables such as age and gender. Should the above functions be non-parallel (and/or cross) for any of the specified variables ($p < 0.01$), the subsequent analysis will be stratified by those variables. Cox proportional hazards models will be stratified for variables that demonstrate non-proportional hazards (crossing or non-crossing). Once determined, we will include these variables as stratification variables in the Cox regression. Analyses for the regressors will be summarized across the strata. Children who are lost to follow-up before the end of the follow-up interval will be censored at the time of their last visit. For events that can occur more than once in a child, the counting processes extension of the Cox model in SAS will be used to measure the relative impact of treating children with HU on these endpoints (Andersen and Gill, 1982).

4.4.3.2 Regression Analyses and Adjustment

We will adjust study results for potential confounding factors in secondary analyses. The addition of confounding variables generally improves the operating characteristics of analyses of the main effect, but given the small study size, the number of confounding variables to be considered in any secondary analysis will be small (limited to five or ten). We will use step-wise regression methods to isolate and include the most important confounding variables in the regression model.

Tests of interaction will be part of regression analyses. Most of these analyses will be designed to determine if BABY HUG Treatment Study treatment effects or other HU exposure effects are consistently observed across different clinical groups of subjects. If an interaction test is significant ($p < 0.01$), we will report that treatment effects differed according to the stratification dictated by the interaction test. Interaction tests such as these have low power to detect specified alternatives (e.g., half the efficiency of a main effects comparison). Additional analyses will be required to support the discovery of a proposed interaction, as a large number of interaction tests will be performed and some, by chance, will be found to be significant. One important analysis will be to incorporate variables that measure the duration of HU open-label treatment over the follow-up interval. This will be done by including variables that measure the duration of use for a cross-sectional analysis or time-dependent covariates for a longitudinal analysis.

We will use SAS procedures to perform adjusted analyses, PROC GLM to perform randomized block analysis of variance, PHGLM to perform stratified and standard Cox proportional hazards analyses, PROC GENMOD and MIXED to perform longitudinal data analyses, and PROC LOGISTIC to perform unconditional logistic regression. The standard output from these procedures provides point estimates for the regression coefficients, standard error estimates and confidence intervals. The results of these analyses are printed into computer files so that they can be directly inserted in progress reports using PROC REPORT. In some instances, the procedures in SAS will not suffice since SAS procedures usually do not include methods to incorporate information about

missing data, nor do they include complex models specifically designed to relate a biological process with the risk of disease progression. We will use PROC IML and PROC NLIN to program the required models if necessary.

4.4.3.3 Longitudinal Data Analysis

Many of the endpoints that will be collected and analyzed in the BABY HUG Follow-up Study will be longitudinal in nature with collection points at entry into the treatment study, two years after the child is enrolled into the treatment study (exit), two years after enrollment into the follow-up study (active group only), and four years or exit from the follow-up study (whichever comes first). Examples of such endpoints are: HJB, pit counts, GFR estimates, liver-spleen scan data, anthropometry, and neuropsychology testing. For each of these endpoints, we will present box plots of the measures according to the three different treatment indicators (BABY HUG Treatment Study assignment to HU, ever treated with HU (see page 4-1), and a time-dependent indicator of HU treatment) at each collection point. For the longitudinal analysis, we will use the baseline collection from the treatment study as an adjustment variable and then test the longitudinal trajectory for differences according to the treatment variable being studied and time-by-treatment interactions. Time trajectories will be analyzed using linear, quadratic and cubic terms. All data will be analyzed using generalized estimating equations (GEE) or mixed model analysis of variance; binary data will be analyzed in GEE using a logit link function and continuous data will be analyzed using the linear link function.

4.4.3.4 Missing Data Analysis

We will generally use the methods of Rubin (Little and Rubin, 1987) to impute missing data from children's records with complete data to complete the records for children with missing data. This method has been accepted by the FDA as a legitimate method for correcting for missing data. We will also use analyses involving rank statistics in which children who die or have bad clinical events are given the worst rank for other dependent variables. This technique has been used

successfully in the Multicenter Study of Hydroxyurea (McMahon et al, 1997). For categorical data or time to event data, the composite endpoint of death or the event (such as occurrence of acute chest syndrome) can be used.

4.4.4 Interim Monitoring

During the course of this study, the BABY HUG OSMB will review interim data analyses to monitor for the emergence of adverse treatment effects and the primary endpoint. No alpha will be spent on these primary endpoint reviews, i.e., the reviews will not be evaluated for early termination of the study. The interim OSMB analysis reports for the OSMB will include, but are not limited to comparisons by treatment group for:

1. Primary endpoint.
2. Child characteristics at baseline, completion of the BABY HUG Treatment Study, four years after randomization into the BABY HUG Treatment Study (two years on the follow-up study), and four years in the follow-up study or study exit.

   Spleen function

   Spleen size

   Pit counts

   HJB quantitation

   Schwartz equation GFR estimates

   Cystatin C GFR estimate

   Urine microalbumin:creatinine ratio

   Neuropsychological Testing Performance

   Height, weight, head circumference

   TCD measurements
3. Blood count toxicities
4. Dose adjustments

5. Safety assessments and clinical events
6. Serious Adverse Events
7. Distribution of baseline characteristics:

   Gender

   Age at Entry

The OSMB will also review Clinical Center study performance including completeness of follow-up, submission of forms and quality of laboratory data submitted, number of children lost to follow-up, and protocol violations.

4.4.5 Safety Related Outcomes

All clinical data will be collected retrospectively every six months. For children who agree to active follow-up, the results of the active assessment studies will be reported as soon as they are performed. Serious adverse event (SAE) reporting for the conditions listed in Table 4.1 will include only events that occur during the first five days following performance of the active assessment studies. Otherwise, a structured listing of clinical events will be submitted to the MCC and tabulated based on the affected organ system according to standardized monitoring procedures (e.g., MedDRA). These will be tabulated and a systematic review will be made to determine if one treatment group (or HU exposure type) has more reports of SAEs than the other treatment group (or HU exposure type). Depending on the evidence accumulated, it will be the responsibility of the OSMB Chair, the Executive Secretary of OSMB and the Project Officer to decide whether a full meeting of the OSMB is necessary to discuss the results and make recommendations, whether a conference call is necessary, or whether the report warrants no further action. Classification and reporting considerations are discussed in Section 9.2.4 of this protocol.

Ninety-five percent confidence intervals for the difference between proportions for the different treatment groups will be used to compare the occurrence of SAEs. If the confidence interval for the difference in these proportions does not cover zero, the Project Officer will be

notified promptly. The Executive Secretary of the OSMB and the Project Officer in consultation with the OSMB Chair will then recommend whether there should be an emergency meeting (or conference call of the OSMB) to determine the appropriate actions for this study.

