PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL
BABY HUG

PROTOCOL

August 2009

.

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PREFACE TO THE BABY HUG PROTOCOL

Decades of observational data have documented that sickle cell anemia (SCA) is a devastating medical disorder. Over 50,000 affected persons in the United States have inherited SCA, and the vast majority of them are African-American. To date, there have been few therapeutic options for persons with SCA. Bone marrow transplantation, which can provide a cure for individuals with a matched sibling donor, and chronic blood transfusions to prevent cerebrovascular disease are possible therapeutic options that are not appropriate or available for all patients. No other treatment has yet been demonstrated to prevent the chronic end organ damage seen in this disorder.

SCA is characterized by acute complications and chronic organ damage that cause lifelong morbidity and early mortality. Acute events result from erythrocyte sickling and vaso-occlusion, and begin early in life with dactylitis, other painful events, and acute chest syndrome. Chronic organ damage from sickling is more insidious but also begins early in life as fetal hemoglobin (HbF) levels begin to decline. During the first two years of life, sickling begins to damage the spleen, rendering the child susceptible to overwhelming infection. The kidney also is affected in infancy and manifests abnormality first as increased glomerular filtration, followed by proteinuria in older children and finally renal failure in young adults. Other organs (e.g., brain, liver, and lungs) are also affected by chronic sickling, but damage that begins in infancy typically does not become manifest until an older age.

The anti-neoplastic agent hydroxyurea has emerged recently as an oral medication that can increase fetal hemoglobin (HbF) production and thereby reduce intracellular sickling. Hematological benefits of hydroxyurea therapy include higher HbF values, higher hemoglobin concentration, and less hemolysis. The clinical efficacy of hydroxyurea therapy in reducing acute events (crisis, acute chest syndrome, hospitalizations, rate of transfusions) in severely affected adults with SCA was demonstrated in a randomized clinical trial -- the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) -- long-term follow-up suggests that hydroxyurea therapy may be associated with reduced mortality as well. The short-term safety of hydroxyurea therapy has been demonstrated in school-aged children (HUGKIDS) and infants and very young children (HUSOFT) with SCA, but its clinical efficacy has not been proven in this younger age group. Importantly, the effects of hydroxyurea on chronic end organ damage in SCA have not been investigated, although children in HUSOFT had the suggestion of reduced splenic dysfunction compared to historical controls, and their observed increases in glomerular filtration rate with age were inversely correlated with the amount of HbF.

With these encouraging results, the challenge now is to determine the appropriate and optimal use of hydroxyurea therapy for children with SCA. Several important questions must be addressed, and the proposed answers must be sensitive to the history of medical investigation involving African-Americans.

Highest priority must be given to patient protection and to assuring that patients' families make their decisions regarding participation in BABY HUG freely, with confidence and trust in the choices offered. Conversely, however, it would be unethical to allow hydroxyurea to be used indiscriminately in this vulnerable patient population without demonstrated benefit in a randomized clinical trial. A recent survey by the American Society of Pediatric Hematology/Oncology noted that 30% of pediatric hematologists already use hydroxyurea in children under age 5 years, and some already use it as early as age 1 year of life. It is essential, therefore, to design and conduct a formal randomized clinical trial to ensure that hydroxyurea will be used appropriately in young children with SCA. The following are three critical study design questions with proposed answers:

1. What is the best age range in which to consider hydroxyurea therapy for the prevention of chronic organ damage?

   The pathophysiology of sickling in SCA begins during the first year of life, and organ damage to the spleen and kidney clearly begins before 2 years of age. The onset of damage appears to be associated with the decline in Hb F levels that begins at 6 months of age. Since the onset of disease begins in infancy, the first year or two of life is the appropriate age range to study. In BABY HUG, enrollment will begin at 9 months of life and continue through age 17 months.

2. Should hydroxyurea be reserved only for patients with severe disease, or might less severely affected infants benefit as well?

   Even though only a subset of infants with SCA will manifest acute clinical sickling complications during the first two years of life, chronic (subclinical) sickling occurs in all patients. Treatment that is limited to severely affected infants might not address the capacity of hydroxyurea to prevent organ damage. Conversely, the exclusion of well-appearing infants would be inappropriate since they might be denied beneficial therapy. Functional asplenia is recognized as a poor prognostic sign, hence the prevention of splenic damage is a worthwhile goal for all infants. In BABY HUG, very young children with SCA will be randomly assigned to either hydroxyurea or placebo therapy.

3. Can hydroxyurea help prevent the chronic end organ damage in sickle cell disease that begins early in life?

   This question will be addressed directly in BABY HUG. The spleen and the kidneys are the organs commonly affected early in childhood; hence these organs will be the primary study endpoints.

<u>Summary</u>. Based on the known pathophysiology of SCA, previous data from studies using hydroxyurea in infants and children, and careful consideration of sociological and ethical factors, we propose that hydroxyurea should be tested in a formal randomized clinical trial for the prevention of chronic organ damage in infants with SCA. We propose a randomized double-blinded placebo-controlled trial to prevent damage to the spleen and kidney, since these organs are the first to be affected early in life. We propose to determine if we can enroll children with SCA who are between 9 and 17 months of age, to prevent chronic damage to the spleen and kidneys. With proper precautions to ensure safety of all participants, we believe that this is the optimal trial design that allows equipoise, ethical patient protection, and the opportunity to determine the true efficacy of hydroxyurea in preventing chronic organ damage in children with SCA.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 1

BACKGROUND AND STUDY RATIONALE

1.1 OVERVIEW OF SICKLE CELL ANEMIA

The term Sickle Cell Disease (SCD) refers to a group of genetic hematological disorders characterized by the predominance of sickle hemoglobin (HbS). SCD is one of the most common inherited diseases in the United States, affecting approximately 1 in 375 African-American live births. Currently it is estimated that there are over 75,000 persons in the United States with SCD. A single inherited amino acid substitution in beta globin results in the formation of HbS (containing beta-globin S instead of beta-globin A). HbS undergoes polymerization in the deoxygenated state, leading to deformation of the cellular membrane and alteration of cellular physiology. Sickle Cell Anemia (SCA) is characterized by homozygous state for HbS and represents the majority of SCD patients.

Clinical manifestations of SCA result primarily from hemolytic anemia and the effects of intravascular sickling, including both acute tissue hypoxia and chronic organ damage. Patients with SCD commonly develop acute vaso-occlusive events due to sickling of erythrocytes within the capillaries and small venules. Acute vaso-occlusive sickling events can manifest in many ways, including painful crisis, priapism, splenic sequestration, acute chest syndrome, or stroke. Over a period of years, patients with SCA develop organ damage from repeated acute and chronic sickling events. The primary organs that are affected chronically by sickling include the spleen, kidneys, brain, and lungs. Data gathered during the Cooperative Study of Sickle Cell Disease (CSSCD) demonstrate clearly that chronic organ damage is a major cause of morbidity and mortality for patients with SCA.

