PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)

FOLLOW-UP STUDY

PROTOCOL

NOTICE

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Prepared by:

Clinical Trials & Surveys Corp.
2 Hamill Road, Suite 350
Baltimore, MD 21210-1874

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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, double-blind randomized placebo-controlled trial including 200 patients at 14 clinical centers. The final patient is expected to enter the BABY HUG treatment protocol by July 2007 and complete the treatment protocol in July 2009. As per the protocol, the parents will not be told whether their children received active drug during the trial until the last patient has exited the study, queries resolved, and the database locked.

The purpose of the BABY HUG follow-up study is to provide structured follow-up of the patients in the BABY HUG treatment study in order to characterize the long-term benefits and toxicities associated with treatment with Hydroxyurea (HU) and to determine whether variations in the duration and age of initiation (early vs. late) of treatment affect the efficacy and toxicity of the drug. This information will help to create an optimal treatment paradigm for the use of HU in sickle cell anemia (SCA). The follow-up protocol is also designed to offer the parents the option to have their children treated with open label HU until the results of the BABY HUG treatment study become available.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
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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 patients enrolled in the BABY HUG treatment study after they have completed their period of randomized treatment. Long-term follow-up is critical for several reasons. Toxicity may only become evident years after treatment. Previous studies have assessed late toxicity associated with HU therapy in only a small number of very young children with SCA. In the HUSOFT extension study 17 patients completed four years of treatment with HU and 11 completed six years. The report by Hoppe et al described eight children with a mean age of 3.7 years (range 2 to 5 years) treated with HU for a mean of 137 weeks. Long term follow-up of patients in the BABY HUG treatment study will provide data on late toxicity in a large number of patients.

The incidence and type of toxicity associated with HU therapy may also vary with the age treatment begins. Data from the HUSOFT extension study suggests that age may be an important factor in the development of hematologic toxicity. Long term follow-up will be necessary to specifically define the range of toxicities that occur.

It will be important to document if the benefits associated with HU therapy *in this age group* persist. Hematologic efficacy, in terms of maintaining elevated levels of HbF, has been reported in all patients treated with HU in HUSOFT, but confirmation of this efficacy and whether it persists with long term treatment in a larger group of patients is needed. In addition, if HU therapy is efficacious in preventing chronic organ damage it will be important to determine the duration of this effect.

Benefit in terms of preventing organ-specific failures may be influenced by the relationship between early treatment and late treatment with HU and the natural progression of organ failure. Decline in renal function, as an example, may not be linear because the mechanism of renal

damage in SCA is interstitial and medullary as well as glomerular. Each of these types of failure leads to loss of function at different rates. If the rate of decline in renal function increases with time then early treatment with HU may not have as great a benefit as late treatment in terms of preservation renal function.

Overall determination of the long-term risks and benefits of treatment with HU in infants and toddlers will allow more specific characterization of the role and timing of treatment with HU in patients with SCA. More specifically, it will help to determine the specific age range for which treatment with HU offers the maximum benefit in terms of reductions in morbidity and mortality.

Long term follow-up may demonstrate that treatment with HU does not cause concordant effects across all organ systems in terms of net benefit versus harm. This would lead to a differential weighting of the risks and benefits relative to each organ system involved and the resultant morbidity associated with it. For example, long-term follow-up may demonstrate a net benefit of treatment with HU in preservation of renal function, but net harm in terms of brain development and neurological function. Preservation of renal function is important because survival after the diagnosis of renal failure in SCA, despite dialysis, is four years (Powars et al, 1991). Therefore, therapy must be aimed at prevention of progression (Wong et al, 1996) and treatment with HU may be the optimal therapy for this. ESRD, however, does not usually occur until the third decade of life (Powars et al, 1991) whereas cognitive defects, if they occur as a result of treatment with HU, would be expected to occur at a very young age. In this case the use of HU in infants and toddlers would be relatively contraindicated depending on the type and severity of insults to the brain relative to the degree of preservation of renal function. In fact one could argue, based on patients' and clinicians' perceptions of qualitative benefits, that even if treatment with HU provided for preservation of function of all three of the other major organ systems affected (spleen, kidneys and lungs) that it would not outweigh the net harm to the brain (patient and or family acceptance of

HU therapy would most likely be poor if it was felt that treatment caused “brain damage” regardless of its’ other benefits).

The risk benefit ratio could be shifted even further toward no treatment if the maximum benefits possible with HU treatment are reduced. In fact, in terms of preservation of organ function, this is already the case as improvements in supportive care continue to reduce morbidity (Brookoff et al, 1992; Ballas et al 2005) and mortality (Quinn et al, 2004) including that associated with ACS (Quinn et al, 1999) and sepsis (Adamkiewicz et al, 2003). In addition, transfusion therapy has been shown to be effective in the primary and secondary prevention of stroke in these patients (Adams et al, 1998) and the availability of oral iron chelators (Okpala, 2005) should make treatment of iron overload easier and presumably safer.

Presumed benefits also may not be bourn out by long term follow-up. In the HUSOFT extension study, after four years of HU therapy in children with a median age of 5 years and despite HbF levels above 20%, the prevalence of silent brain infarcts was 21%, similar to the prevalence reported among untreated SCA children (Moser et al, 1996). In addition, a concern was raised that preservation of splenic function with HU therapy in young children could prolong their risk of acute splenic sequestration. This would increase the cumulative probability of a first attack leading to an increased mortality (Emond, AM et al, 1995).

Long term follow-up may also show an association between HU treatment and the development of malignancy in this age group. Large studies have shown that long-term therapy with HU is leukemogenic in elderly patients with MPD (Najean et al, 1997b). Available data do not suggest, however, that there is a significant increase in the risk of malignant disease in adult patients with SCD treated with HU. The Medical College of Georgia recently reported their experience with HU therapy in SCD over a 15-year period (Bakanay et al. 2005). Leukemia was not reported among the 226 patients treated. After nine years of follow-up, the US multi-center trial

(Steinberg et al. 2003) observed two cases of cancer among 204 sickle cell subjects who took HU, and one case among 96 subjects who did not.

Despite the probable lack of carcinogenic potential in adults the inhibition of DNA synthesis and repair caused by treatment with HU, especially during the period of the rapid growth and development and relative immunologic incompetence in infants and toddlers, makes an increased risk of malignancy very plausible. Hydroxyurea has been shown experimentally to have teratogenic (Alvierti et al, 1981) and in some settings mutagenic (Ziegler-Skylakakis et al, 1985) effects, although its' carcinogenic potential at therapeutic doses has not been established. Like the possible risks and benefits described above, the increased risk of malignancy and its' magnitude may be dependent on the timing of treatment with HU in patients with SCA.

In terms of brain development, children with SCA can have significant neurocognitive deficiencies as a result of both clinically overt and silent infarctions. Treatment with HU may reduce the incidence of recurrent strokes (Ware et al. 2004) and thus prevent neurocognitive decline. In fact, a recent abstract has suggested that HU treatment is associated with improvement in neurodevelopmental scores (Bernaudin et al, 1999). As with the possible increased risk of malignancy however, treatment with HU during the same time frame as a period of rapid brain growth and development may cause significant developmental delay or permanent cognitive deficits.

Data in rats suggests that prenatal HU treatment can be toxic to the developing brain resulting in impaired locomotor activity and maze learning (Butcher et al, 1973) and that postnatal treatment impairs weight gain and organ size (Horiuchi et al, 1998). It should be noted, however, that no studies have documented embryo or severe neurodevelopmental toxicity with the therapeutic doses of HU currently used in children with SCA. In addition, previous studies suggest that the effects on rodents do not accurately reflect the effects of HU in primates (Wilson et al,

1975). If the results of long-term follow-up confirm that treatment with HU causes significant neurodevelopmental toxicity it would clearly be contraindicated in infants and toddlers with SCA.

Other questions about the effects of HU treatment remain. While studies suggest that growth and development are either not affected or improved by HU treatment concern remains about growth and pubertal maturation. Concern has also been raised about the effects of HU on gametogenesis (Scott et al, 1996). Compliance with HU treatment in children and adolescents is a major concern. There are many reports of patients who became pregnant during treatment with HU despite careful counseling to reinforce the importance of contraception while receiving HU. (Scott et al, 1996 and MSH).

As is apparent from the above discussion, the determination of the overall risk benefit ratio for treatment with HU in this group of patients is extraordinarily complex. It can not be defined without the collection of clinical long-term 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). Even if the BABY HUG treatment study provides evidence of benefit in preventing chronic organ damage in children with SCA, long term follow-up may clearly demonstrate that the toxicity associated with HU treatment makes its' use unwarranted. As such, the Baby HUG treatment study will not provide the answer to the question of the role of HU in the treatment of very young children with SCA until the long term effects are defined.

2.2 SPECIFIC AIMS

The specific aims of this follow-up study are:

1. To identify any long-term toxicities in those babies randomized to treatment with HU in the BABY HUG treatment study.
2. To assess the optimal age for initiation of treatment with HU (early vs. late treatment).
3. To determine if prolonged treatment with HU changes the risk and benefits of its use.