Table 4.1

Definition of Serious Adverse Events

A **serious adverse event** is any one of the following.

1. Death
2. Life-threatening event
3. Prolonged hospitalization (greater than 7 days)
4. Splenic sequestration crisis
5. Stroke, TIA
6. Acute chest syndrome
7. ICU admissions

Serious Adverse Events that are SCD-related have been added to the list, as defined by the FDA. Item #3 has been modified from the FDA definition because frequent hospitalizations occur as a consequence of having sickle cell anemia without being enrolled in a study.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 5

OPEN-LABEL TREATMENT WITH HYDROXYUREA

5.1 OVERVIEW

Treatment with open-label HU during the follow-up study is at the discretion of the parent or guardian after consultation with the child's primary care physician. Regardless of the follow-up group in which the family chooses to have their child participate, all children on open-label HU will be followed according to local center hydroxyurea standards to monitor for toxicity. All blood tests performed for this purpose will be collected and processed locally, except for those noted as "core" measurements in Appendix A. For use of open-label HU, the dose, formulation, monitoring intervals, and toxicity levels stated below are guidelines only. Actual treatment is in accordance with routine local clinical care. Cost of treatment and monitoring will be borne by the family and their child's insurer.

5.2 DOSE TITRATION OF OPEN- LABEL HYDROXYUREA

The use of open-label HU will not be specifically regulated as was done in the BABY HUG Treatment Study. The dose of HU prescribed will be carefully recorded and reported semi-annually. Local criteria for dosing, dose escalation, and toxicity values will be used. The following guidelines for local adaptation are suggested:

- Children should begin open-label treatment with HU at the same dose as the dose of the BABY HUG Treatment Study drug which was 20 mg/kg..
- Dose escalation of 5 mg/kg every 6-8 weeks should be strongly considered if there is no toxicity to a maximum tolerated dose or 35 mg/kg.
- A CBC with differential white blood cell count and reticulocyte count should be monitored monthly while on HU.

- Predetermined toxicity levels should be utilized to monitor blood counts. The toxicity values should be no lower than an ANC<1250/mm^3, platelet count <80,000/ mm^3, a hemoglobin level below 6 gm/dl or greater than 20% fall in hemoglobin concentration from a three-month rolling average. Local clinical criteria with higher cut off points for declaring a toxic value may be used if desired.
- Dose should be reduced for severe or recurrent toxicities, with an attempt to re-escalate the dose if six months pass without subsequent toxicity.

5.3 MONITORING FOR TOXICITY

Children enrolled in the follow-up study within two months after completion of the BABY HUG Treatment Study must have all laboratory data from visits during this time reviewed only by the Primary Endpoint Person (PEP) and the MCC staff in order to ensure that masking accomplished in the treatment study is maintained. The PEP will determine if toxicities are present. The MCC staff will also screen local CBC reports for toxicities. If there are toxicities, the PEP will report them to the local Clinical Center staff. The Clinical Center will treat the child in accordance with routine local clinical care. Children who are enrolled in the follow-up study more than two months after completion of the BABY HUG Treatment Study will not require review of their initial laboratory data by the BABY HUG Treatment Study PEP or MCC staff.

All subsequent laboratory data as well as the labs done for each study visit will be reviewed and determination of toxicities will be made by the local Clinical Center BABY HUG PI and appropriate other clinical staff at each Clinical Center. The results will be reported retrospectively every six months by structured abstraction of the medical record. The PI, nurse coordinator and all clinical staff may view all of the laboratory data from each study visit.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 6

LABORATORY ANALYSES AND SPECIMENS

6.1 OVERVIEW

All clinical hematology specimens will be processed locally. The Hematology Core laboratory will process specimens for fetal hemoglobin, creatinine and BUN. The other core or central laboratories in the BABY HUG Follow-up Study will evaluate and report the appropriate levels: pit count, HJB, VDJ, Microalbumin:creatinine ratio-urine and Cystatin C as described in the BABY HUG Treatment Study Protocol and Manual of Operations. The TCD Core Laboratory will review a clinical TCD done about two years after the end of randomized treatment. The NHLBI Specimen Repository will be utilized for processing study specimens. The amount of blood and times of collection are specified in Appendix A.

6.2 PITTED CELL CORE LABORATORY

After four years or exit of follow-up, a pit count will be obtained using one drop of blood preserved in gluteraldehyde. An additional sample will be obtained for children in active follow-up at two years. Specimens will be stored and refrigerated locally, and shipped in batches to the Pitted Cell Core Laboratory at UT Southwestern-Children's Medical Center Dallas Clinical Laboratory. The technique for sample collection is described in the MOO for the BABY HUG Treatment Study and the BABY HUG Treatment Study Protocol.

6.3 CYSTATIN C CORE LABORATORY

Specimens for measurement of Cystatin C will be processed at the St. Jude Clinical Laboratory according to Section 9.11 of the BABY HUG Treatment Study Protocol.

6.4 VDJ/DNA MUTATION CORE LABORATORY

DNA will be isolated using a standard commercially available kit (Puregene DNA Isolation Kit, Gentra Systems Inc.). The purified DNA will be quantitated using a spectrophotometer and

used directly in the VDJ mutation assay. The overall goal of the VDJ studies is the investigation and quantification of the mutagenic and carcinogenic risks of HU therapy for very young children with SCD enrolled in the BABY HUG study, To accomplish this goal, we will analyze peripheral blood for the presence of changes in chromosomal integrity that indicate unrepaired genetic damage. The specific aims of the VDJ mutation study are: to quantitate the frequency of "illegitimate" VDJ recombination events that occur between the T cell receptor gamma (TCD-gamma) and beta (TCR-beta) gene loci located on chromosome 7; and to compare using serial measurements the frequency of VDJ mutational events among subjects depending on their time of HU exposure (treatment study, follow-up study, never).

6.5 HJB CORE LABORATORY

The sample will be collected and processed in the research laboratory of Dr. Russell Ware at St. Jude's Children's Research Hospital as detailed in section 9.4 of the BABY HUG Treatment Study Protocol. A small aliquot of RBC will be fixed in ice-cold methanol and frozen at -85^{o}C according to a previously published protocol. This sample will be shipped frozen to Litron Laboratories, Inc. in Rochester, NY and analyzed by flow cytometry for quantitation of HJB (micronuclei) in both immature and mature erythrocytes. As above, serial measurements of the quantitative HJB measurement will be compared amongst subject groups depending on their time of HU exposure (treatment study, follow-up study, never).