1.2 CHRONIC ORGAN DAMAGE IN SICKLE CELL ANEMIA

1.2.1 Spleen

Of all internal organs affected by chronic sickling, the spleen is the one damaged most severely early in life. The slow circulation within the spleen provides an ideal milieu for sickling, which leads to tissue hypoxia and organ infarction. In most children with sickle cell anemia, the spleen is non-functional by the age of 2 years (Pearson et al, 1979). This acquired state of functional asplenia (Diamond, 1969) leads to a susceptibility to infection by encapsulated bacteria (Zarkowsky et al, 1986; Gill et al, 1995; Kabins and Lerner, 1970; Pearson, 1977). Splenic damage can be identified by the absence of radioactive tracer uptake, or the presence of increased numbers of pitted erythrocytes (Pearson et al, 1979; Rogers et al, 1982; Pearson et al, 1985; Fatunde and Scott, 1986). Transfusion therapy has been associated with a “reversal” of splenic hypofunction (Buchanan et al, 1989; Barrios et al, 1993), suggesting that splenic damage may be reversible during the first few years of life. Similarly, bone marrow transplantation in children with SCD may be able to correct splenic dysfunction (Ferster et al, 1993). Splenic regeneration may also occur with hydroxyurea (HU) therapy in patients with SCA (Claster and Vichinsky, 1996).

1.2.2 Kidneys

The term “sickle nephropathy” refers to the constellation of chronic renal damage that occurs in patients with SCA. For decades, it has been known that defects in renal tubular function, specifically in acidification (Oster et al, 1976) and concentrating ability (Francis and Worthen, 1968) begin early in childhood, along with papillary necrosis (Eknoyan et al, 1982). More recently, sickle nephropathy has been characterized by proteinuria, occasionally with urinary protein loss in the range of the nephrotic syndrome (Tejani et al, 1985). The prognosis of nephrotic syndrome is poor, with chronic azotemia and acute renal failure occurring frequently (Bakir et al, 1987). The estimated prevalence of proteinuria in SCA is about 6% for children (Wigfall et al, 2000) and 25% for adults (Falk et al, 1992). Focal and segmental glomerulosclerosis and mesangial proliferation

have been described histologically, and probably result from glomerular hyperfiltration (Tejani et al, 1985).

An elevated glomerular filtration rate (is a common feature in patients with sickle nephropathy that begins very early in life (Allon et al, 1988), and may portend later severe renal damage. Therapy to reduce glomerular capillary hypertension significantly can reduce urinary protein excretion in adults with SCA (Falk et al, 1992). Renal damage from chronic sickling is a significant cause of morbidity and mortality; in the CSSCD, 18% of deaths in adults with SCA occurred secondary to overt organ failure, primarily renal (Platt et al, 1994).

1.2.3 Brain

The CSSCD has collected prospective data on chronic organ damage to the brain in over 300 children with SCD. Using brain magnetic resonance imaging (MRI), 22% of children age 6-12 years had infarction/ischemia and/or atrophy, including 13% who had no history of a clinical CVA (Leong et al, 1997). The lesions in these latter children are referred to as “silent infarcts” that reflect subclinical organ damage to the brain. Most of the lesions were present at entry into the study (age 6 years). More recently, 39 infants with SCA, 7 - 48 months of age, were found to have an 11% prevalence of brain abnormalities on MRI (Wang et al, 1998).

Organ damage to the brain in SCD is often associated with changes in the large intracranial arteries, most commonly stenosis within the distal internal carotid artery (ICA) and proximal middle cerebral artery (MCA). Abnormal cerebral blood flow can be identified in some children by magnetic resonance angiography (MRA), while transcranial doppler (TCD) has been shown to identify children with an increased risk of developing stroke. An abnormal time averaged maximum TCD velocity (>200 cm/sec) in the distal ICA or proximal MCA is associated with a high risk of stroke (Adams et al, 1992). In the Stroke Prevention (STOP) Trial, children with SCA and an abnormal TCD who received monthly blood transfusions had significantly fewer strokes than children who were simply observed (Adams et al, 1998).

Taken together, these data suggest that chronic organ damage to the brain from sickling begins early in life. Moreover, therapeutic intervention may help prevent the development of organ damage to the brain. Even in the absence of overt neurological disease, SCA puts some children at risk for neuropsychological sequelae including lowered intellectual functioning, academic skills deficits, impaired fine-motor functioning, and attentional deficits (Bonner et al, 1999).

1.2.4 Lungs

The lungs are also frequent target organs in patients with SCA. Episodes of acute chest syndrome (ACS, with intrapulmonary sickling) can result in obstructive lung disease with reactive airways (Leong et al, 1997). However, repeated organ damage from ACS and chronic sickling in the lungs most frequently leads to a restrictive pattern of lung disease with diminished lung compliance (Bowen et al, 1991). Recent studies using pulmonary function tests (PFTs) have suggested that abnormal lung function in SCA may begin in early infancy (Koumbourlis et al, 1997).

1.3 SICKLE CELL ANEMIA AND FETAL HEMOGLOBIN

1.3.1 Fetal Hemoglobin

There is great clinical heterogeneity observed in SCD, even for patients with an identical hemoglobin phenotype (Platt et al, 1991; Powars, 1991; Seward et al, 1993; Steinberg et al, 1995). This clinical variation is partly explained by differences in the hemoglobin concentration, mean cellular hemoglobin concentration, proportion of dense cells, erythrocyte rheology, % adhesive cells, presence of alpha-thalassemia, and the beta-globin haplotype (Platt et al, 1994; Platt et al, 1991; Steinberg et al, 1984; Baum et al, 1987; Powars, 1991; Phillips et al,1991; Embury and Steinberg, 1994). The amount of fetal hemoglobin (% HbF) is perhaps the most important parameter influencing clinical severity in SCA (Charache, 1990). Normal adults have <1% HbF (Wood, 1993), while patients with SCA have 1-20% HbF (Serjeant, 1975). Patients with hereditary persistence of fetal hemoglobin (HPFH) can have fetal hemoglobin (HbF) levels that reach 30-40% (Wood et al, 1975). Higher % HbF is associated with decreased clinical severity and fewer painful events,

transfusions, and hospitalizations in sickle cell anemia (Platt et al, 1991; Rucknagel et al, 1987; Odenheimer et al, 1987). A threshold of 10-20% HbF has been postulated, above which patients experience fewer clinical events (Powars et al, 1984). The % HbF also predicts early mortality in patients with SCD (Platt et al, 1994; Leikin et al, 1989).