2.3 DESIGN OF THE STUDY

Upon completion of active participation in the BABY HUG treatment study, each family will be asked to consent to participate in a long-term follow-up study and to have their child's retrospective data collected since they exited the BABY HUG treatment study (if applicable). Follow-up of these children will continue to a common termination point on December 31, 2011. This will yield patient follow-up intervals of approximately 4.5 to 8 years (see section 9.1). As part of providing informed consent, families will be able to participate in the follow-up study in one of two ways; a *passive follow-up* that will only involve the abstraction of clinical data from the medical record, or an *active follow-up* that will involve the performance of many of the laboratory tests and procedures done during BABY HUG. These include, but are not limited to, serial laboratory parameters that are not part of routine clinical care such as Hgb F levels, pitted cell count, Howell-Jolly Body determination, a liver-spleen scan, DTPA GFR measurement, creatinine clearance (and the Schwartz measurement of GFR), Cystatin C, urine concentrating ability, transcranial Doppler (TCD), and neuropsychological testing. The time table for the performance of these tests is presented in Appendix A.

All families will be offered the option to place their child on open label HU, regardless of the follow-up group in which they choose to participate. The intention to take open label HU will be a clinical decision of the family and their physician. BABY HUG Clinical Center staff will present the issues related to taking HU in the designated age range to the family in an unbiased fashion.

For patients electing to take open-label HU during the follow-up study, the recommendations for dose, monitoring intervals and toxicity levels are listed in section 5.2 Details of the prescription and monitoring schedule, however, will be at the discretion of the Clinical Center Principal Investigator and may be based on local clinical care standards for patients receiving HU.

The combination of randomized treatment assignments and open-label self-selected treatment decisions will create an unbalanced factorial study with factors represented by an early

treatment (the BABY HUG treatment study randomized comparisons) and a late treatment (the self-selected treatment decisions of the parents whose children are in the Follow-up Study). Voluntary choice of open-label treatment may lead to an uneven distribution of patients into the four comparison groups. Uneven distribution will lower the power of the follow-up study design to address some of the proposed questions. The four groups can be represented in the following way:

		Late Treatment: Yes	Late Treatment: No
Early Treatment	Yes	Continuous HU Treatment	Early HU Treatment Only
	No	Late HU Treatment Only	No HU Treatment

The standard main effects and interactions to estimate from this design are: effects pertaining to the short term effects of HU, effects pertaining to the late treatment effects of HU, and whether early plus late treatment provide extra benefit or risk than either alone (the interaction test). Study size and power assessments are presented in Section 4.4. The proposed sample size of 200 should allow investigators to detect moderate effects for the two primary endpoints.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)

FOLLOW-UP STUDY

CHAPTER 3

PATIENT ELIGIBILITY, RECRUITMENT, ORIENTATION, AND INFORMED CONSENT

3.1 INTRODUCTION

All patients who have successfully completed the BABY HUG treatment study will be eligible for this study. Each patients's current address and telephone number will be updated and maintained in the Clinical Center files. Consent will be required for participation in the follow-up study.

Before they were enrolled in the BABY HUG treatment study, parents were made aware that there would be a follow-up study and that the investigators' wished to maintain contact with the patient after the clinical trial ended. This information was repeated during the course of the study and will be considered at the infant's 24-month visit. During the follow-up study, passive data collection and retrospective data abstraction (if applicable) will be carried out for all infants whose parents (or guardians) give consent to be in the study. For those parents (or guardians) who elect to allow their child to be actively followed, more than minimal risk studies will be performed on the infants to gain more information about the BABY HUG treatment trial primary organ endpoints. With the family's consent this will include information from other health care providers.

3.2 PARENT/GUARDIAN ORIENTATION

The rationale and the importance of the follow-up study will be presented and explained to the parents (or guardians). The protocol will be discussed in detail. Parents (or guardians) will be given a list of the laboratory evaluations and tests that would be performed as part of the active follow-up. (Appendix A). Explanations of these evaluations will be provided to the parents in terms of their relevance to the follow-up study and their risks in a fashion similar to the way in

which it was discussed during the BABY HUG treatment trial. They will be advised that they will be required to sign a new consent form in order to participate in the follow-up study. As part of the consent process, the requirements for both the active and passive follow-up groups will be explained to the parents (or guardians) and they will be offered participation in the active and passive follow-up groups simultaneously. If they do not agree to be part of the "active" follow-up group, they will be able to participate in the "passive" follow-up group if they so choose. In addition, they will be asked if they want to initiate two-year open-label treatment with HU. If they elect to initiate HU treatment, details of the treatment and monitoring schedule, will be at the discretion of the Clinical Center Principal Investigator and may be based on local clinical care standards for patients receiving HU. They will also be advised that it will be their responsibility to pay for this treatment.

Families may continue to participate in the study even if they move out of the area. Families will be encouraged to notify the coordinator in advance of the move so that plans for data collection can be developed. If funds are available, the coordinator will arrange for the family to return to the clinical site at designated intervals for clinical and laboratory evaluation as per protocol. If not, the coordinator will arrange to contact the family at designated intervals to obtain as much follow-up data as is available.

3.3 INFORMED CONSENT

Individual Clinical Center consent forms will be prepared based on the model informed consent form presented below, and will be approved by the Data and Safety Monitoring Board. Each final consent form will be reviewed by a member of the DSMB to ensure all appropriate issues are addressed.

The Clinical Center Principal Investigator will obtain the consent from each family entering the study by contacting the parents (or guardians) at the start of each child's long-term follow-up. The family will be given adequate time and privacy to review the consent form. They

should be advised that a list and description of the additional tests required, if they choose to have their child participate in active follow-up, is contained in the consent form. They will be given a copy of the consent to review at home. They will have the opportunity to have all of their questions and concerns addressed by the PI. Copies of the signed consent form will be given to the parents (or guardians) and placed on 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 a BABY HUG follow-up study in which children will continue to make regular clinic visits with the study doctors until December 2011. The BABY HUG treatment study was designed to see if treatment with the drug hydroxyurea (also referred to as 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, heart failure, stroke and blood infection by reducing the number of sickle-shaped cells in you child's bloodstream. If treatment with hydroxyurea provides a benefit, it will be important to determine how long that benefit continues. Another important part of this study is to investigate the long-term safety of HU. Follow-up of your child will provide important information concerning these issues.

Families may choose to participate in one of two ways. The first is known as "active follow-up". Choosing this type of follow-up means that your child will come for clinic visits at least every 4 months until age 5 and then at least every 6 months. The same laboratory testing, imaging procedures and behavioral testing that were done during the BABY HUG treatment study will be done in the follow-up study, except that the frequency of testing will be less. You may accept or decline any test and still remain in the "active follow-up" group.

The other follow-up group is known as the "passive follow-up" group. In this group the schedule of clinic visits and the testing done will again be at least every 4 months until age 5 and then at least every six months. However, we will only collect information from routine tests which usually include a complete blood count and measurement of your child's height, weight and head circumference at each visit. We will also collect information about illnesses that your child has during that time and how they were treated.

If you choose to participate in either group, we will go to your child's medical record to collect clinical information about your child since he/she exited the BABY HUG treatment study, if this is necessary.

Regardless of which follow-up type you choose, you will be able to decide with your doctor's advice, whether you want your child to be treated with hydroxyurea during this follow-up period. The decision to treat your child is a clinical decision made only by your family and your doctor. Once the decision is made, you, your doctor, and the doctors working in the study will know that your child is taking hydroxyurea. You can decide to stop HU treatment of your child at any time. We will not tell you which treatment your child received during the BABY HUG treatment study at this time. We plan to tell you the results of the BABY HUG treatment study after the last child completes it, about July 2009. We will also tell you at that time, whether your child received hydroxyurea or placebo. At the end of the follow-up study your family will be informed of the results and any new recommendations from the study doctors.

STUDY TESTS TO BE PERFORMED

Passive Follow-Up Group

At each clinic visit every four to six months, we will ask you questions concerning your child's health since the last visit. We will record the results of physical examinations and laboratory tests performed as part of routine clinical care. If your child has any major health problems between clinic visits including the usual complications of sickle cell disease and especially those that require hospitalization, please tell the BABY HUG study staff immediately.

If your family plans to move from the area, the study coordinator will contact you at the appropriate intervals to obtain information on clinical events. We will request any information relevant to the study regarding your child's health from other health care providers. You will have 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, you agree to allow the reporting of the same clinical information as in passive follow-up, as well as additional tests to check the function of your child's spleen and kidneys, and neuropsychological development. These tests will be performed only once, approximately four years after your child was enrolled in the BABY HUG treatment study. Each of these tests may require a separate visit. In addition, a test will be done to measure the blood supply to the brain. This test will be performed twice, at approximately 3 and 4 years after you child was enrolled in the BABY HUG treatment study.

Tests requiring radiation to evaluate the function of the spleen and kidneys will be performed in exactly the same way they were done in the BABY HUG treatment trial. For each test, small doses of radioactive material will be given in 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. At the time of the kidney test, we will take three blood samples over four hours. In addition, the function of the kidneys will be checked by collecting a sample of urine for analysis.

In order to monitor your child's brain growth and development, neuropsychological testing will be performed. In the event your child develops symptoms suggestive of brain dysfunction, a Magnetic Resonance Imaging (MRI) test will be done. This test uses magnetic waves to form a picture of the brain. There is no exposure to radiation with this study. This test would be done even if your child was not part of the study at the time the symptoms occurred.

RISKS: For those in the active follow-up group.

The needle used to take blood or give radioactive material will cause a sharp pain at the time it goes into the skin. Sometimes a bruise will form at the place the needle goes into the skin. There is also a small chance of infection from the IV line or venous stick.