6.6 NHLBI SPECIMEN REPOSITORY

Five milliliters of blood will be collected for future use by BABY HUG and other sickle cell disease investigators. The sample will be divided into serum, plasma and cell pellet aliquots and stored at the Medical College of Georgia. In addition, this laboratory will conserve residual plasma, serum, and cell pellets. At the end of the BABY HUG Follow-up Study, these specimens will be shipped to the NHLBI Specimen Repository (BioLINCC - Biologic Specimen and Data Repository Information Coordinating Center) provided informed consent has allowed for their long-term storage and use and the submitted application is approved. At the end of the follow-up study, a public-use

database will be delivered to the NHLBI that will allow clinical data to be linked to the specimens to aid investigators in carrying out future SCA-related studies. No DNA will be extracted or saved. Anonymized specimens will not provide any identifier to the individual from whom the specimen(s) was (were) obtained.

6.6.1 Specimen Storage

Parents/guardians must provide consent to have their child's specimens stored in a central repository. The planned specimens and potential future studies are outlined in Appendix A. Specimens will be prepared as outlined in the BABY HUG Follow-up Study Manual of Operations. Labels, provided by the MCC, will be applied to the specimens that will be stored at -80 and then sent to the NHLBI repository. Labels will be coded so that the specimens can be linked to relevant clinical data (e.g. clinical events, liver/spleen scan results, etc) in the future.

6.6.2 Obtaining Stored Specimens

The process involved in requesting biospecimens is determined by the "Proprietary Period" or "Open Period" status of the study collection. The "Proprietary Period" lasts until the clinical study data are made available for sharing following the NHLBI Limited Access Data Sharing Policy timeline. During the "Proprietary Period" only centers involved in the BABY HUG Follow-up study will be permitted access to the specimens. During the "Open Period" specimens will be available to all investigators successfully completing the application process as described below.

Investigators will need to provide a design of the proposed research and evidence of the qualification to perform the research. In addition, evidence of the availability of funding to perform the requested research must also be provided. Furthermore, investigators wishing to obtain specimens must address ethical and legal considerations, including consistency with the terms of the informed consent and compliance with human subjects and HIPAA regulations. Investigators requesting biospecimens during the "Proprietary Period" will need permission of the BABY HUG Follow-up study Steering Committee prior to obtaining the specimens.

6.7 HEMOGLOBIN F, BUN and MICROALBUMIN : CREATININE RATIO CORE LABORATORY

A 0.5 mL blood sample and a 5.0 mL urine sample will be collected at each Clinical Center and shipped to the Medical College of Georgia (MCG), a core laboratory. The MCG will analyze the blood samples to provide HbF, creatinine, and BUN measurements. The MCG will also analyze the urine sample to supply the microalbumin : creatinine ratio. The BUN measurement was added to this protocol in August 2009 and is required for each blood draw. If a blood draw has already been performed before August 2009, a small amount of blood from the stored blood samples or the remaining blood samples can be used to obtain the BUN measurement.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 7

GUIDELINES FOR STANDARD CLINICAL CARE

7.1 INTRODUCTION

The basic principles of supportive care for infants enrolled in the follow-up study are similar to those in the BABY HUG Treatment Study. The cooperation of all medical staff involved in the clinical care of study children will be solicited to enhance child adherence to the follow-up study protocol. Parent education and guidelines for the diagnosis and treatment of common clinical events are addressed in the BABY HUG Treatment Study Protocol Section 8.4. At no time should the performance of the BABY HUG Follow-up Study protocol be allowed to compromise the elements of good clinical care of the children enrolled in the study.

7.2 IMMUNIZATIONS

All routine pediatric immunizations should be given as per standard clinical recommendations as noted in the BABY HUG Treatment Study Protocol Section 8.2 and in accordance with local routine clinical care. (American Academy of *Pediatrics Red Book 2006*)

7.3 PROPHYLACTIC MEDICATIONS

Twice daily prophylactic penicillin should have already been initiated prior to enrollment in the BABY HUG Treatment Study and continued until at least five years of age. The dose, formulation and use of an alternative antibiotic are in accordance with routine local clinical care. Reminders about the need for this prophylactic agent should be offered at each clinical contact.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 8

SPECIAL STUDIES AND READING GROUPS

8.1 INTRODUCTION

Special studies and event reports that will be centrally evaluated by individuals independent of the BABY HUG Clinical Centers include: liver-spleen scans, abdominal ultrasound, pitted cell counts, HJB, creatinine clearance, and Cystatin C measurements.

8.2 LIVER-SPLEEN SCANS

Tc99m sulfur colloid liver-spleen scans will be performed once, only in children whose parents/guardians gave consent to active assessment, at two years into the follow-up study (four years after the child's enrollment in the BABY HUG Treatment Study) according to standard techniques. The results of this scan will be compared to the scans performed at screening and completion of treatment in the BABY HUG Treatment Study. Thus, pretreatment, post treatment and follow-up scans performed at time zero, two and four years respectively, will be available for review and comparison. Results will be assessed by a panel of three pediatric radiologists who are unaware of the original treatment assignment of the child. The reading process has previously been described in Section 9.5 of the BABY HUG Treatment Study Protocol. The proportion of children in each treatment group over the four years of combined treatment and follow-up will be compared according to spleen function (normal, decreased, or absent). In addition, the number of times that there is a decline in splenic function from one category to another will be compared in each treatment group. Scans that demonstrate an improvement in uptake will be scored as not demonstrating a decline.

8.3 PITTED CELL COUNTS (Pit Counts) and HOWELL JOLLY BODIES (HJB)

Pit counts will continue to be done in a single laboratory, the Pitted Cell Core Laboratory. Tubes containing the glutaraldehyde buffer and directions for specimen collection will be provided to

the Clinical Centers by the Pitted Cell Core Laboratory as in the MOO of the treatment study. HJB will be performed in the single laboratory that performed them in the BABY HUG Treatment Study. Pitted cell counts and HJB will be performed from specimens collected from all follow-up study children once, at four years or exit after the child's enrollment in the BABY HUG Follow-up Study, whichever comes first. Children whose parents/guardians gave consent for active follow-up will have an additional (third) measurement two years after entry into the follow-up study (four years after enrollment in the BABY HUG Treatment Study).