Except in the case of HPFH, HbF is not found in all erythrocytes, but rather is located in a subset known as HbF-containing cells or “F cells” (Dover et al, 1978; Boyer et al, 1975). In normal adults, the percentage of F cells ranges from 0.5% to 7%, while in patients with SCA, the % F cells has a much broader range (Wood et al, 1975; Dover et al, 1978). Because F cells have a decreased tendency toward sickle formation, they survive preferentially in the peripheral blood of patients with SCA (Dover et al, 1978). The number of F cells, therefore, may be of equal or greater importance than the absolute amount of HbF in influencing the clinical severity of an affected individual with SCA. F cells can be quantitated accurately by several methods, including immunological staining of HbF by monoclonal antibodies, followed by enumeration by visual methods (Horiuchi et al, 1995) or flow cytometry (Dover and Boyer, 1987; Campbell et al, 1999; Marcus et al, 1997).

1.3.2 Physiologic Decline of HbF

Fetal hemoglobin (HbF), the predominant hemoglobin produced in utero, comprises approximately 80-90% of the total hemoglobin at birth. In normal persons, the % HbF declines to adult levels during the first year of life (Wood, 1993). For patients with SCA, this physiologic decline occurs more slowly, and the HbF nadir may not be reached until age 5 years (Mason et al, 1982; Brown et al, 1994). Clinical events from SCA rarely occur in the first 6 months of life, due primarily to high HbF levels. Events occur during the first two years of life, however, including splenic hypofunction (Pearson et al, 1979; Rogers et al, 1982), pneumococcal sepsis (Zarkowsky et al, 1986), splenic sequestration (Topley et al, 1981), dactylitis (Gill et al, 1995), and acute chest syndrome (Gill et al, 1995). These observations suggest that maintaining high HbF levels might prevent acute and chronic sickling damage.

The physiologic decline in % HbF and % F cells in a cohort of infants homozygous for HbSS between birth and 24 months of age was recently investigated (Marcus and Ware, 1999). The % HbF was measured by 2-minute alkali denaturation and % F cells by flow cytometry (Marcus et al, 1997). The amount of HbF per F cell was calculated using the formula: (mean corpuscular hemoglobin) x (% HbF)] / (% F cells). Over this period of time, the HbF parameters declined in an exponential fashion (Figure 1-1). At 24 months of age, the average % HbF was 14.6 ± 7.3% and the % F cells was 64.7 ± 16.9%. The average amount of HbF per F cell fell below 15 pg/cell by age 12 months, confirming data from a previous study (Maier-Redelsperger et al, 1994). Previous in vitro studies have suggested a threshold value of 15 pg HbF per F cell, below which sickling occurs (Sunshine et al, 1979; Poillon et al, 1993). These data demonstrate clearly that HbF parameters decline significantly during the first 1-2 years of life, below levels sufficient to inhibit sickling. These results support the concept of early pharmacologic intervention for very young children with SCA, with the intention to increase HbF parameters to levels that inhibit in vivo sickling.

1.4 EFFICACY OF HYDROXYUREA IN SICKLE CELL ANEMIA

1.4.1 Induction of HbF

The pharmacologic enhancement of HbF can be achieved using a variety of agents including cytotoxic drugs (e.g. 5-azacytidine, hydroxyurea), hematopoietic growth factors (e.g. erythropoietin), and short chain fatty acids (e.g. butyrate and derivatives). Each of these therapeutic agents has been shown to have efficacy for increasing the % HbF in patients with sickle cell anemia (Goldberg et al, 1990; Charache et al, 1992; Perrine et al, 1993; Dover et al, 1994; Charache et al, 1995). However, side-effects and toxicities vary considerably and no direct comparisons of efficacy have been reported. Hydroxyurea is a prototypic therapeutic agent due to its efficacy, ease of administration, and modest toxicity profile.

1.4.2 Hydroxyurea For Adults With SCA

Hydroxyurea has been tested in adults with sickle cell anemia, and in most patients will increase both the absolute amount of HbF as well as the number of F cells (Goldberg et al, 1990; Charache et al, 1992). Charache and co-workers (Charache et al, 1992) reported a mean increase in HbF of 11% in a Phase I/II study of adults receiving daily HU treatment. In the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) Phase III clinical trial (Charache et al, 1995), adult patients were randomized prospectively either to daily HU or placebo. The study results demonstrated that HU therapy led to a significant reduction in the annual number of painful events, episodes of acute chest syndrome, and erythrocyte transfusions (Charache et al, 1995). The mean increase in % HbF was 8.6%, although the range of HbF responses was substantial and some patients did not respond, possibly due to either non-compliance or exhaustion of marrow reserves (Steinberg et al, 1995). Even when compliance was assured, approximately 20% of adults with SCA did not respond to HU therapy (Rodgers et al, 1990). The primary toxicity of HU therapy was dose-dependent and reversible bone marrow suppression (Charache et al, 1992; Charache et al, 1995).

1.4.3 Hydroxyurea For Children With SCA

After the Phase I/II adult HU trial was completed, several anecdotal reports suggested that HU therapy might be beneficial for children with SCA (Jayabose et al, 1996; Scott et al, 1996; Ferster et al, 1996; de Montalembert et al,1997). To determine the safety and efficacy of HU therapy for children, investigators from seven institutions administered HU therapy to a total of 84 school-aged children with SCA in an NHLBI-sponsored Phase I/II clinical protocol (HUG-KIDS). HUG-KIDS provided convincing evidence that HU therapy is associated with improved hematological parameters in this younger patient population, including increased % HbF and % F cells (Kinney et al, 1999). The toxicity profile of HU was mild and included primarily reversible myelosuppression. Clinical efficacy was not a stated goal of this Phase I/II study, but it was clear that the pediatric patients with SCA had a less severe acute clinical course while on HU therapy. Based on these encouraging results in older children, it is reasonable to consider HU therapy for very young children

with sickle cell anemia, to allow therapeutic intervention before in vivo sickling leads to acute clinical events and chronic organ damage.

1.4.4 Hydroxyurea For Very Young Children With SCA

A recent report provided short-term data regarding HU therapy for young children with SCA. Eight children, age 2-5 years, received HU with evidence of both hematological and clinical efficacy, and with minimal toxicity (Hoppe et al, 2000). To determine the feasibility of HU therapy for very young children with SCD, a Phase I/II pilot trial (HUSOFT) was recently performed. Children between age 6-24 months were eligible for enrollment from four institutions, including Duke University Medical Center, St. Jude Children's Research Hospital, University of Texas Southwestern Medical Center, and Medical College of Wisconsin. A total of 21 completed two years of therapy at 20 mg/kg/day. All patients then on study desired to continue treatment for an additional two years at 25 and 30 mg/kg/day. Laboratory studies and physical examinations were performed every four weeks. Patients were closely monitored for toxicities, especially of the blood counts and growth parameters, and for compliance. Routine testing of the complete blood count, %HbF and %F cells were used to document hematological efficacy in before-after comparisons and compared to untreated children with sickle cell anemia. Additional studies included brain MRI/MRA, neurodevelopmental testing, and liver-spleen scans. Hematological toxicities in HUSOFT were well-tolerated, and laboratory efficacy was demonstrated (Wang et al, 2001). Recently, follow-up of the majority of patients from the HUSOFT study who were continued on hydroxyurea treatment for an additional 2-4 years, showed that these patients tolerate prolonged hydroxyurea therapy with sustained hematologic benefits, fewer acute chest syndrome events, improved growth, and possibly preserved organ junction (Hankins et al, 2005).