If your child takes part in this research, he or she may have one or more medical imaging studies. The tests your child may have include a liver-spleen scan and a DTPA scan. These tests involve a small amount of radiation which has been compared to the amounts that people encounter naturally in daily life from space and from rocks in the soil. This natural radiation is greater at higher altitudes. Your child would receive about the same amount of radiation as he or she would get from living in a high altitude city such as Denver for 1 ¼ years. The radiation dose we have discussed is what your child will receive from this study only and does not include any exposure he or she may have received or will receive from other tests.

BENEFITS

No one knows whether or not the study treatments given in BABY HUG treatment study 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 this medication to young children with sickle-cell anemia and how long to give it.

FREE CHOICE

Your child's participation in the long-term follow-up study is up to you and is your free choice. You are free to take your child out of this study at any time. If you take your child out of the study or do not take part in the study, we will still be willing to take care of your child as always. The choice other than to continue follow-up in the study is to go on with standard care for sickle cell anemia with your child's doctor. You and your child's doctor may plan to use or not use hydroxyurea. Some children with sickle cell anemia get other treatments that may be possible for your child. These treatments include blood transfusions and bone marrow transplants. Your choice to continue on in the study will not change the way your child is treated in our clinic. 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 laboratory tests or 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 your health insurance provider.

PAYMENT

Each family with a child in active follow-up at this clinic will receive $ 50.00 for each visit required to perform the additional tests needed, to cover the cost of travel, meals, other expenses to the family and use of time and resources for being in the study.

PRIVACY

You have a right to privacy. All facts in this study that can single out your child or family will remain private. A number system is used for patient files that does not allow patients to be known to anyone outside this center. 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. At the end of the study a computer file of the study results will be made for future use. 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.

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

Data may be given to the National Institutes of Health, the Food and Drug Administration or other U.S. or state agency as required.

LIMITS

The <<insert Clinical Center name>> is not set up to provide compensation for subjects who may incur injuries as a result of being in this research. 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.

You can discuss the rights of research subjects 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 with 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.

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 choose to have my child participate in the "passive follow-up" group.

Initials

_____ I choose to have my child participate in the "active follow-up" group.

I understand that I may withdraw my child from the study, should I so desire and that in so doing, I will in no way hurt my child's ongoing medical care at this medical center or elsewhere."

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

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

CHAPTER 4

STUDY ENDPOINTS

4.1 INTRODUCTION

The primary objective of the follow-up study is to monitor the continued safety and efficacy of HU treatment during the BABY HUG treatment study in the prevention of splenic and renal injury. Secondary endpoints include evaluation of hematological parameters, measures of renal and splenic function and evaluation of growth and neuropsychological development. The follow-up study will provide additional data for these primary and secondary endpoints for at least two years after the child's participation in the BABY HUG treatment study. The fact that some patients will be on open-label HU during follow-up will allow the assessment of the effects of early, late and continuous treatment with HU.

4.2 PRIMARY ENDPOINTS

4.2.1 Spleen Scintigraphy

Liver-spleen scan will be performed after two years of additional follow-up. The results of this scan will be compared to the two scans performed at initial screening and completion of the BABY HUG treatment study. Thus, pre-treatment, post-treatment and follow-up scans performed at time zero, two and four years respectively 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 they will be blinded to treatment assignment and not be involved in the acquisition of the images.

4.2.2 Kidney-Glomerular Filtration Rate (GFR)

GFR will be assessed in 3 ways during the follow-up period. Measurement of the GFR will be determined by the performance of a DTPA clearance study four years after the child's enrollment in the BABY HUG treatment study. The results of this measurement will be

compared to previous measurements of the GFR made by DTPA clearance studies done at the initiation and completion of the BABY HUG treatment study. This will give an assessment of the rate of change in GFR and the effects of HU on that change over a four-year period that includes points along the combined continuum of treatment and follow-up studies at times zero, two and four years.

Central laboratory measurement of serum creatinine will be performed once at four years after the child's enrollment in the BABY HUG treatment study. This will be used to estimate the GFR according to the Schwartz formula, GFR=kL/Pcr, where L = the body length in centimeters, Pcr = the plasma creatinine concentration in mg/dl, and k = 0.55 mg creatinine/100 min x cm x 1.73 m^2.

Measurement of Cystatin C will be performed at the Clinical Chemistry Laboratory at St. Jude's and will also be performed once at four years after the child's enrollment in the BABY HUG treatment study. This will also be used to estimate the GFR.

4.3 SECONDARY AND SAFETY ENDPOINTS

4.3.1 Hematologic Parameters

Patients on passive follow-up will have a CBC done at each clinic visit and those patients on open label HU will have additional laboratory monitoring in accordance with routine local clinical care.

Patients on active follow-up will have a local CBC done at each regular clinic visit and those on open label HU will also have a fetal hemoglobin level measured. Patients in the active follow-up group who are not on open label HU will have HbF measured during scheduled visits as noted in Appendix A. They will be compared to pretreatment and treatment levels during BABY HUG. The hemoglobin and mean corpuscular volume (MCV) will be evaluated based on the child's comparison group.

4.3.2 Spleen

Serial pitted red cell counts and quantitation of Howell-Jolly bodies will be performed once, four years after the child's enrollment in the BABY HUG treatment study. The results of the pitted red cell counts will be compared with those performed during the BABY HUG treatment study at screening, 6, 12, 18 months and at completion. This will provide an assessment of changes that have occurred over a four-year period along the combined continuum of treatment and follow-up studies that includes points at times zero, 6, 12, 18, 24 and 48 months. The results for the Howell-Jolly bodies will be compared with those performed at screening and completion of the BABY HUG study. This will provide an assessment of changes that have occurred over a four-year period along the combined continuum of treatment and follow-up studies that include points at times zero, 24 and 48 months.

4.3.3 Kidney Function

Urinary concentrating ability will be measured once, four years after the child's enrollment in the BABY HUG treatment study. The results of these measurements will be compared to the measurements made at screening and completion of the BABY HUG treatment study. This will provide an assessment of the rate of decline in renal papillary function over a four-year period that includes points along the combined continuum of treatment and follow-up studies at times zero, two and four years.

4.3.4 Central Nervous System

Evaluation of the cerebrovascular circulation will be done by TCD performed at 3 years and 4 years after the child's enrollment in the BABY HUG treatment study. The results of these measurements will be compared to the studies performed at screening and completion of the BABY HUG treatment study. This will provide an assessment of the changes that have occurred over a four-year period along the combined continuum of treatment and follow-up studies that includes points at times zero, two, three and four years.

Neuropsychological evaluation with the WPPSI test will be performed once, four years after the child's enrollment in the BABY HUG treatment study. These test results will be compared with the Bayley and Vineland tests performed during the BABY HUG treatment study at screening, 12 months and at completion. This will provide an assessment of changes that have occurred over a 4-year period along the combined continuum of treatment and follow-up studies that includes points at times zero, 12, 24 and 48 months.

4.3.5 Safety Endpoints and Clinical Events

Patients treated with open-label HU will be monitored locally for bone marrow depression with complete blood counts as needed consistent with local clinical standards. Other adverse reactions and toxicity associated with HU treatment will be recorded at each visit.

All patients will be monitored for the occurrence and severity of clinical events. These will be identified by asking standardized history questions at each clinical visit. At each visit, parents will be asked to describe any illnesses experienced since the last visit. If illness is reported, a directed history will be obtained to allow its characterization. Only serious adverse events will be collected in the follow-up study. These are listed in Table 4.2. They will be reported on adverse event (AE) forms to the MCC within seven days. Documentation (discharge summaries, clinic/emergency department records, local laboratory values or radiology reports) for all fatal or life threatening events will be collected by Clinical Center staff for review. Life threatening events include, but are not limited to, bacteremia, meningitis, osteomyelitis, and any event for which the patient receives a transfusion or undergoes surgery. Selected events and supporting documentation collected by Clinical Center staff will be reviewed centrally by two pediatric hematologists who are not a part of the BABY HUG Clinical Center.

4.4 STATISTICAL ANALYSES OVERVIEW

The analysis of the primary study outcomes of the full Phase III trial will be conducted according to the randomized and self-selected treatment groups to which they have been assigned.

The combinations of randomized treatment assignments and open-label self-selected treatment decisions will create an unbalanced factorial study with factors represented by an early treatment (the BABY HUG treatment study randomized comparisons) and late treatment (the self-selected treatment decisions of the parents whose children are in the follow-up study). The four groups can be represented as shown in table 4.1.

Table 4.1

Four Comparison Treatment Groups*

		Late Treatment: Yes	Late Treatment: No
Early Treatment	Yes	Continuous HU Treatment	Early HU Treatment Only
	No	Late HU Treatment Only	No HU Treatment

*As described in the text above

The main effects and interactions to estimate from this design are: the short term effects of HU, the late treatment effects of HU, and whether early plus late treatment provide extra benefit than either alone (the interaction test). All statistical comparisons will be carried out using this design model. Statistical testing will be performed by testing each main effect and interaction. To limit the number of spurious associations, alpha levels of 0.02 will be used to indicate statistical significance when performing spleen comparisons and 0.01 for the GFR comparisons. All tests will be performed with two-sided alternatives. There will be more than

90% power to detect a 50% reduction in the incidence of categorical worsening of spleen function, if 60% or more of patients assigned to placebo experience categorical worsening of spleen function (80% are expected to experience this effect by 3 to 4 years of age). There will be more than 90% power to detect differences of GFR of 5.5 ml/min/1.73 m^2, if the standard deviation is 10 ml/min/1.73m^2. A more detailed presentation of anticipated analyses is provided in Section 4.5.