8.4 CLINICAL EVENTS

Clinical events (hospitalizations and specific other sickle cell related events) will be retrospectively abstracted from the medical records of study participants at specified intervals (two times per year). Clinical Centers will be asked to classify the event based upon BABY HUG criteria using simple yes/no data forms. Definitions of clinical events can be found in Appendix F of the BABY HUG Treatment Study Protocol except for the post-hoc Steering Committee definition of splenic sequestration which is defined as follows:

> *Splenic Sequestration:* 2 cm or more increase from last visit in palpable spleen size AND a decrease in Hb of 2 g/dL or more below the last steady state value as determined by the investigator.

8.5 CREATININE CLEARANCE AND CYSTATIN C

Creatinine clearance will be estimated from Cystatin C measurements and the Schwartz equations as indicated in the BABY HUG Treatment Study MOO. These studies will be done at the times indicated in Appendix A.

8.6 ABDOMINAL ULTRASOUND

An abdominal ultrasound will be performed by standard clinical techniques to estimate the size of the spleen and the presence of gall stones according to the techniques outlined in the MOO of the BABY HUG Treatment Study. This assessment will document if the spleen is enlarged and the subject therefore at risk for splenic sequestration or if it has involuted. The presence or

absence of gall stones is a secondary endpoint assessment for the degree of hemolysis occurring over time.

8.7 TRANSCRANIAL DOPPLER (TCD)

Transcranial Doppler studies will be performed as part of routine clinical care. They will be performed by technicians certified by the Medical University of South Carolina (MUSC) and the Medical College of Georgia (MCG) for the BABY HUG Treatment Study. As annual TCD evaluation is standard of care in the BABY HUG Follow-up Study age group, it is anticipated that most children will have at least one TCD study during the follow-up period. The study performed for clinical indications closest to two years in the follow-up study will be copied and sent for review and analysis by the TCD reading committee.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 9

FOLLOW-UP PROCEDURES

9.1 INTRODUCTION

Children in both the passive and active follow-up groups will have data from clinic visits no less than every six months in the first four years of the follow-up study and then not less than once per year until the common termination date, December 2011, is reached. The first child was enrolled into the BABY HUG Treatment study in October 2003 and the last child was enrolled in September 2007. Therefore, the total follow-up period including randomized treatment time will range from 4 years and 3 months to 8 years and 2 months.

9.2 FOLLOW-UP VISITS

Once informed consent for the follow-up study is obtained, retrospective data abstraction will be carried out for all medical visits that occur between the treatment study and enrollment in the follow-up study. The type of data to be collected is shown in Appendix B.

All follow-up visits will be performed at the Sickle Cell Clinic of the local Clinical Center and children will be evaluated in accordance with routine local clinical care. At each visit during this period the evaluation for those children on HU will include an interval medical history and information regarding adverse events and toxicity. In addition, height and weight will be recorded. Head circumference will also be measured and recorded until the age of five years. Results of all testing obtained for clinical indications, including but not limited to TCDs, neuroimaging, and medical consultations, will be collected in a systematic manner in the follow-up study.

9.2.1 Passive Follow-up Group

All children (active and passive) in the follow-up study will have Cystatin C (if not done at exit from the treatment study), BUN, urine microalbumin: creatinine ratio and a stored blood sample collected at entry to the follow-up study. At four years or exit from the follow-up study (whichever

occurs first), a repeat Cystatin C, creatinine, BUN, VDJ, Pit count, HJB, urine microalbumin: creatinine ratio, and a stored blood sample will be collected (see Appendix A). All other laboratory testing will be limited to those tests performed in accordance with routine local clinical care, including HU monitoring for those children being treated with HU. These later tests will be performed locally. Study data will be abstracted from the medical record using structured forms collected semi-annually.

9.2.2 Active Follow-up Group

In addition to the collections specified in Section 9.2.1 for the passive follow-up group, active follow-up will involve the collection of many of the same primary and secondary endpoint measurements as in the BABY HUG Treatment Study. These will include: Hgb F, pit counts, HJB, liver-spleen scan information (quantitative and qualitative), multi-digit serum creatinine, BUN (for the calculation of the Schwartz estimates of GFR), Cystatin C, and neuropsychological testing. The schedule of child visits during which specimens will be collected for laboratory testing is shown in Appendix A. Most primary and secondary endpoints will be measured four years after the child's enrollment in the BABY HUG Treatment Study. The Vineland neuropsychological test will be measured four years after the child's exit from the BABY HUG Treatment Study. This schedule is designed to measure all endpoints along the combined continuum of treatment and follow-up at zero, two and four years.

The timing of the active follow-up group special studies will depend upon the subject's exit date from the BABY HUG Treatment Study. The ideal time window is 24 months (-1 month/+2 months). However, some subjects will be entering the Follow-up Study greater than two years after they have exited from the BABY HUG Treatment Study. Therefore, the special studies will need to be performed within 3 months of entry. Consequently, the timing of the "24 month" special studies is actually staggered across many months contingent upon the subject's lapse of time from the BABY HUG Treatment Study. For example, if a subject enters the BABY HUG Follow-up Study three (3)

years after exiting BABY HUG, the subject will have his “24 month” special studies done within 3 months of entry, which is approximately 36 months post BABY HUG.

9.2.3 Collection of Laboratory Data

As noted in section 5.3, only the PEP will review the local laboratory results for children enrolled in the follow-up study within two months after the completion of the randomized BABY HUG Treatment Study. The PEP will enter these data into the BABY HUG Follow-up Study database via the Internet Data Entry System maintained by the MCC within seven days of receiving them and keep these in a locked file with no access by BABY HUG Follow-up Study Clinical Center staff. For all study visits after this two-month period, any local Clinical Center staff member may collect and enter laboratory data.

9.2.4 Adverse Event Reporting

Clinical events reported on the semi-annual follow-up form (Form 10) will be tabulated by the MCC based on the affected organ system. If any child dies while on study, efforts will be made to obtain complete post-mortem information. Narratives of fatal events will be sent with study forms to the MCC.

The NHLBI OSMB reviews the protocol at six-month intervals. A progress report showing results according to the different treatment types (see Chapter 4) will be forwarded by the MCC to the OSMB at these times and their recommendations will be expeditiously implemented. The OSMB members will also be provided with annual reports documenting each child’s growth, development and progress and other analyses as requested. The OSMB may recommend early termination of the study for considerations of safety.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 10

CLOSE-OUT PROCEDURES

10.1 OVERVIEW

As noted in Section 9.1, the anticipated common termination date for this proposal is December 31, 2011, although every attempt will be made to secure follow-up funding for this unique group of children beyond this date. Because the last child recruited into the BABY HUG Treatment Study will complete the study by September 2009, it is estimated that the treatment study will be completed and results disseminated during the follow-up study. Whether HU is found to be efficacious in the BABY HUG Treatment Study or not, and whether or not toxicities are found in the treatment study, the follow-up study will be performed. If interim or final analysis of the BABY HUG Treatment Study demonstrates that HU is not safe and/or efficacious, parents/guardians with children on the follow-up study will be advised to stop HU treatment if their children are currently receiving HU. Data processing and analysis of final study data will be continued according to the original schedule. As mentioned in Chapter 2, regardless of efficacy outcome it is important to determine a complete long-term toxicity profile for the subjects in the BABY HUG Treatment Study. If HU treatment is found to be efficacious, it will be essential to determine how long this effect is preserved.