Preliminary results of clinical efficacy in HUSOFT relate to the potential prevention of chronic organ damage, specifically the possibility that hydroxyurea can help preserve splenic and renal function in some infants with SCA (Wang et al, 2001). Splenic function, as evidenced by uptake of radioactive tracer, was present in eight of 17 HUSOFT children (47%) after two years on study. This

compares favorably with data from CSSCD (Pearson et al, 1985) that document elevated pitted cell counts (consistent with functional asplenia) in >80% of children with SCA at the same median age of 39 months. Renal function was assessed primarily by GFR estimation, calculated using the formula [(height) x k] divided by (creatinine), as described by Schwartz (Schwartz et al, 1987). The estimated GFR is normal (100 ± 20 ml/min) in infants with SCA during the first 6-12 months of life, but quickly rises to 150 ml/min by age 2-4 years and >200 ml/min by age 6-10 years (Wigfall et al, 2000; Kinney et al, 1999; Russell Ware, unpublished observations). Analysis of HUSOFT data reveal that the estimated GFR was 121 ± 20 ml/min/1.73 m^2 at study entry (median age 15 months, n=28) and 162 ± 43 ml/min/1.73 m^2 at study exit (Franca Barton and Russell Ware, unpublished observations). The absolute change in GFR was strongly associated with change in %HbF (Pearson coefficient -0.60, p=0.004), suggesting that preservation of HbF can help prevent the elevation over time in untreated children with sickle cell anemia.

Recently two groups of investigators have presented evidence that hydroxyurea therapy in children is associated with lower TCD velocities (Zimmerman et al, 2002; Bernaudin et al, 2001).

1.5 TOXICITIES OF HYDROXYUREA THERAPY

1.5.1 Organ Damage

The short-term toxicities of HU for patients with SCA have been carefully studied in two large Phase I/II trials. In the safety trial for adults with SCA (Charache et al, 1992), the only observed short-term toxicity was bone marrow depression. Neutropenia was most common (76%), followed by reticulocytopenia (22%), anemia (4%), and thrombocytopenia (1%). The myelosuppression was typically mild, dose-dependent, and reversible. In the safety trial (HUG-KIDS) for children with SCA (Kinney et al, 1999), laboratory toxicity occurred at approximately 8% of the clinic visits. Neutropenia was the most common hematological toxicity (63%), followed by reticulocytopenia (19%), anemia (13%), and thrombocytopenia (4%). Hepatic toxicity (>2-fold increase in ALT levels) was observed at only 0.3% of clinic visits, and was not associated with HU dose or additional medications. No episodes of renal toxicity were noted, nor other significant clinical adverse events during this

pediatric HU safety trial. Growth (assessed by height and weight) and development (menarche, puberty) also were not adversely affected by HU therapy (Kinney et al, 1999).

1.5.2 Neurodevelopmental Effects

There are limited data in children with SCA regarding the effects of HU therapy on neuropsychological development. Previous small studies of HU therapy for children with SCA did not report any obvious neurodevelopmental decline. Similarly, the Phase I/II HUG-KIDS pediatric safety trial did not specifically test for neurodevelopmental progress, although no obvious declines in neurocognitive function were noted (Kinney et al, 1999). In the HUSOFT infant pilot trial, a subset of patients had full neurodevelopmental testing both at study entry (median score = 93) and at study exit (median score = 89). These values were not statistically different by t-test or Wilcoxon rank sum test (Wang et al, 2001). A recent abstract has suggested that HU therapy is associated with improvement in neurodevelopmental scores (Bernaudin et al, 1999).

Animal data suggest, however, that HU therapy can be toxic for the developing brain. Almost thirty years ago, prenatal treatment with HU was found to cause substantial postnatal effects on rats: perinatal mortality was increased (Fritz and Hess, 1980), weight gain was inhibited (Butcher et al, 1973; Adlard and Dobbing, 1975), locomotor activity was reduced (Fritz and Hess, 1980; Butcher et al, 1973), and maze learning was impaired (Butcher et al, 1973; Adlard and Dobbing, 1975). Given early in gestation, HU therapy also can be embryolethal or have embryotoxic effects on the eyes, face, brain, heart, and limbs (Aliverti et al, 1980). Neuronal cells such as the dorsal root ganglia may be especially susceptible to impairment of DNA synthesis by HU (Theisen, 1979). A recent abstract suggests that post-natal HU therapy also impairs weight gain, organ size, and brain development of rats (Horiuchi et al, 1998). It must be emphasized that all of these studies used doses of HU up to 1000-2000 mg/kg/day. No studies have documented embryotoxicity or severe neurodevelopmental toxicity using pharmacologic HU doses (20-40 mg/kg/day). Also, effects of HU exposure may be considerably different in rats than in primates (Wilson et al, 1975). Differences in HU plasma levels, half-life clearance, and tissue penetration exist; previous studies suggest that the

effects on rodents do not accurately reflect the effects of HU in primates (Wilson et al, 1975). Taken together, the available laboratory and clinical data neither establish nor exclude the possibility that pharmacologic HU doses in early childhood are related to any marked neurodevelopmental toxicity for the human brain, although very high doses of HU could be.

1.5.3 Mutagenic And Carcinogenic Potential

Although the short-term toxicities of HU are typically well-tolerated, the long-term risks associated with HU therapy are unclear. Specifically, the risk of developing leukemia or other malignancies following HU exposure has not been determined. Hydroxyurea has been shown experimentally to have clastogenic (Gebhart, 1981; Oppenheimer and Fishbein, 1965), teratogenic (Murphy and Chaube, 1964; Aliverti et al, 1980) and in some settings mutagenic effects (Ziegler-Skylakakis et al, 1985), but its potential as a carcinogen at therapeutic doses has not been established. Since HU is a potent inhibitor of ribonucleotide reductase and reduces intracellular dNTP pools, HU interferes not only with DNA synthesis but also with DNA repair mechanisms (Snyder, 1984). In vitro, DNA damage that develops either spontaneously or from environmental mutagens cannot be fully repaired in the presence of HU, leading to the accumulation of somatic mutations and chromosomal damage (Li and Kaminskas, 1987). These laboratory observations provide a plausible biochemical mechanism by which in vivo HU therapy could lead to somatic DNA mutations and eventual carcinogenesis.