4.5 STATISTICAL CONSIDERATIONS IN DESIGN AND STUDY SIZE

4.5.1 Primary Treatment Comparisons

Most of the studies conducted in the BABY HUG follow-up study will compare the early and late effects of treatment with hydroxyurea (HU) to no treatment. The early treatment comparisons will be equivalent to continued comparisons of the randomized groups created in the BABY HUG treatment study. The late treatment comparisons will be accomplished by comparing the infants whose parents elect to start HU treatment after completion of the BABY HUG treatment study to the infants whose parents elect not to have their infants take HU after completion of the BABY HUG treatment study. This follow-up study will have two primary endpoints: 1) the proportion of infants with decreased or absent spleen function and 2) kidney damage measured by glomerular filtration rate (GFR).

The primary analyses will be carried out using a factorial design with two factors representing treatment with HU (yes or no) in the early and late treatment periods. The spleen endpoint will be tested at an overall alpha = 0.06 and the kidney endpoint will be tested at overall alpha = 0.03 (0.01 for each factorial analysis comparison). There will be no interim monitoring plan proposed for either of these endpoints. An interim monitoring plan will be constructed to monitor the appearance of adverse events and potential toxicities.

The features common to power evaluations for these two endpoints are the critical values used to determine the alpha level and power of each test. We have designated these

values as Z_α and Z_β respectively. "N" is the number of children in the study. We have presented the formulas for study size calculations, but each of these formulas can be algebraically rearranged to provide corresponding power calculations.

4.5.2 Spleen Endpoint

Study size calculations for analyses 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 proportion of events in the different groups. We intend to use a logistic regression model to analyze the three different contrasts associated with the factorial design. It is anticipated that the interaction test will have very low power. Therefore, we will focus on testing and estimating the main effects of the analysis which will seek to determine: 1) if early treatment with HU affects the likelihood that a spleen will be functioning 48 months after a child has entered the BABY HUG treatment study and 2) if late treatment with HU affects the likelihood that a spleen will be functioning 48 months after a child has entered the BABY HUG treatment study. Although a logistic regression model will be used, a study size calculation for a two-group comparison provides similar power and sample size calculations for the proposed analyses. The power analysis is calculated using an equivalence hypothesis strategy. In our example we will carry out the power analysis for early treatment and then discuss the differences that may be in place for testing the late treatment hypothesis.

Let p_1 be the probability that the spleen is not functional four years after treatment initiation for patients assigned to receive HU during the BABY HUG trial (early HU treatment) and p_2 be the probability that the spleen is not functional four years after treatment initiation for patients assigned to receive placebo. The overall alpha level of this comparison will be alpha = 0.02 (two-tailed). An equivalence hypothesis requires that the new treatment be no worse than the old (placebo) treatment. In establishing an equivalence hypothesis, one must specify a

difference “d” that is not significant clinically, and the hypothesized spleen failure rate by four years of follow-up in infants treated with HU (p_1). We estimate this difference to be 40%.

We will test the null hypothesis:

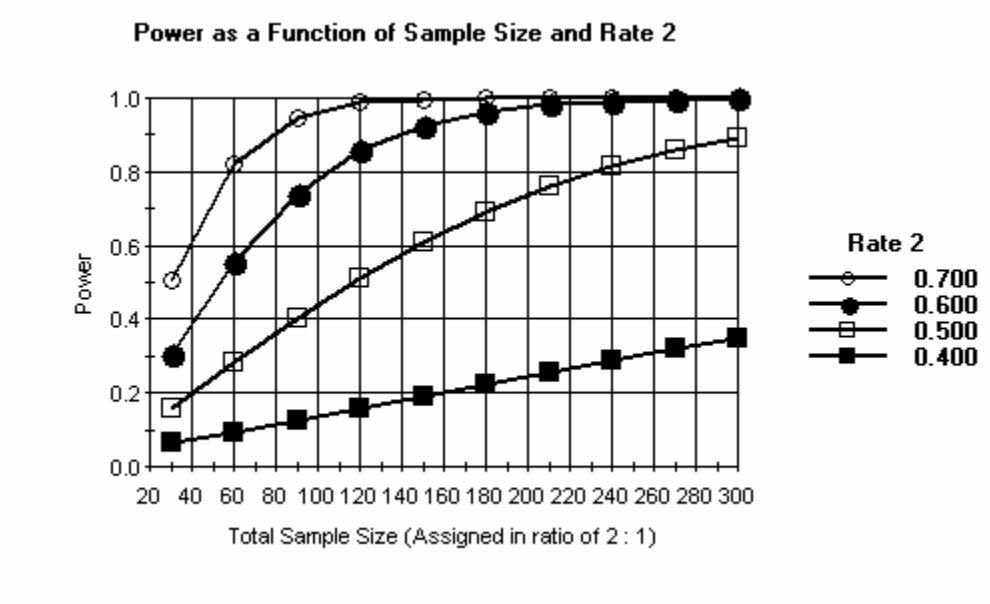
H_O: $p_1 - p_2 - d \leq 0$

versus the alternative:

H_A: $p_1 - p_2 - d > 0$.

Figure 4.1 shows that the spleen failure rate in the placebo group will have to be large (greater than 0.55) for the proposed study design to show with adequate power that postponing HU treatment is not an equivalent strategy to early administration of HU to the infant.

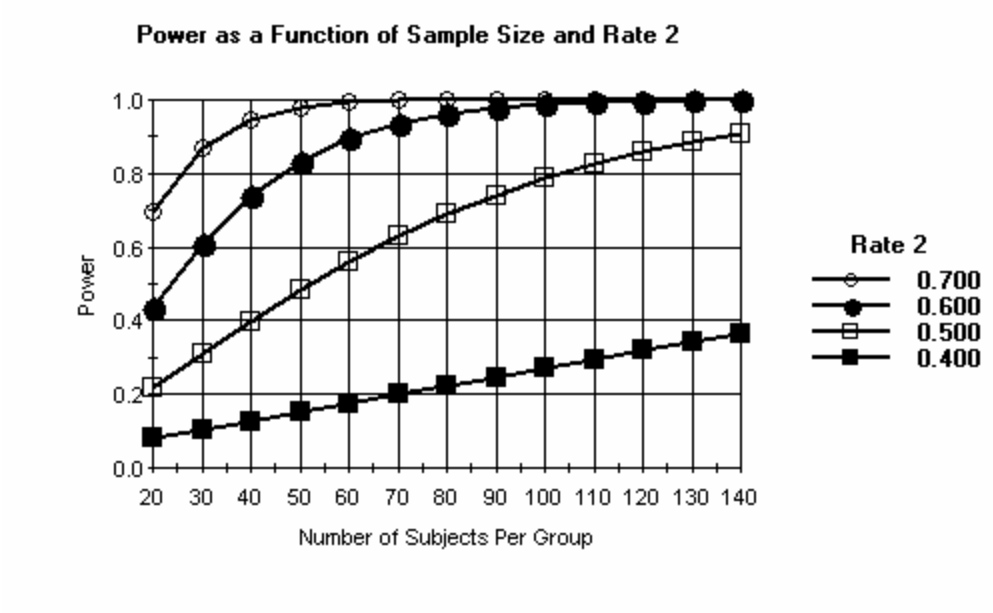
Figure 4.1
Power to show that the event rate for placebo treatment is at least as high as the event rate for HU treatment
Power as a Function of Sample Size, Rate 2 (PBO Treatment)



Alpha = 0.020, Tails = 1, Rate 1 (Early HU) = 0.400,
Acceptable Difference = 0.100

An analogous argument can be made for late treatment with HU. The only difference is that the sample size may be unbalanced for this comparison. If there are equal numbers of parents who elect for late treatment and no late treatment, Figure 4.1 can also be used to review the operating characteristics for the late treatment hypothesis if there is equal allocation to the open-label HU (late treatment) ones. If the sample is unbalanced in allocation for this hypothesis, Figure 4.2 can be used to address the hypothesis for the circumstance in which twice as many parents opt for late HU treatment as opt for no late HU treatment. Again, the no clinical difference effect is assumed to be 0.1.

Figure 4.2
Power to show that the event rate for no late HU treatment is at least as high as the event rate for late HU treatment
Power as a Function of Sample size, Rate 2 (no late HU treatment)



Alpha = 0.020, Tails = 1, Rate 1 (Late HU) = 0.400,
Acceptable Difference = 0.100

The operating characteristic of a comparison with unequal allocation is similar to equal allocation so long as the imbalance does not exceed more than 3 to 1. In both evaluations, it

will be necessary for the no treatment group to have a slightly worse failure rate (higher) in order to disprove the equivalence hypothesis

4.5.3 Analysis of GFR

The glomerular filtration rate, (GFR) is a continuous variable and will be evaluated from the DTPA GFR measurement taken four years into the infant's follow-up interval (two years after the infant exited the BABY HUG treatment study). A factorial design will be used in which means can be compared for early HU use (in the BABY HUG treatment study), late HU use (elective use of HU after BABY HUG follow-up is complete) and the interaction of the two factors. Sample size formulas for each of these two comparisons are similar to a two-sample t-test of independent samples which is presented below:

$$N = \frac{(Z_a + Z_b)^2}{a\ (1-a)(\mu_c)^2}$$

In the above equation: $\mu_t - \mu_c$ is the expected difference between two means (e.g., μ_t = GFR for children assigned to HU in the BABY HUG treatment study and μ_c = GFR for children assigned to PLBO in the trial) and σ^2 is the common variance of the continuous variable; N = the total study size, a = the proportion of infants assigned to group t and (1-a) = the proportion of infants assigned to group c. In a balanced design, a = 0.5.