10.2 DEBRIEFING CONTACT

After final long-term follow-up data have been collected and final reports on the results have been prepared for presentation or submitted for publication, each child's family will be scheduled for a debriefing contact. Families will be informed of the results of follow-up study and any recommendations of the investigators.

10.3 FINAL STUDY DATA AND DISSEMINATION OF RESULTS

Data processing and analysis of final follow-up study data will proceed on a "time-of-the-essence" basis. Clinical Centers will implement the following procedures for finalization of study data. All queries for data clean-up including resolution of forms/procedures expected but not completed, as determined by the MCC, will be addressed within two months of the last follow-up visit. Clinical Centers will be responsible for archiving records that document reported events and specified outcomes. The MCC will archive all electronic study data. Data from the Core Laboratories, Endpoints Evaluation Committees and medical records serve as the definitive sources for child outcomes in the study.

The OSMB will review the final data analyses regarding the main findings of the study, including analyses for efficacy and safety, at a planned final meeting. These data analyses will form the basis of the final consensus recommendations from the OSMB, Steering Committee and the NHLBI. These consensus recommendations will be shared first with the study children' families and will be made public as soon as possible thereafter. The final data analysis report will be available for submission to the FDA and for archival as will any databank studies.

Archival of central source data, including Core Laboratory results, will be consistent with requirements for a study conducted under an Investigational New Drug (IND) Exemption and sponsored by the NHLBI and NICHD. Storage of frozen and preserved specimens will be maintained according to the requirements of the NHLBI specimen repository as outlined in Chapter 6, following approval of the application to establish a biorepository. The MCC will archive study data in accordance with FDA guidance and NHLBI requirements. Public data files will be made available according to NHLBI policy.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY PROTOCOL

CHAPTER 11

ORGANIZATIONAL STRUCTURE AND PARTICIPATING UNITS

11.1 INTRODUCTION

The BABY HUG Follow-up Study will be conducted by the same participating units as in the BABY HUG Treatment Study except for the Pharmacy Distribution Center and Clinical Center Pharmacies which are no longer necessary. All open-label HU will be prepared and distributed by the pharmacy at each Clinical Center. Descriptions of the various participating units can be found in Chapter 13, Section 2, of the BABY HUG Treatment Study Protocol.

Administration of this study will involve the same structure, personnel, and reporting procedures as in the BABY HUG Treatment Study. Descriptions of these functions can be found in Chapter 13, Section 3, of the BABY HUG Treatment Study Protocol. Exhibit 11.1 details the organizational chart for the follow-up study. A list of participating centers is found in Exhibit 11.2.

11.2 CLINICAL CENTER STAFF

The Clinical Center staff will be trained in accordance with the procedures set out in the follow-up study protocol, many of which are the same as those in the BABY HUG Treatment Study protocol. The objective is to standardize all study procedures carried out in the Clinical Centers. Treatment of study children whether on open-label HU or not, will be in accordance with routine local clinical care.

11.3 MONITORING AND ENDPOINT EVALUATION

Study monitoring will be carried out by the OSMB, Steering Committee and Operations Committee based on this protocol. The Endpoints Evaluation Committees will perform their respective functions according to the BABY HUG Treatment Study Protocol.

Exhibit 11.1
Pediatric Hydroxyurea Phase Clinical Trial (BABY HUG)
Follow-up Observational Study Organizational Chart