The carcinogenic potential of HU therapy has been investigated most carefully in patients with myeloproliferative disorders (MPD). The Polycythemia Vera Study Group (PVSG) reported a 5.9% incidence of acute leukemia in 51 adults with PV treated with HU (Fruchtman et al, 1994; Landaw, 1986) compared to 1.5% of historical counterparts who received phlebotomy alone ($p=0.12$). Reports of acute leukemia in adults with MPD treated with HU alone (Sedlacek et al, 1986; Lofvenberg et al, 1990; Holcombe et al, 1991; Weinfeld et al, 1994; Furgeson et al, 1996) have added concern regarding the long-term leukemogenic potential of HU therapy in this clinical setting. Recently, large studies have provided some evidence that long-term HU therapy for patients with

MPD is associated with an increased risk of developing acute leukemia (Najean et al, 1997a; Sterkers et al, 1998; Najean et al, 1997b). Taken together, these data suggest that hydroxyurea therapy may have a mutagenic and carcinogenic potential for patients with MPD, especially with long-term usage.

The carcinogenic potential of HU therapy is not evident in the setting of other hematological diseases. Sixty-four patients with erythrocytosis secondary to cyanotic congenital heart disease were treated with HU (mean 5.6 years) and had no cases of secondary malignancy (Triadou et al, 1994). In the United States, some adults with SCA have received HU therapy for over 10 years; no cases of secondary leukemia from the MSH trial have been observed (Steinberg et al, 1999). However, anecdotes of malignancy or myelodysplasia in patients with SCA on hydroxyurea therapy have been reported. These cases need to be evaluated in light of the incidence of cancer in the African-American population in general, and specifically in patients with SCD.

The incidence of cancer among African-American children in the US is about 11 new cases per 100,000 children per year (Gurney et al, 1995), of which one-third are leukemia or lymphoma. The incidence among African-American children under age four years, however, is at least 15 cases per 100,000 children per year (Gurney et al, 1995), due primarily to leukemia, neuroblastoma, and Wilms' tumor (Miller et al, 1993). In the setting of SCA, the incidence of malignancy is not known. The CSSCD identified 1 child with Wilms' tumor in the original study period and 14 subsequent cases of cancer were reported in children and adults with SCD (Dianne Gallagher and Duane Bonds, unpublished observations). In a large retrospective survey performed by members of the International Association of Sickle Cell Nurses and Physician Assistants (IASCNAPA), a total of 41 cases of cancer were reported in patients with sickle cell anemia. These cases included children and adults of all ages, and a wide variety of cancer types were reported (Schultz et al, 1999).

1.5.4 Acquired DNA Mutations In Association With Hydroxyurea Therapy

The inhibitory effects of HU on DNA repair mechanisms could lead to an accumulation of acquired DNA mutations that eventually could result in malignant transformation. Two in vitro

assays of DNA damage can measure the mutagenic effects of in vitro and in vivo hydroxyurea exposure: the hypoxanthine phosphoribosyltransferase (HPRT) assay that measures the frequency of mutations at the selectable *hprt* gene locus (Albertini et al, 1982; O'Neill et al, 1987), and the VDJ gene locus assays that detect "illegitimate" interlocus recombination events between the T-cell receptor Vγ and Jβ gene loci within chromosome 7 (Stern et al, 1989; Lipkowitz et al, 1992). Using these two quantitative assays, the mutagenic effects of in vitro and in vivo hydroxyurea exposure were measured (Hanft et al, 2000). In vivo HU exposure was not associated with more DNA mutations in adults with SCD or myeloproliferative disorders (MPD), but was associated with a suggestively higher numbers of VDJ events in children with SCD (Table 1). These results suggest that the mutagenic potential of HU exposure is low, and serial studies should be performed in young SCD patients on HU therapy.

Patient Population	# patients	Mean Age (years)	Median HU exposure	HPRT M_f (x 10^{-6})	VDJ events (per µg DNA)
Adults with MPD	27				
Low HU exposure	15	57 ± 17	0 months	37.3 ± 37.6	1.06 ± 0.73
Prolonged HU exposure	12	62 ± 16	11 years	41.1 ± 29.3	0.64 ± 0.29
Adults with SCD	30				
No HU exposure	15	27 ± 12	0 months	19.1 ± 19.1	1.07 ± 0.38
Short HU exposure	15	29 ± 9	24 months	16.7 ± 10.9	1.14 ± 0.38
Children with SCD	38				
No HU exposure	21	11 ± 3	0 months	11.5 ± 18.7	1.06 ± 0.45
Shorter HU exposure	17	11 ± 3	7 months	11.2 ± 6.7	1.58 ± 0.87
Longer HU exposure	17	13 ± 3	30 months	9.2 ± 7.8	1.82 ± 1.20
Normal Controls	32	43 ± 15	0 months	25.8 ± 24.8	1.04 ± 0.38

Table 1. Patient characteristics and quantitation of acquired DNA mutations after in vivo hydroxyurea exposure. Patient and control PBMC were tested for DNA mutations in both the HPRT and VDJ assays. The 27 adults with MPD, with either low or prolonged HU exposure, had no significant differences in *hprt*$^-$ M_f or number of VDJ recombination events. Adults with SCD also had no significant differences, according to HU exposure. Children with SCD and HU exposure had more VDJ events compared to those with no HU exposure, P=0.04 by ANOVA (Hanft, 2000).

1.6 SUMMARY

Decades of observational data, including landmark studies from the CSSCD, have documented that sickle cell anemia is a severe, debilitating hematological disorder. The morbidity and mortality of SCA arise from both acute vaso-occlusive events and chronic organ damage. Protection from HbF is typically lost in infancy and early childhood, with the physiologic decline of HbF. Accordingly,

therapy designed to prevent chronic organ damage in SCA should be considered early in life. Hydroxyurea has emerged as an exciting therapeutic agent for patients with SCA, due to its ease of oral administration, modest toxicity profile, laboratory efficacy with increased %HbF, and clinical efficacy for acute vaso-occlusive events. The efficacy of HU in preventing chronic organ damage has not been tested, but data from the pilot HUSOFT trial suggest that HU may help prevent damage to the spleen and kidneys compared to expectations from the CSSCD and nonrandomized groups (Wang et al, 2001; Hankins et al, 2005) not given HU. Finally, hematological and neurodevelopmental toxicities from HU in infants and young children with SCA appear to be mild or absent. Taken together, the available data make a compelling case for the proposed Phase III trial of hydroxyurea in very young children (9 through 17 months of age) with SCA, designed to prevent chronic organ damage.