Two differences in this unbalanced factorial design and a simple two-sample t-test are: 1) the allocation percentages in the late treatment groups may be different from 0.5. and 2) the expected values for the factors being compared are averaged over other factors in the design. For the latter difference, the "averaging operator" will be represented by the symbol ".". Thus the average effect that can be expected from infants randomized to the early treatment HU arm of the BABY HUG study will be "$\mu_{t.}$" and the average effect that can be expected from the early treatment PLBO group is represented by "$\mu_{c.}$" (see next page). Both of these means are averaged across the subsequent treatment groups determined by the parent-physician

determinations of the treatment regimen for the infant after the BABY HUG study treatment is stopped. The interaction test in this design addresses the question about whether the four-year follow-up difference between the group randomized to HU and the group randomized to PLBO among the group who keep taking HU in the late-treatment interval is the same as the difference between the two corresponding groups among the infants whose parents elect not to take HU in the late-treatment follow-up interval. The null and alternative hypotheses for each of the three tests that will be performed in this analysis plan are presented below. Each contrast in this analysis of GFR will be tested at an alpha of 0.01 (two-tailed). Letting the indicies t, c, h, and n indicate: early randomized treatment with HU, early randomized treatment with placebo (control), late physician/parent decision to treat the child with HU, and late physician/parent decision to not treat with HU respectively:

We will test for the early treatment effect,

H_O: $\mu_{t.} = \mu_{c.}$

versus the alternative:

H_A: $\mu_{t.} \neq \mu_{c.}$;

for the late treatment effect:

H_O: $\mu_{.h} = \mu_{.n}$ versus the alternative:

H_A: $\mu_t \neq \mu_c$,

and for the interaction effect:

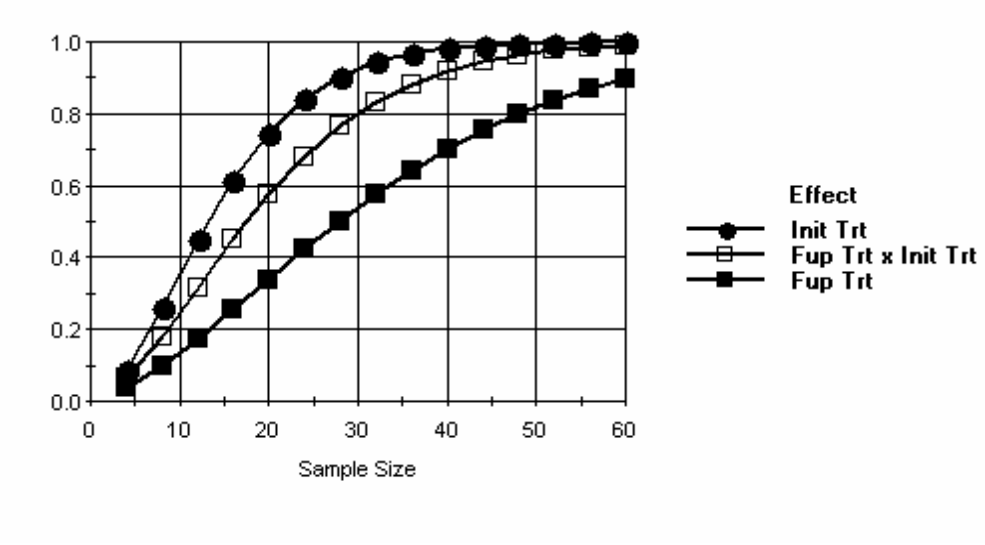
H_O: $\mu_{th} - \mu_{tn} = \mu_{ch} - \mu_{cn}$

versus the alternative:

H_A: $\mu_{th} - \mu_{tn} \neq \mu_{ch} - \mu_{cn}$.

Below we present Figure 4.3 showing the power to detect two different treatment effect sizes and an interaction effect size.

Figure 4.3*
Power of a 2 X 2 Factorial as a Function of Per-Cell Sample size



Initial Treatment Effect = 0.75 SD
Follow-up Treatment Effect = 0.5 SD
Initial x Follow-up Treatment Effect = 0.64 SD

Alpha = 0.010, Tails = 2

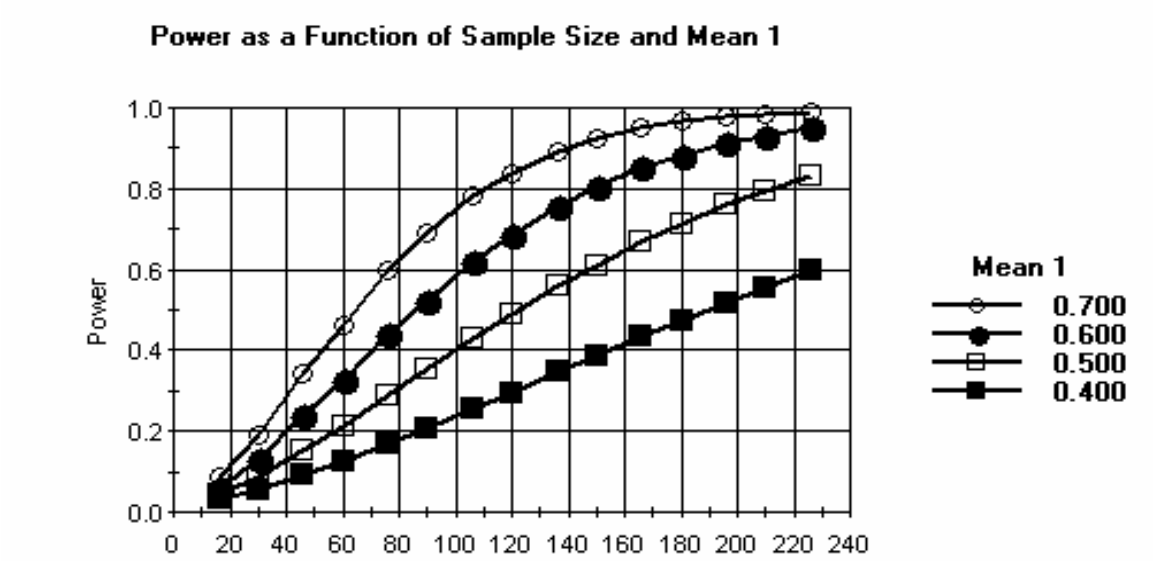
*This assumes there is equal allocation in the late treatment groups.

There will be adequate power (greater than 80%) to detect moderate to large effect sizes (greater than 0.5) when testing each contrast at the 0.01 alpha level.

As has been mentioned, however, this study will not have the luxury of assigning infants to the late-treatment groups and the elective nature of the late-treatment assignments could result in unequal numbers in the HU late treatment group and the no late treatment group. To address this issue, Figure 4.4 presents a power analysis for a simple t-test with unbalanced allocation. In this example, the allocation has been skewed 2 to 1 in favor of the HU late-treatment group. Comparison of the two figures shows that there is little difference between the operating characteristics whether or not equal allocation occurs. As long as the elective

allocation to late treatment is not severely skewed (greater than 3 to 1 in favor of either late treatment group) adequate power will remain to detect moderate to large effects sizes using the proposed design.

Figure 4.4*
Power as a Function of Sample size, Mean 1



*Total Sample Size Assigned in ratio of 2:1. Alpha = 0.010, Tails = 2, Mean 2 = 0.000, SD = 1.000

The estimated mean GFR for PLBO children in this study is 162 ml/min/1.73m^2 and the estimated standard deviation is approximately 10 ml/min/1.73 m^2. Using these estimates, the proposed study size of 100 children per group would be sufficient to detect a real difference of means within each factor of 5.0 to 6 ml/min/1.73 m^2.

4.5.4 Data Analysis

4.5.4.1 Introduction

Primary analyses for the BABY HUG follow-up study will focus on estimating treatment effects on the designated co-primary endpoints: loss of spleen function and GFR. Assessment of treatment differences will be based on pooling data across all participating Clinical Centers using all patients entered. Secondary analyses will develop statistical models to determine associations and relationships between dependent variables, risk factors and the treatment variable. 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 serial neuropsychological tests, weight, height, etc. will use longitudinal data analysis models (Laird and Ware, 1982; Schlucter, 1992; Liang and Zeger, 1986; Andersen and Gill, 1982). 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 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 primary 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. Patients who are lost to follow-up before the end of the follow-up interval will be censored at the time of their last visit.

4.5.4.2 Regression Analyses and Adjustment

We will adjust study results for potential confounding factors in secondary analyses. The most important of these analyses may test whether the distributions of the primary and secondary endpoints differ by the age of enrollment of the infant. For these evaluations, we will divide the infant population into those who are less than 15 months of age at the time of enrollment into the Baby Hug treatment study and those who are 15 months of age or more at the time of enrollment.