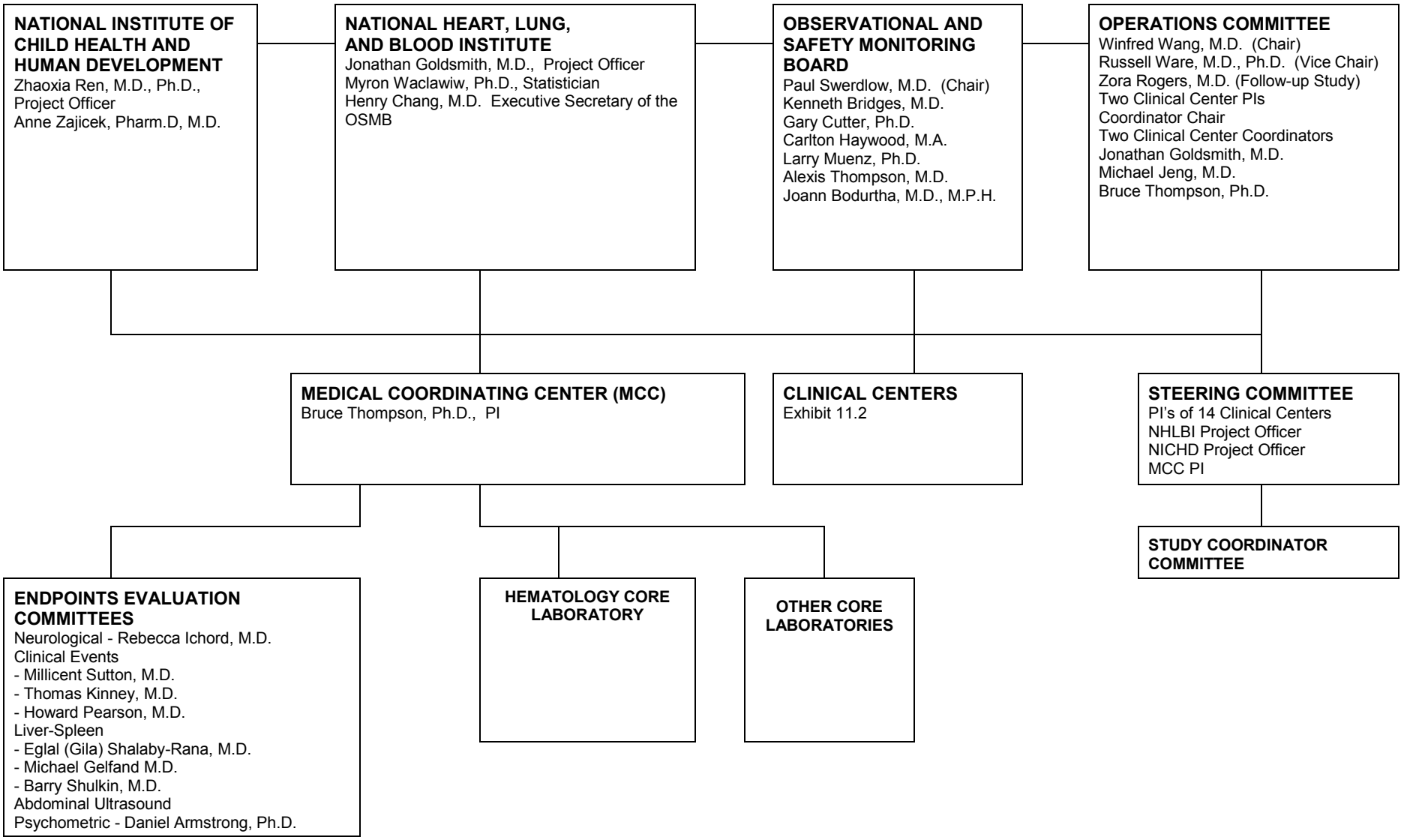


Exhibit 11.2
PARTICIPATING CLINICAL CENTERS

CLINICAL CENTERS

Children's Research Institute, Lori Luchtman-Jones, M.D. - 01
(Washington, DC)

Duke University Medical Center, Courtney Thornberg, M.D. - 02
(Durham, NC)

Howard University College of Medicine, Sohail Rana, M.D. - 03
(Washington, DC)

Johns Hopkins University School of Medicine, James F. Casella, M.D. - 04
(Baltimore, MD)

Medical University of South Carolina, Sherron Jackson, M.D. - 05
(Charleston, SC)

St. Jude Children's Research Hospital, Winfred C. Wang, M.D. - 06
(Memphis, TN)

State University of New York - Brooklyn (SUNY), Scott T. Miller, M.D. - 07
(Brooklyn, NY)

University of Miami School of Medicine, Ofelia Alvarez, M.D. - 08
(Miami, FL)

University of Mississippi Medical Center, Rathi V. Iyer, M.D. - 09
(Jackson, Mississippi)

University of Texas Southwestern Medical Center, Zora R. Rogers, M.D. - 10
(Dallas, TX)

University of Alabama, Birmingham, Thomas Howard, M.D. - 11
(Birmingham, AL)

Drexel University, Norma Lerner, M.D. - 12
(Philadelphia, PA)

Emory University School of Medicine, R. Clark Brown, M.D., Ph.D. - 13
(Atlanta, GA)

Wayne State University, Ingrid Sarnaik, M.D. - 14
(Detroit, MI)

MEDICAL COORDINATING CENTER

Clinical Trials & Surveys, Corp. (Baltimore, MD)
Bruce W. Thompson, Ph.D., Principal Investigator

PROJECT OFFICE

Division of Blood Diseases and Resources
National Heart, Lung, and Blood Institute (Bethesda, MD)
Jonathan Goldsmith, M.D., Project Officer
Myron Waclawiw, Ph.D., Statistician
Henry Chang, M.D., Executive Secretary of the OSMB

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APPENDIX A
Schedule and Volume of Blood and Urine Collection and Schedule of Special Studies
BABY HUG Follow-up Observational Study

Time Point	CLINICAL DATA REPORTS	Blood Sample Collection (All volumes in ml)						Other Tests						
		HbF (Core)		HJB (Core)	Pitted Cells (Core)	STORED BLOOD SAMPLE	VDJ	Cystatin C (ml) (Core)	Creatinine and BUN (core)	Liver Spleen Scan	Abdominal Ultrasound	†Neuropsych (WPPSI)	Microalbumin/ Creatinine Ratio Urine (Core)	Vineland
CONSENT (end of randomized study)		**		**	**	5.0^	**	0.5	**	**	**	**	5.0	
+6 Months	X													
+12 Months	X													
+18 Months	X													
+24 MO (Active Follow-Up Only) -1 month/+2 months	X (all)	0.5		0.1	0.1			0.5	0.5	Y	Y	Y		
+30 MO	X													
+36 MO	X													
+48 MO -1 month/+2 months	X			0.1	0.1	5.0^	3.0	0.5	0,5				5.0	Y (for active follow-up only)
+60 MO	X													
+72 MO or Exit	X													

CBC with diff and reticulocyte count will be done at each clinic visit as a clinical parameter. All CBCs performed for medical management within two months after completion of the BABY HUG Treatment Study should be reviewed only by the PEP.

** Done on exit from BABY HUG Treatment Study

^ The stored blood sample will be collected, separated and aliquots made and stored for future study

The entry visit has a time window of 3 months from consent signing for those who exited the BABY HUG Study > 6 weeks before enrolling in BABY HUG Follow-up and a time window of 6 weeks for those subjects who are entering the BABY HUG Follow-up Study immediately following the BABY HUG Treatment Study.

The 24 month Active special studies visit has a time window of -1month/+2 months.

The 48 month visit has a time window of -1month/+2 months.

Appendix B
BABY HUG FOLLOW-UP OBSERVATIONAL STUDY DATA COLLECTION SHEET
For child visits between the treatment and follow-up studies

BABY HUG FOLLOW-UP OBSERVATIONAL STUDY

CLINICAL DATA REPORT

PART I: IDENTIFYING INFORMATION

1. Patient's ID Number: [ ][ ][ ][ ] 2. Current Clinic: [ ][ ]

3. Patient's Letter Code: [ ][ ][ ]

4. Abstraction Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

PART II: INTERVAL INFORMATION

1. Visit: [ ][ ][M]

2. Interval Start Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

3. Interval End Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

	Yes	No*
4. Any patient contact during this interval?	(1)	(2)

*A. If no, reason _____

*If No, Skip to Part IX.

PART III: HU USE

	Yes**	No*
1. Was the patient prescribed HU at any time during this interval?	(1)	(2)

*If No, Skip to Part IV.

**A. If yes, what was the:

1. Dose at the first time it was prescribed this interval: [ ][ ][ ] . [ ] mg/kg

2. Dose form: Liquid (1) Capsules (2)

	Yes* (1)	No** (2)
2. Was the patient still being prescribed HU at the end of the interval?		