BABY HUG Protocol
Figure1-1

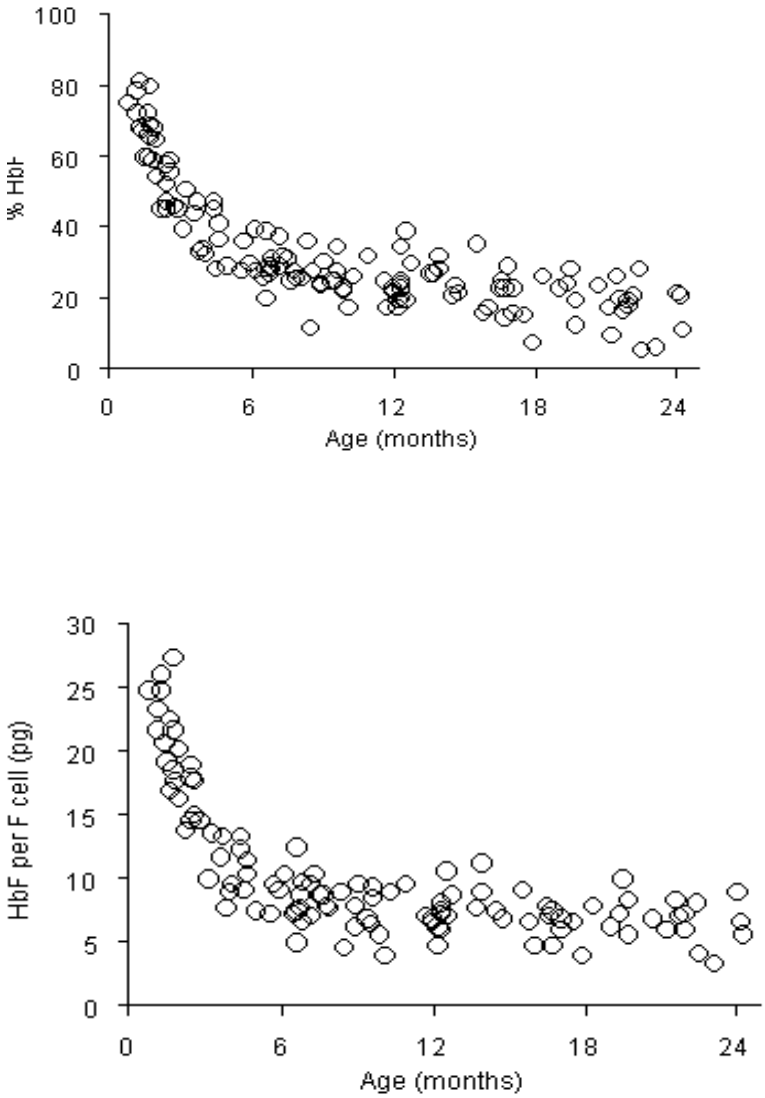


Figure 1.1. Measurement of HbF parameters in infants with HbSS over the first 24 months of life (Marcus and Ware, 1999). The top panel illustrates the exponential decline in % HbF as a function of age. At 12 months, the average HbF (mean ± 1SD) is 24.5 ± 5.0 %, and at 24 months is 14.6 ± 7.3 %. The lower panel plots the calculated value of HbF per F cell versus age, showing a exponential decrease to below 15 pg/cell at age 12 months and below 10 pg/cell by age 24 months, below the threshold of HbF per cell that inhibits in vitro sickling (Sunshine et al, 1979; Poillon et al, 1993).

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 2

OBJECTIVES AND DESIGN OF THE TRIAL

2.1 INTRODUCTION

Hydroxyurea (HU) has demonstrated laboratory and clinical efficacy for adults with sickle cell anemia (SCA). In this patient population, several studies have demonstrated that HU increases the hemoglobin concentration, mean corpuscular volume, and fetal hemoglobin (HbF) parameters including %HbF and % F cells. Toxicities are mild and primarily include transient and reversible neutropenia. In the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) clinical trial, which was an NHLBI-sponsored randomized, double-blinded, placebo-controlled trial in adults with SCA, HU reduced the number of acute vaso-occlusive episodes such as painful events and acute chest syndromes, transfusions, and hospitalizations (Charache et al, 1995).

Pediatric patients with SCA also benefit from HU therapy. A Phase I/II trial (HUG-KIDS) involving 84 children who were 5-15 years old concluded that HU was well tolerated by pediatric patients with SCA, and the laboratory effects were similar to those observed in adults (Kinney et al, 1999). Several smaller studies have further suggested that HU has clinical efficacy in this younger age group (Hoppe et al, 2000; Bernaudin et al, 1999), and reduces the number of acute vaso-occlusive events. A pilot trial involving infants and very young children with SCA (HUSOFT) has provided preliminary data demonstrating that HU is well-tolerated in this very young age group, with laboratory effects similar to those for older children (Wang et al, 2001; Hankins et al, 2005).

Although HU therapy has emerged as an exciting and efficacious therapeutic agent for patients with SCA, important issues remain regarding its use, especially for children less than 2 years old. Perhaps the most important issue is whether or not HU therapy can prevent chronic organ damage secondary to vaso-occlusive sickling. HUSOFT data are suggestive, but not conclusive, regarding the potential for hydroxyurea to prevent chronic organ damage, specifically

in the spleen and kidney. Ideally, HU therapy to prevent organ damage would begin early in life, before repeated sickling events begin to damage the spleen, brain, kidneys, and lungs. Another important issue regarding HU therapy relates to its mechanism of action, e.g., whether or not HbF parameters mediate the prevention of chronic organ damage. Finally, there are questions about the long-term safety of HU administration for young patients with SCA, including its effects on growth and development, as well as its mutagenic and carcinogenic potential.

At this time, however, there are limited data regarding the toxicities, effects on growth and development, and occurrence of adverse events in this very young age group (9-17 months). Moreover, the accurate and reproducible quantitative measurements of splenic and renal function have not been fully validated. For these reasons, a Feasibility and Safety Pilot Study has been performed to (1) determine the feasibility of the protocol design, (2) provide additional safety and toxicity data, and (3) validate the proposed methods of evaluating splenic and renal function.

2.2 SPECIFIC AIMS

The primary aim of this trial is:

1. To determine whether daily oral hydroxyurea can reduce by $\geq$50% chronic organ damage that develops in young children with sickle cell anemia.

Secondary objectives of this trial include:

1. To determine the relationship between fetal hemoglobin (HbF) levels and chronic organ damage in young children with sickle cell anemia;
2. To investigate the safety of HU for young children with sickle cell anemia regarding
    a. physical growth and development,
    b. neuropsychological development,
    c. immunological responses, and
    d. mutagenic effects on DNA.

2.3 DESIGN OF THE TRIAL

2.3.1 Overview of the Trial

This NHLBI- and NICHD-sponsored Phase III therapeutic trial will be a randomized, placebo-controlled double-blinded study and will involve 14 Clinical Centers, a Medical Coordinating Center, and eight core laboratories within the United States. The Phase III study will include 200 children with sickle cell anemia (SCA) aged 9-17 months. A Feasibility and Safety Pilot Study has enrolled 40 children from 12 to 17 months of age. Each child was randomly assigned to either hydroxyurea or placebo and will receive study treatment for no more than two years (see Figure 2-1). Selected data from all 40 children in the Feasibility and Safety Pilot Study have been presented to the DSMB and a decision has been made regarding reopening recruitment for the full study. After the children's treatment period has ended, they will remain in study follow-up to determine whether or not there are untoward effects of discontinuing study treatment. After study treatment ends, renewed consent will be requested for continued follow-up clinic visits for up to five years after the end of study treatment.