Tests of interaction will be performed to determine if treatment effects are different in these two groups of infants. We plan to use regression methods to evaluate the significance of the hypothesized interactions. For instance, this will be done by multiplying an indicator variable for the birth cohort (1 = less than 15 months, 0 = 15 months or older) times treatment (1 = HU, 0 = PLBO) and evaluating the beta coefficient associated with this variable. If significant ($p < 0.01$), we will report that treatment effects differed according to the age of enrollment. Interaction tests such as these are low in power to detect specified alternatives (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.

The addition of confounding variables generally improves the operating characteristics of analyses of the main effect, but given the small sample size, the number of confounding variables will be small in any secondary analysis. Given the small sample size proposed for this analysis, it may be necessary to limit the number of confounders in any one analysis to five or ten. We will use step-wise regression methods to isolate and include the most important confounding variables in the regression model.

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.

4.5.4.3 Missing Data - Prevention and Analysis

We will generally use the methods of Rubin (Little and Rubin, 1987) to impute missing data from patients' records with complete data to complete the records for patients with missing data. This method has been accepted by the U.S. Food and Drug Administration (FDA) as a legitimate method for correcting for missing data. We will also use analyses involving rank statistics in which patients who die or have bad clinical events are given the worst rank for other dependent variables. This technique has been used successfully in the analysis of the MSH (McMahon et al, 1992; 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.

4.5.5 Interim Monitoring

During the course of this study, the BABY HUG Data and Safety Monitoring Board (DSMB) will carry out interim data analyses to monitor for evidence of beneficial and adverse treatment effects. The interim analysis reports for the DSMB will include, but are not limited to comparisons by treatment group for:

1. Primary, secondary and safety endpoints.
2. Serious Adverse Events
3. Distribution of baseline characteristics:

    Gender

    Age at Entry

    Initial (BABY HUG 2- year) outcome of:

        Spleen and Kidney Function

        Transcranial Doppler Measurements

        Neuropsychological Testing Performance

The DSMB will also review semi-annual reports on Clinical Center study performance including recruitment, completeness of follow-up, complications of treatment, submission of forms and quality of laboratory and forms data submitted, patients lost to follow-up, and protocol violations.

Monitoring Safety and Adverse Events

The DSMB Chair, Executive Secretary of DSMB, and NHLBI and NICHD Project Officers will review semiannual reports including:

1. Patient characteristics at baseline (at completion of the BABY HUG treatment study)

   Age, race and gender

   Spleen function

   Spleen size

   Pitted cell counts

   DTPA clearance for GFR quantitation

   Schwartz equation GFR estimates

   Cystatin C estimate of GFR

   Urine concentrating ability

   Neuropsychological Testing Performance

   Height, weight, head circumference

   Transcranial doppler (TCD) measurements

2. Blood count toxicities
3. Dose adjustments
4. Safety assessments and adverse events

   Serious adverse events: update tallies, rates and individual summaries

   (case reports) for immediate review by DSMB Chair, Executive Secretary of DSMB and Project Officer

4.5.6 Safety Related Outcomes

Adverse event (AE) forms for SAEs will be submitted to the MCC and tabulated based on the affected organ system according to standardized monitoring procedures (e.g., MEDRA). These will be tabulated and a systematic review will be made to determine if one treatment group has more reports of SAEs than the other treatment groups. Depending on the evidence accumulated, it will be the responsibility of the DSMB Chair, the Executive Secretary of DSMB and the Project Officer to decide whether a full meeting of the DSMB 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 9.2.5 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 DSMB and the Project Officer in consultation with the DSMB Chair will then recommend whether there should be an emergency meeting (or conference call of the DSMB) to determine the appropriate actions for this trial.

Table 4.2

Definition of Serious Adverse Events

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

1. Death
2. Life-threatening events
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 sickle cell 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 clinical trial.

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

CHAPTER 5

OPEN-LABEL TREATMENT WITH HYDROXYUREA

5.1 OVERVIEW

Treatment with open-label HU during the follow-up trial is at the discretion of the parent or guardian after consultation with the child's primary care physician. Administration and monitoring of open-label treatment with HU will follow all accountability and reporting requirements of the FDA. Regardless of the follow-up group in which the family chooses to have their child participate, all patients on open-label HU will be systematically followed to monitor for toxicity. All blood tests performed for this purpose will be collected and processed locally, except for those noted in Section 5.3 below. 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.

Each child's supply of HU will be provided by the Clinical Center before the patient's scheduled visit. Cost of treatment and monitoring will be borne by the family and their child's insurer.

5.2 DOSE TITRATION OF OPEN- LABEL HYDROXYUREA

While the use of open-label HU will not be specifically regulated as is done in the BABY HUG treatment study, attempts should be made to standardize its use. The following guidelines are suggested:

- Patients should begin open-label treatment with HU at the same dose as the dose of the BABY HUG study drug.

- The dose of HU should be increased by 5 mg/kg every 6-8 weeks if there is no toxicity to a maximum tolerated dose of 27-35 mg/kg in accordance with local clinical care.
- A CBC should be checked at least monthly while receiving hydroxyurea.
- Predetermined toxicity levels should be utilized to evaluate the monitoring blood counts. The toxicity values should be no lower than an ANC<1250, platelet count <80,000, or a hemoglobin level below 6 gm/dl or greater than a 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.
- Interval to repeat blood count after toxicity develops should be at least 5 days and no more than 14 days.
- Other toxicity is defined as:
    1. doubling of the baseline serum creatinine level with a current value >1.0 mg/d
    2. increase in alanine aminotransferase (ALT) to >150 IU/L
    3. bilirubin >10
    4. unexplained gastrointestinal disturbance
    5. unexplained rash or hair loss.
- If toxicity occurs 2 times at the same dose consideration should be given to reducing the dose by 2.5 mg/kg when it is restarted. An attempt should be made to re-escalate the dose if the patient is without subsequent toxicity for 6 months.

5.3 MONITORING FOR TOXICITY

Prior to the first study visit at 4 months, CBCs with differential and reticulocyte counts for HU monitoring and monthly assessments for toxicity will be performed as needed in accordance with local care standards. Patients enrolled in the follow-up study within two months after completion of the BABY HUG treatment study must have all lab data from visits during this time reviewed only by the Primary Endpoint Person (PEP) and the MCC staff in order to ensure that blinding accomplished in the treatment study is maintained. The PEP will determine if toxicities

are present. The MCC staff will also screen these local CBC reports for toxicities. If there are toxicities, the PEP will report these to the local Clinical Center. The Clinical Center will treat the patient in accordance with routine local clinical care or may follow the paradigm described above in section 5.2. Information related to the treatment given will be reported to the PEP. The MCC will also review this treatment.

Patients enrolled in the follow-up study more than 2 months after completion of the BABY HUG treatment study will not require review of their labs by the PEP.

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 BABY HUG PI at each Clinical Center. The results will be reported to the MCC for review. The nurse coordinator and all clinical staff may view all of the laboratory data from each study visit.

5.4 DURATION OF STUDY TREATMENT

For those parents (or guardians) who decide to have their child treated with open-label HU, the goal of the follow-up study treatment plan will be to maintain the patient on HU for at least 24 months.

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

CHAPTER 6

LABORATORY ANALYSES AND SPECIMENS

6.1 OVERVIEW

All routine hematology and chemistry specimens will be processed locally. For patients on active follow-up, the Hematology Core laboratory will process specimens for fetal hemoglobin. Three other core laboratories; the Pitted Cell Core, TCD Core and HU Assay Core and the NHLBI Specimen Repository will be utilized for the processing of study specimens. Specimens for Howell-Jolly bodies will be processed according to section 9.4 of the BABY HUG treatment study protocol. The amount of blood and times of collection are specified in Appendix A.

6.2 PITTED CELL CORE LABORATORY

After 24 months of follow-up, one drop of blood will be preserved in gluteraldehyde for each child enrolled. These specimens will be stored and refrigerated locally, and shipped in batches to the Pitted Cell Core Laboratory.

6.3 CYSTATIN C LABORATORY

Specimens for measurement of Cystatin C will be processed according to section 9.11 of the BABY HUG treatment study protocol.

6.4 TCD CORE LABORATORY

A TCD Core Laboratory supported by funds from a separately awarded grant will be responsible for the performance and central evaluation of TCD studies performed at 12 and 24 months after the child's completion of the BABY HUG treatment study.

6.5 NHLBI SPECIMEN REPOSITORY

The Hematology and Biochemistry Core Laboratory will conserve residual plasma, serum, and cell pellets for shipment to the NHLBI Specimen Repository where they will be kept in a bank of samples for the purpose of ancillary studies approved by the Steering Committee. At the end of the study, a limited amount of data will be provided to be linked to the specimens (e.g., whether they come from a male or female child). After appropriate IRB review, the specimens will be available

for anonymized use provided consent was given at the time the child was recruited into the follow-up study. No DNA will be extracted or saved. Anonymized specimens will not provide any link to the individual from whom the specimen(s) were obtained.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP 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, whether or not the patient is receiving open label HU. The cooperation of all medical staff involved in the clinical care of study patients will be solicited to enhance patient adherence to the follow-up study protocol. Parent education and guidelines for the diagnosis and treatment of common clinical events is addressed in the BABY HUG treatment study protocol section 8.4. At no time should the performance of the 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.

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 STUDY PROTOCOL

CHAPTER 8

SPECIAL STUDIES AND READING GROUPS

8.1 INTRODUCTION

Special studies and event reports that will be centrally evaluated by individuals blind to treatment assignment and independent of the BABY HUG Clinical Centers include liver-spleen scans and pitted cell counts.