* A. If **Yes**, what was the:

1. Dose at the end of the interval: [ ][ ][ ] . [ ] mg/kg

2. Dose form: Liquid (1) Capsules (2)

B. If **No, what was the date the patient stopped being prescribed HU?

[ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

		Yes* (1)	No (2)
3.	Did the patient have HU held because of possible drug toxicity during this interval?		

*A. If **Yes**, check all that apply:

1. Low ANC (1)
2. Low Hgb (1)
3. Low PHs (1)
4. Other bacterial or viral infection (1)
5. Other (1)
   a. Specify _____

4. Estimate how many weeks during this interval the patient actually took HU: [ ][ ]

This is the number of weeks HU was taken minus the number of weeks HU was stopped due to toxicity, if applicable. If there was no toxicity, it is the number of weeks HU was taken in this time period.

ID Number [ ][ ][ ][ ] Visit [ ][ ][ ] - Seq [ ][ ]

PART IV: BLOOD RESULTS

		Yes	No*
1.	Were any blood specimens collected for clinical reasons during this interval?	(1)	(2)

*If NO, reason: _____

If No, Skip to Part V.

2. First CBC in interval:

A. Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

B. Hemoglobin [ ][ ] . [ ] gm/dl

C. MCV [ ][ ][ ] . [ ] fL

D. Reticulocyte (% of RBC) [ ][ ] . [ ] % (1) Not Done

E. White Blood Cell Count [ ][ ] . [ ] K/mm^3

F. Absolute Neutrophil Count [ ][ ] . [ ][ ] K/mm^3 (1) Not Done

G. Platelet Count [ ][ ][ ][ ] K/mm^3

H. Red Blood Cell Count [ ] . [ ][ ] M/mm^3

3. Last CBC in interval:

A. Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

B. Hemoglobin [ ][ ] . [ ] gm/dl

C. MCV [ ][ ][ ] . [ ] fL

D. Reticulocyte (% of RBC) [ ][ ] . [ ] % (1) Not Done

E. White Blood Cell Count [ ][ ] . [ ] K/mm^3

F. Absolute Neutrophil Count [ ][ ] . [ ][ ] K/mm^3 (1) Not Done

G. Platelet Count [ ][ ][ ][ ] K/mm^3

H. Red Blood Cell Count [ ] . [ ][ ] M/mm^3

Yes No*

ID Number [ ][ ][ ][ ] Visit [ ][ ][ ] - Seq [ ][ ]

4. Were any of the following laboratory values obtained during this interval? (1) (2)

*A. If No, reason:
Not a part of routine care (1)
Other (2)
a. If other, Specify: _____

*If No, Skip to Part V.

B. Creatinine:

1. Date: [ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

2. Value: [ ][ ] . [ ] mg/dL

C. ALT

1. Date: [ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

2. Value: [ ][ ][ ] IU/L

D. GGT

1. Date: [ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

2. Value: [ ][ ][ ] u/L

E. Fetal Hemoglobin:

1. Date: [ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

2. Value: [ ][ ] . [ ] %

ID Number Visit Seq
[ ][ ][ ][ ] [ ][ ][ ] - [ ][ ]

PART V: IMAGING RESULTS

		Yes	No*
1.	Were any TCD's performed during this interval?	(1)	(2)

*If No, Skip to Part V, 4.

A. **TCD Date**	B. ***Results**
1. ☐☐☐ - ☐☐ - ☐☐☐☐ (Month - Day - Year)	(1) (2) (3)
2. ☐☐☐ - ☐☐ - ☐☐☐☐ (Month - Day - Year)	(1) (2) (3)
3. ☐☐☐ - ☐☐ - ☐☐☐☐ (Month - Day - Year)	(1) (2) (3)
4. ☐☐☐ - ☐☐ - ☐☐☐☐ (Month - Day - Year)	(1) (2) (3)
5. ☐☐☐ - ☐☐ - ☐☐☐☐ (Month - Day - Year)	(1) (2) (3)
6. ☐☐☐ - ☐☐ - ☐☐☐☐ (Month - Day - Year)	(1) (2) (3)

*Results
1. Normal (all mean velocities less than 170)
2. Conditional (highest mean velocity 170-199)
3. Abnormal (any mean velocity over 200)

COMPLETE TRANSMITTAL FORM 105 AND FAX ALONG WITH A COPY OF ALL TCD REPORTS TO THE MEDICAL COORDINATING CENTER.

ID Number ☐☐☐☐ Visit ☐☐☐ - Seq ☐☐

2. MRI Date* [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

*A. If MRI done, result:

CHECK THE MOST SEVERE RESULT

Normal	(1)
Silent Infarct(s) (gliosis)	(2)
Stroke (CVA or thrombosis)	(3)
Hemorrhage (subarachnoid or subdural)	(4)
Other	(5)^

1. ^If Other, Specify: _____

COMPLETE TRANSMITTAL FORM 105 AND FAX ALONG WITH A COPY OF THE REPORT TO THE MEDICAL COORDINATING CENTER.

3. MRA Date* [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

	Yes	No
*A. If MRA done, any result abnormal?	(1)	(2)

COMPLETE TRANSMITTAL FORM 105 AND FAX ALONG WITH A COPY OF THE REPORT TO THE MEDICAL COORDINATING CENTER.

4. CT Date* [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

	Yes	No
*A. If CT done, any result abnormal?:	(1)	(2)

COMPLETE TRANSMITTAL FORM 105 AND FAX ALONG WITH A COPY OF THE REPORT TO THE MEDICAL COORDINATING CENTER.

ID Number [ ][ ][ ][ ] Visit [ ][ ][ ] - Seq [ ][ ]

PART VI: OTHER PROCEDURES

1. EEG Date* [ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

	Yes	No
*A. If EEG done, any result abnormal?	(1)	(2)

2. PFTs Date* [ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

	Yes	No
*A. If Pulmonary Function Tests done, any result abnormal?	(1)	(2)

3. Neuropsych Date* [ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

	Yes^	No
*A. If neuropsychology testing done, any results abnormal?	(1)	(2)

^1. Specify test: _____

COMPLETE TRANSMITTAL FORM 105 AND FAX ALONG WITH A COPY OF THE REPORT TO THE MEDICAL COORDINATING CENTER.

	Yes*	No
4. Other clinical tests done:	(1)	(2)

*A. If Yes, specify: _____

ID Number [ ][ ][ ][ ] Visit [ ][ ][ ] - Seq [ ][ ]

PART VII: CLINICAL EVENTS

1. Clinic Visits

A. During this interval how many times was this patient seen in clinic (not ER, day unit, or hospital)? ☐☐

If zero, Skip to Part VII, Item 2.