The target dose of hydroxyurea will be 20 mg/kg/day in liquid formulation or equivalent volumes of placebo. A Medical Coordinating Center and Pharmacy Distribution Center will ensure that patients and investigators are blinded to the treatment assignments of individual patients. A battery of laboratory tests and special studies will be performed at entry and exit. Clinical events (including death, acute chest syndrome, and stroke) and other outcomes will be classified by a central evaluation panel, blind to treatment assigned and under the direction of the Medical Coordinating Center. Interim results will be monitored by an NHLBI-appointed Data and Safety Monitoring Board according to statistical plans outlined in the Protocol (Chapter 4) and elaborated in a separate document prior to the start of recruitment. Long-term follow-up in an observational study after study treatment is terminated in BABY HUG is anticipated so that children enrolled in

BABY HUG will be observed for growth and safety outcomes for at least five years, if resources for the follow-up can be obtained.

2.3.2 The Feasibility and Safety Pilot Study

The 40 children enrolled in the Feasibility and Safety Pilot Study will be on study treatment for two years. The DSMB authorized immediately continuing recruitment for the remaining 160 children in the full Phase III BABY HUG Clinical Trial. The anticipated recruitment and follow-up periods are presented in Figure 2-1. The Primary Aims of the Feasibility and Safety Pilot Study were:

1. To determine the feasibility of BABY HUG in terms of recruitment, follow-up, adherence to study treatment, and compliance with the study schedule of procedures;
2. To assess hydroxyurea toxicity, effect on growth and development, and occurrence of severe/unexpected adverse events;
3. To establish the distribution and inter-observer and intra-observer variability of spleen function based on dual, independent readings of liver-spleen scans;
4. To evaluate the validity and variability of glomerular filtration rate (GFR) as estimated by serum creatinine and height (the Schwartz formula) compared with a "gold standard" such as DTPA clearance; and,
5. To assess some pharmacokinetic parameters of hydroxyurea in the BABY HUG age group.

At study entry, plasma specimens for pharmacokinetics were collected from the blood collected as part of the radionuclide (DTPA) study (at 1, 2 and 4 hours following radionuclide injection), in addition to plasma specimens collected at time 0 and 8 hours from the start of the test. After counting radioactivity in the plasma for the renal function study, the plasma from each specimen was saved, frozen (-70° C) for at least a month (well after all radiation decays) and shipped on dry ice for pharmacokinetic evaluations. The 8-hour specimen was collected after the

DTPA study and was handled similarly to the other four specimens. Measurements of hydroxyurea levels were made on specimens from all 40 children in a commercial laboratory recommended by the manufacturer for regulatory purposes. Children assigned to placebo were also tested for DTPA according to the same schedule; their plasma was prepared and shipped for HU determination just as the HU-assigned specimens.

The DSMB Chair, Executive Secretary of DSMB, the NHLBI Project Officer and the Steering Committee reviewed monthly and semi-annual Feasibility and Safety Pilot Study reports including information on:

1. Recruitment: Expected vs. Actual (overall), and reasons for ineligibility
2. Patients screened, eligible and randomized
3. Patient characteristics at baseline (overall)
    a. Age, race and gender
    b. Spleen function (scan reading)
    c. Spleen and kidney size (by ultrasound evaluation)
    d. Pitted cell counts
    e. Schwartz equation GFR estimates
    f. DTPA clearance for GFR quantitation
    g. Urine concentrating ability
    h. CBC
    i. Presence of gallstones
    j. Blood chemistries
    k. Microalbuminuria
    l. Transcutaneous O_2% saturation
    m. Physical examinations
    n. Neurological examination and neuropsychological tests

o. Height, weight, head circumference

p. Transcranial doppler (TCD) measurements (supported by grant funds independent of BABY HUG contracts)

4. Blood count toxicities

5. Dose adjustments

6. Intra- and Inter-observer agreement of liver-spleen scan readings

7. GFR estimated from the Schwartz equation versus measured by DTPA

8. Immunological impairment

9. Safety assessments and adverse events

a. Height, weight and head circumference

b. Neurological examination and neuropsychological development

c. Unexpected and serious adverse events: counts and percentages. Individual cases were summarized also for immediate review by the DSMB Chair, the Executive Secretary of the DSMB, and the NHLBI Project Officer.

Based on individual patient and group safety monitoring reviews, the Executive Secretary of the DSMB, the NHLBI Project Officer and/or DSMB Chair could recommend full DSMB review or individual treatment interruptions. All individuals whose treatment was interrupted continued to be monitored. There were monthly reviews of accumulating data.

If height, weight, or Bayley scores were worse among children assigned to HU than children assigned to placebo through the early months (at least three) of randomized study treatment, the DSMB could have recommended to the NHLBI that the full Phase III trial not proceed in children less than 18 months of age. If serious adverse events (e.g., death, stroke, or splenic sequestration) were more frequent among children assigned to HU than among children assigned to placebo, to a greater degree than could be expected by chance, the DSMB could have recommended to the NHLBI that the study be discontinued. If spleen function according to liver-spleen scan readings

was absent at entry for more than 40% of the children enrolled, the DSMB could have recommended to the NHLBI to re-design the primary outcome or other features of the study design (e.g., eligibility criteria) as necessary. See Chapter 4 for detailed study stopping rules.

2.4 ENDPOINTS

1. The primary endpoints are chronic organ damage to the spleen and kidney.
    a. Spleen -- organ damage will be defined as decrease or loss of radionuclide ^{99m}Tc uptake (relative to the liver) at 2 years from baseline; and,
    b. Kidney -- Deleted as of May 29, 2009.
2. Secondary endpoints for this study include the following:
    a. Spleen -- pitted cell count, size as measured by ultrasound;
    b. Kidney--urine specific gravity, osmolality, urinalysis including microalbuminuria, size as measured by ultrasound;
    c. Lung -- oxygen saturation (percutaneous monitor);
    d. Hepatobiliary – cholelithiasis (ultrasound evaluation), serum bilirubin (direct);
    e. Hematology and Chemistry -- hemoglobin F, serum bilirubin (indirect);
    f. Clinical events -- e.g., hospitalization, pain, splenic sequestration, splenomegaly, and acute chest syndrome occurrence; and,
    g. Transcranial doppler – time averaged maximum flow velocity in the distal internal carotid/proximal middle cerebral arteries.
3. Safety endpoints include the following:
    a. Death, stroke, TIA, splenic sequestration, prolonged hospitalization (greater than 7 days), life threatening events, acute chest syndrome, ICU admissions;
    b. Growth -- weight, height, head circumference;
    c. Brain -- neurodevelopmental testing, neurological examination;

d. Mutagenesis -- VDJ recombination events (first 140 patients), chromosomal karyotype and breakage studies;

e. Hematology and Chemistry -- hemoglobin, platelets, liver function, etc.; and

f. Immune System -- antibody response to immunizations, T-cell counts and antigen-specific responses.