8.2 PITTED CELL COUNTS

Pitted cell 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. Pitted cell counts will be performed from specimens collected from active follow-up patients once, at four years after the child's enrollment in the BABY HUG treatment study.

8.3 LIVER-SPLEEN SCANS

Tc99m sulfur colloid liver-spleen scans will be performed once, at 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 initial screening and at the completion of treatment in BABY HUG. 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 3 pediatric radiologists who are unaware of the treatment assignment of the patient. The reading process has previously been described in section 9.5 of the BABY HUG treatment study protocol. The proportion of patients in each treatment group over the 4 years of combined treatment and follow-up classified by spleen function (normal, decreased, or absent) will be compared. In addition, the number of comparisons between the three scans in each patient demonstrating a decline from one category to another (0, 1 or 2) in splenic function will be compared in each

treatment group. Triplet scans that demonstrate an improvement in uptake will be scored as not demonstrating a decline.

8.4 CLINICAL EVENTS

Description of Clinical Events will be submitted on Clinical Event Forms to the MCC where they will be classified by independent pediatric hematologists or neurologists. Independent review for adjudication by a third physician will be performed in the case of disagreement. Definitions of clinical events can be reviewed in Appendix C.

8.5 TRANSCRANIAL DOPPLER (TCD)

Transcranial Doppler studies will be performed by trained technicians from a Central Laboratory located in the Medical College of Georgia (MCG), Augusta, Georgia, or by local Clinical Center examiners trained by the MCG technicians. The Central Laboratory will perform standardized readings of the studies.

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

CHAPTER 9

FOLLOW-UP PROCEDURES

9.1 INTRODUCTION

Patients in both the active and passive follow-up groups will have clinic visits every four months until age 5 then every six months until the common termination date, December 2011, is reached. The first patient was recruited into the BABY HUG treatment study in October 2003 and it is anticipated that the last patient will be recruited in July 2007. Therefore, the total follow-up period will range from 4 years and 6 months to 8 years and 2 months.

9.2 FOLLOW-UP VISITS

Retrospective data abstraction will be carried out for all patient visits that occur between the treatment study and enrollment in the follow-up study. The 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 the patients will be evaluated in accordance with routine local clinical care. At each visit during this period the evaluation will include an interval medical history and information regarding adverse events and toxicity for those patients on HU. In addition, height and weight will be recorded as well as head circumference up until the age of 5. Testing performed during follow-up clinic visits will depend on whether the patient is in the active or passive follow-up group.

9.2.1 Active Follow-Up Group

The active follow-up protocol will involve the collection of many of the primary and secondary endpoint measurements that were collected in the BABY HUG treatment study. These will include: Hgb F, Pitted Cell Counts, Howell-Jolly Bodies, liver-spleen scan information (quantitative and qualitative), DTPA GFR measurements, multi-digit serum creatinine (for the calculation of the Schwartz estimate of GFR), Cystatin C, urine concentrating ability, TCD, and

neuropsychological testing. The schedule of patient visits during which specimens will be collected for laboratory testing is shown in Appendix A. All primary and secondary endpoints will be measured four years after the child's enrollment in the BABY HUG treatment study. This schedule is designed to measure all endpoints along the combined continuum of treatment and follow-up at times zero, two and four years.

9.2.2 Passive Follow-up Group

Patients in this group will have laboratory testing limited to those tests performed in accordance with routine local clinical care including HU monitoring for those children being treated with HU. These tests will be performed locally. Study data will be abstracted from the medical record.

9.2.3 Collection of Laboratory Data

As noted in section 5.3, only the PEP will review the local laboratory results for patients enrolled in the follow-up study within 2 months after the completion of the BABY HUG treatment study. The PEP will enter these data into the BABY HUG database via the Internet Data Entry System within seven days of receiving them and keep these in a locked file with no access by BABY HUG Follow-Up staff. For all study visits after this 2 month period, the local Clinical Center staff will collect and enter laboratory data.

9.2.4 Adverse Event Reporting

As noted in Section 4.3.5 of the protocol only SAEs including, but not limited to, those as defined in Table 4.2 will be reported on the AE form (Form 50).

Event forms will be submitted to the Medical Coordinating Center (MCC) and tabulated based on the affected organ system. Each SAE will be reported to the MCC within 7 days of the event and supporting information will be provided by the Clinical Center. MCC staff will annotate the report regarding whether the patient is on open label HU and send the information to the NHLBI and NICHD Project Officers, the FDA, the Executive Secretary of the DSMB, and the DSMB Chair

for review. The occurence of an SAE will be reported to the Clinical Center IRB and the FDA within 7 days of NHLBI review.

If any study patient dies, efforts will be made to obtain complete post-mortem information. Discharge summaries and narratives of fatal events will be sent with study forms to the MCC.

Any serious adverse events (as defined by the FDA) which are not included in the above list will be summarized and reported semi-annually. The Clinical Centers will be required to provide supporting information using a MedWatch Form 3500A for the events listed in Table 4.2.

In addition to this reporting mechanism, a centralized over-ride system will be carried out by individuals not involved with the study. These individuals will review adverse events that are not thought to be serious by the study investigators and make an independent judgments about whether an adverse event is "serious" and reportable to the FDA. The two central review individuals will be the NHLBI Project Officer and MCC Medical Consultant. Either of these individuals will have the ability to elevate an adverse event being reported to the MCC to the "serious" category which will precipitate the collection of the required information for the Med Watch Form 3500A and a subsequent report to the FDA.

Serious adverse events will be listed individually and according to body system, degree of severity (severe, life-threatening, or fatal), and likelihood of relation to HU treatment (not related, possibly, probably or definitely related), and classified according to action taken (none, treatment stopped or interrupted, specific treatment instituted) and outcome (recovery without change in previous condition, some impairment, significant impairment, or death).

The NHLBI Data and Safety Monitoring Board will review the protocol at six month intervals. A progress report showing results according to each of the four groups based on their treatment combinations (see section 2.3) will be forwarded by the MCC to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB members will also be provided

with reports documenting each child's growth, development and progress after each visit. The DSMB may recommend early termination of the study for considerations of safety or efficacy**.**

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

CHAPTER 10

CLOSE-OUT PROCEDURES

10.1 OVERVIEW

As noted in section 9.1, the anticipated common termination date is December 2011. Because the last patient recruited into the BABY HUG treatment study will complete the trial by July 2009, it is estimated that the treatment trial will be completed and results disseminated during the follow-up study. In the event that HU is found to be safe and efficacious the follow-up study will be completed. Data processing and analysis of final study data will be performed.

If the interim or final analysis of the BABY HUG treatment study demonstrates that HU is not safe and or efficacious the follow-up study will be terminated early, patients on open label HU will be advised to stop treatment and follow-up study event data processing and analysis will be performed.

10.2 DEBRIEFING CONTACT

After final long-term follow-up data have been collected and final reports on the results prepared for presentation or submitted for publication, each patient's family will be scheduled for a debriefing contact. The families will be informed of the results of long-term follow-up and any reconsideration of the recommendation 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. The Data and Safety Monitoring Board will review the final data in each of the four comparison groups represented in table 4.1, including analyses for efficacy and safety, at a planned final meeting. The data, upon which the determination of long-term safety and efficacy of HU and of the optimal time of initiation and duration of treatment is made, will form the basis of the final

consensus recommendations from the DSMB, Steering Committee and the NHLBI. These consensus recommendations will be shared first with the study patients' families and will be made public as soon as possible thereafter.

A final data closure, based on cleaned and complete data reporting, will be available for submission to the FDA and for final archival and databank studies. 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 patient 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 patient outcomes in the study.

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 NHLBI/NICHD subcontracts. The MCC will archive study data in accordance with FDA guidance and National Heart, Lung, and Blood Institute requirements. Public data files will be made available according to National Heart, Lung, and Blood Institute policy. The NHLBI/NICHD will finalize the disposition of the IND report(s) according to their agreement with the FDA under the IND.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP 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.

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 and at the operational central units. Treatment of study patients, however, 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 Data and Safety Monitoring Board (DSMB), 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. An organizational chart for this study is presented in Exhibit 11.1. A list of participating centers is found in Exhibit 11.2.