B. Enter the number of visits for which the following were the main reasons for each visit in this time period:

1. Routine Clinical Visit (physical examination by sickle cell team) ☐☐

2. HU toxicity assessment (blood count check to monitor HU therapy and possible side effects) ☐☐

3. Other clinical service (including follow-up of crisis event and general pediatrics) ☐☐

4. Other ☐☐ *

*a. If other, Specify: _____

2. Hospitalization

A. How many times was this patient seen in an ER or day hospital during this interval (in your facility or another): ☐☐

If zero, Skip to Part VII, Item 3.

B. Reasons for visits:		Yes	No
1.	Acute splenic sequestration crisis	(1)	(2)
2.	Acute chest syndrome	(1)	(2)
3.	Neurologic event (stroke or seizure)	(1)	(2)
4.	Aplastic Crisis	(1)	(2)
5.	Urinary tract infection	(1)	(2)
6.	Fever or febrile illness including URI/sinusitis/cold/flu	(1)	(2)

ID Number ☐☐☐☐ Visit ☐☐☐ - Seq ☐☐

7.	Other acute illness, no fever	(1)	(2)
8.	Trauma including broken bones and sprains	(1)	(2)
9.	Sickle Cell Pain Crisis (including dactylitis)	(1)	(2)
10.	Other	(1)*	(2)

*a. If other, specify: _____

3. How many times was the patient admitted to the hospital during this interval (in your facility or another)? ☐☐

If zero, Skip to Part VII, Item 4.

A. What was the primary discharge diagnosis for each of these admissions?

1.	Neurologic event (stroke or seizure)	☐☐
2.	Acute splenic sequestration crisis	☐☐
3.	Acute chest syndrome	☐☐
4.	Aplastic Crisis	☐☐
5.	Urinary tract infection	☐☐
6.	Fever or febrile illness including URI/sinusitis/cold/flu	☐☐
7.	Other acute illness, no fever	☐☐
8.	Trauma including broken bones and sprains	☐☐
9.	Sickle Cell Pain Crisis (including dactylitis)	☐☐
10.	Surgery (see Part VII, Item 5 below)	☐☐
11.	Other	☐☐ *

*a. If other, specify: _____

ID Number ☐☐☐☐ Visit ☐☐☐ - Seq ☐☐

4. Pain

	Yes* (1)	No (2)
A. Has the child experienced pain (defined as pain lasting four hours or more without other obvious cause for which medication such as ibuprofen, acetaminophen, or acetaminophen with opioid was taken for relief) even if not seen by a medical professional during the interval?		

*1. If yes, how many episodes of pain has the patient experienced during this interval? [ ][ ][ ]

5. Surgery

	Yes* (1)	No (2)
A. Did the patient have at least one surgery during this interval?		

*1. If yes, identify the type of each surgery and give date:

a. Tonsillectomy, Adenoidectomy or both (1) Not Done

Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

b. Splenectomy (open or laproscopic) (1) Not Done

Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

c. Cholecystectomy and/or ERCP (1) Not Done

Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

d. Ear tubes, hernia repair, dental rehabilitation (1) Not Done

Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

	Yes^ (1)	No (2)
e. Other		

^1. If other, specify: _____

6. Transfusion

		Yes*	No
A.	Was the patient on a chronic transfusion program during this interval (meaning scheduled transfusions every two-six weeks for three months or more)?	(1)	(2)

*1. If yes, what was the main reason for the chronic transfusion program:

Stroke (clinical neurologic deficit lasting 24 hours or more)	(1)
Elevated TCD velocity	(2)
TIA or other neurologic events	(3)
Splenic Sequestration	(4)
Recurrent Acute Chest Syndrome	(5)
Recurrent Painful Events	(6)
Other	(7)^

^a. If other, specify: _____

		Yes*	No
B.	Did the patient receive an episodic transfusion during this interval (meaning a transfusion, scheduled or not that was for a specific problem or to prepare them for surgery)?	(1)	(2)

*1. If yes, what was the main reason for the episodic transfusion?

Acute Splenic Sequestration	(1)
Acute Chest Syndrome	(2)
Neurologic Event or Stroke	(3)
Aplastic Crisis	(4)
Peri Operative Preparation	(5)
Other	(6)^

^a. If other, specify: _____

PART VIII: PHYSICAL EXAMINATION

If there are more than three sets of collected physical examination data, use the first and last in the sequence and select the one closest in days to the midpoint of the interval. Sickle Cell or Hematology clinic examinations are preferred over ER/hospital/general pediatric visits if there is a choice.

1. Was a physical examination performed during this interval? Yes (1) No (2)

If No, Skip to Part IX.

2. Growth Parameters:

A. First Encounter Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Month Day Year

1. Height [ ][ ][ ] . [ ] cm (1) Not Done
2. Weight [ ][ ][ ] . [ ] kg (1) Not Done
3. Head Circumference [ ][ ][ ] . [ ] cm (1) Not Done

B. Second Encounter midpoint Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done*
Month Day Year

*If Not Done, Skip to Part VIII, Item 2C.

1. Height [ ][ ][ ] . [ ] cm (1) Not Done
2. Weight [ ][ ][ ] . [ ] kg (1) Not Done
3. Head Circumference [ ][ ][ ] . [ ] cm (1) Not Done

C. Last or latest Visit Date: [ ][ ][ ] - [ ][ ] - [ ][ ][ ][ ] (1) Not Done
Month Day Year

*If Not Done, Skip to Part VIII, Item 3.

1. Height [ ][ ][ ] . [ ] cm (1) Not Done
2. Weight [ ][ ][ ] . [ ] kg (1) Not Done
3. Head Circumference [ ][ ][ ] . [ ] cm (1) Not Done

ID Number [ ][ ][ ][ ] Visit [ ][ ][ ] - Seq [ ][ ]

3. A. Was the spleen reported to be palpable below the costal margin at any time during this interval? Yes (1) No (2)

If No, Skip to Part IX.

B. On what date was it the largest (most centimeters below costal margin): ☐☐☐ - ☐☐ - ☐☐☐☐
Month Day Year

Write the largest value below:

1. Mid-clavicular line: ☐☐ . ☐ cm below costal margin (1) Not Done
2. Anterior anxillary line: ☐☐ ☐ cm below costal margin (1) Not Done

C. Was the child diagnosed with acute splenic sequestration during this interval? Yes (1) No (2)

PART IX: COORDINATION

1. Checked for completeness and accuracy:

A. Certification number: ☐☐ - ☐☐

B. Signature: _____

C. General Comments: _____

_____

ID Number ☐☐☐☐ Visit ☐☐☐ - Seq ☐☐