Exhibit 11.1
Pediatric Hydroxyurea Phase Clinical Trial (BABY HUG)
ORGANIZATIONAL CHART

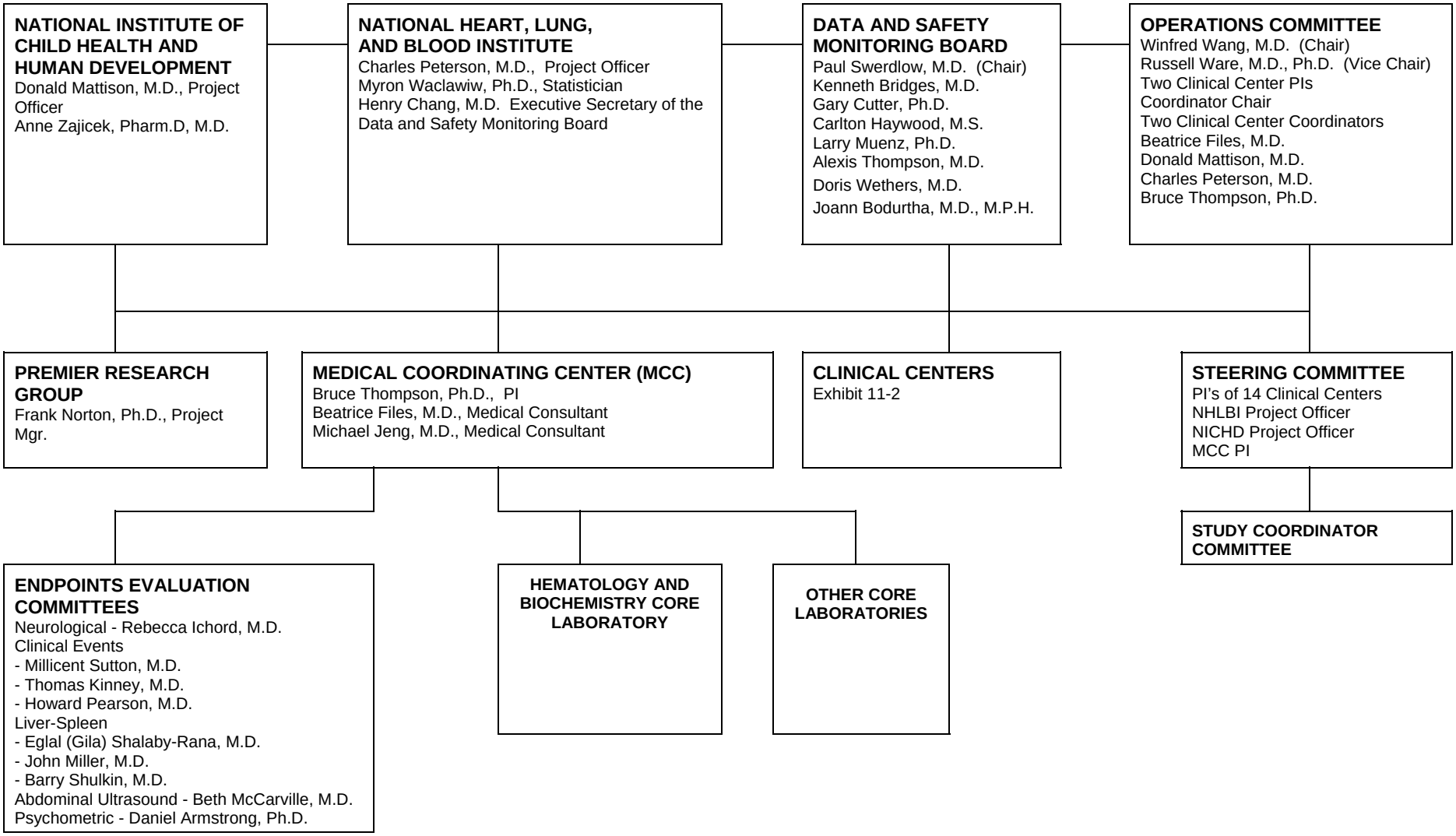


Exhibit 11.2
PARTICIPATING CLINICAL CENTERS

CLINICAL CENTERS

Children's Research Institute, Caterina Minniti, M.D. - 01
(Washington, DC)

Duke University Medical Center, Sherri Zimmerman, 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, Mary Ellen Cavalier, 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, Stuart Toledano, 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, Carlton Dampier, M.D. - 12
(Philadelphia, PA)

Emory University School of Medicine, Peter Lane, M.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
Renee C. Rees, Ph.D., Co-Principal Investigator

PROJECT OFFICE

Division of Blood Diseases and Resources
National Heart, Lung, and Blood Institute (Bethesda, MD)
Charles Peterson, M.D., Project Officer
Myron Waclawiw, Ph.D., Statistician
Henry Chang, M.D., Executive Secretary of the Data and Safety Monitoring Board

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APPENDIX A.
Schedule and Volume of Blood and Urine Collection and Schedule of Special Studies

	Blood Sample Collection (All volumes in ml)				URINE	Special Studies						
WEEKS	*Heme (Local) HbF (Core)	HJB (Core)	Pitted Cells (Core)		Creatinine	Urinalysis/ Urine Concentr (ml)	Liver-spleen scan	Anthropometry	† Neuropsych	DTPA Clearance (ml)	Cystatin C (ml)	TCD
4 MO	0.5							Y				
8 MO	0.5							Y				
+12 MO	0.5							Y				Y
+24 MO	0.5	0.1	0.1		0.5	5.0	Y	Y	Y	6.0	0.5	Y
+36 MO	0.5							Y				
+48 MO	0.5							Y				
+60 MO	0.5							Y				

* CBC with diff and reticulocyte count will be done at each clinic visit.

** All CBCs performed for medical management within 2 months after completion of the BABY HUG treatment study should be reviewed only by the PEP.

† WPPSI

Appendix B
BABYHUG DATA COLLECTION SHEET
For patient visits between the treatment and follow-up studies

Date of Visit _____

Interim History

HU treatment _____ If yes, specify dose _____

Fever > 101.5^{o} _____ Transfusions _____

Vaccinations since last visit _____ Prophylactic Penicillin taken _____

If no, did the child take another antibiotic _____ If yes, specify: _____

Hospitalizations since last visit _____ If yes, specify : admit date _____ discharge date

Brief summary of Hospital Course(s) (Attach additional sheet if needed):

_____

_____

_____

_____

Serious Adverse Events (as listed in Table 4.2; to be reported within 7 days) since last visit?

If yes, specify: start date _____ stop date _____

Diagnosis: _____

Physical Exam

VS P- _____ R- _____ Temp - _____ 0_2 sat _____

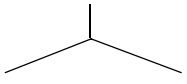
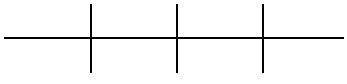
Height _____ Weight _____ Head Circumference _____

Spleen palpable _____

If yes, specify size at MCL _____ at AAL _____

Other abnormal PE _____

If yes, please specify: _____

Labs from last visit CBC  Chem 

Other, specify _____

Appendix C

Clinical Event Definitions

Introduction

In the BABY HUG follow-up study, clinical event definitions will be applied for consistency with other important NHLBI-sponsored clinical studies of sickle cell anemia such as the Clinical Study of Sickle Cell Disease (CSSCD) and the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH).

Definitions

Anemia: A reduction of hemoglobin level by at least 30% from the steady-state level ***OR*** a reduction by at least 20% accompanied by an acute increase in spleen size. Acute anemic events should be classified into one of the following categories.

1. Splenic sequestration crisis: The event is characterized by an increase in spleen size and firmness, reduction of hemoglobin level by at least 20% and may include drop in platelet or white counts. Splenic sequestration is defined in BABY HUG by the findings of a palpable, large spleen with hemoglobin less than 5 gm/dL for nonfatal occurrences.
2. Aplastic Crisis: This event is characterized by a substantial decrease in reticulocyte count to below 1.5 % before or concurrent with a reduction in hemoglobin level to a level greater than 30% below the steady-state level. It usually results from acute infection with parvovirus B19. Check the patient's parvovirus IgM titer; usually it should be positive.
3. Other anemia: Reduction of hemoglobin because of blood loss, transfusion reaction or hyper-hemolysis will be classified as an other anemia. A hyper-hemolytic episode is characterized by normal or increased reticulocyte counts and nucleated red cell count during an episode of falling hemoglobin associated with an increase in indirect

bilirubin level over the usual value. The latter finding is important to allow discrimination from a recovering aplastic crisis.

Aplastic Crisis: See Anemia.

Bacteremia: Febrile illness with blood culture positive for bacteria. Organism must be specified.

Cerebrovascular Accident (CVA): Acute neurologic syndrome secondary to occlusion of an artery or hemorrhage with resultant ischemic and neurologic symptoms and signs.

1. Stroke, hemorrhagic: Injury to brain tissue resulting from disturbance of blood supply to the brain due to hemorrhage. The area of the hemorrhage should also be reported (e.g., subarachnoid, subdural, intracerebral, aneurysm).
2. Stroke, infarctive: Injury to brain tissue consistent with occlusion of vessel(s) by thrombus or embolus which results in neurologic abnormalities on physical examination that persist beyond 24 hours.
3. Transient Ischemic Attack (TIA): Temporary interference with blood supply to the brain. The symptoms include neurologic signs that clear within 24 hours (48 hours if basilar system is involved). After the attack, no evidence of residual neurologic damage remains on physical examination.

Chest Syndrome: Also known as acute chest syndrome (ACS). A clinical syndrome that includes at least 3 of the following symptoms: chest pain, temperature elevation over 38.5^{o}C/101.5^{o}F , tachypnea, wheezing or cough. A new pulmonary infiltrate must be present on x-ray involving at least one complete lung segment to be consistent with alveolar consolidation instead of atelectasis.

Meningitis: Inflammation of the membranes of the spinal cord or brain usually caused by and infectious agent, as demonstrated by lumbar puncture abnormalities and culture. The causative agent should be listed if known.

Osteomyelitis: Bacterial infection of bone requiring long-term antibiotics. The causative agent should be listed if known.

Sepsis: Severe febrile illness with unstable vital signs or shock associated with positive blood culture. Organism must be specified. Positive blood culture in stable patients reported as bacteremia.

Splenic Sequestration: See anemia.

Surgery: Any operative procedure will be listed.

Transient Ischemic Attack (TIA): See Cerebrovascular Accident (CVA).

Transfusion: The provision of red blood cells to correct anemia. The reason for the transfusion should also be specified. Simple or Exchange transfusion should be specified.