2.5 SAFETY MONITORING

1. Individual patients will be monitored according to routine hematology and biochemistry parameters (see Section 6.5). Patients will have treatment interruptions if any of the following occur:

   a. a “toxicity” level, as defined in Chapter 10;

   b. the DSMB confirms a central review recommendation that a decline across two major percentile lines, or drop below the 1.5th percentile on age-specific standard growth curves based on confirmed measurements in height, or weight or head circumference growth warrants a treatment interruption (central reviewers for this aspect of the study are two physicians who are not seeing patients and are not associated with any of the Clinical Centers);

   c. Bayley standardized Mental score that falls to < 70 (confirmed on repeat evaluation within three months); or

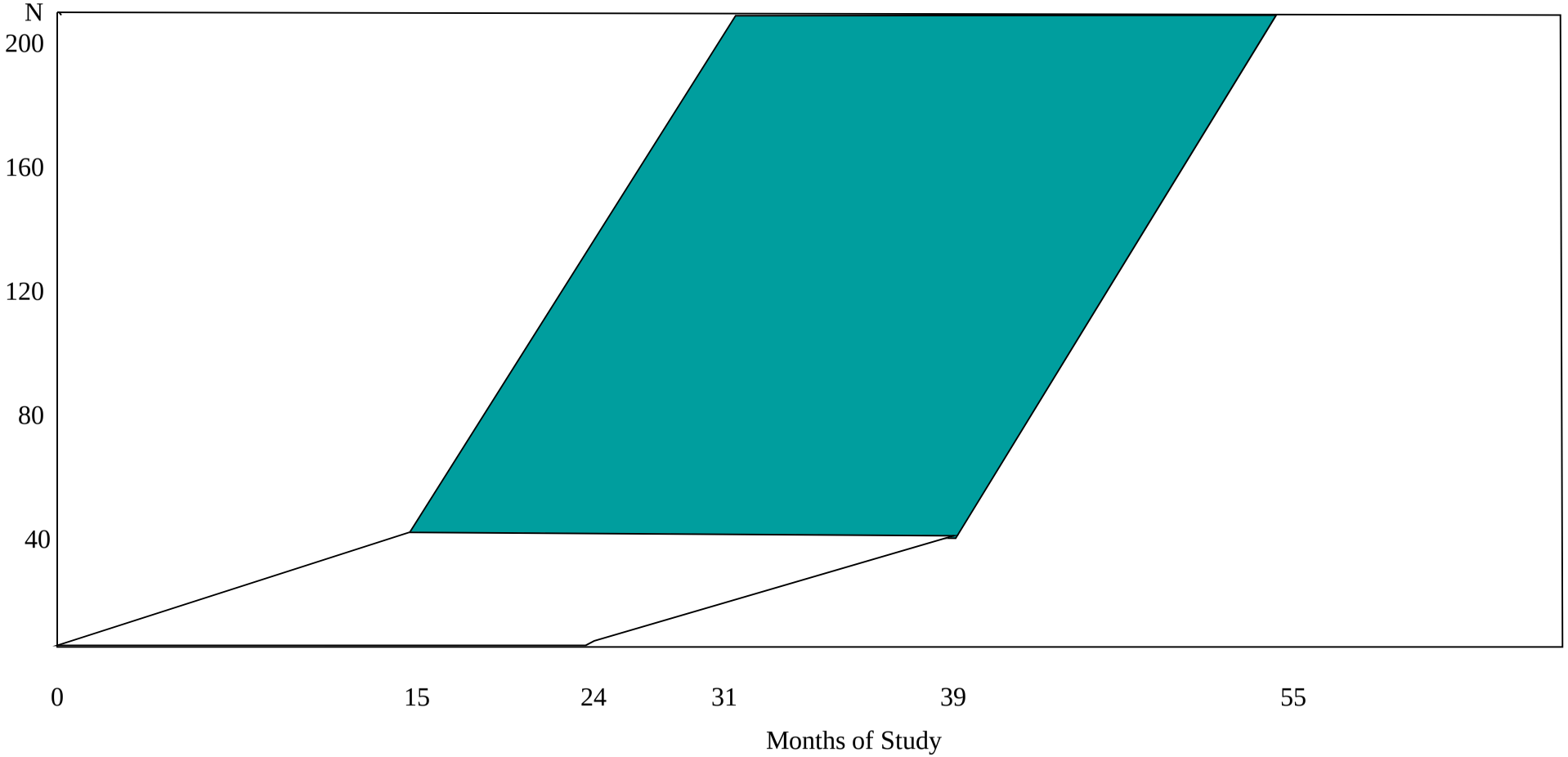
   d. clinical stroke, or failure of head circumference to increase according to normal growth curves.

   Treatment will not be terminated unless a toxicity condition persists or therapy that contraindicates study treatment – such as chronic transfusion – is initiated, to allow the determination of primary, secondary and safety endpoints in that patient. Children whose treatment is terminated, whether assigned to hydroxyurea or placebo will complete as much follow-up in BABY HUG as possible (e.g., all primary endpoint assessments and growth and development assessments will be expected).

2. Group comparisons between hydroxyurea-assigned patients and placebo-assigned patients will be included in semi-annual reviews of the data by the Data and Safety Monitoring Board (see Section 4.4). These reviews will include all secondary and safety endpoints defined in the study. Safety comparisons and inferences based on them will be discussed by the DSMB as data accrue in the study.

3. Monitoring for unanticipated adverse clinical effects will be done using adverse event (AE) forms that will be submitted to the Medical Coordinating Center (MCC) and tabulated based on the affected organ system. A central review group (consisting of two pediatric hematologists with expertise in sickle cell disease and without association with the Clinical Centers) will designate each event as serious or non-serious. Each suspected serious AE (SAE) will be reported to the MCC within 24 hours of the event. MCC staff will immediately review the material and forward it to the central review group. If the central review group finds that an event is serious, MCC staff will send the information to the NHLBI and NICHD Project Officers for review. The NHLBI Project Officer will forward the information to the FDA. The Clinical Centers will report the occurrence of serious AEs that occur at their institution according to the requirements of their local IRB. Further plans for adverse event detection and assessment are in Sections 4.3, 4.4 and Chapter 8.

Figure 2-1
Projected Enrollment, Maintenance of Assigned Study Treatment
(Gray Areas) and Follow-up to Common Termination N



INTERIM MONITORING LOOKS FOR EFFICACY:	1	2	3	4	(Final)
(# on HU / # on PLBO)	(20/20)	(40/40)	(60/60)	(80/80)	(100/100)

BABY HUG Projected Enrollment, Maintenance of Assigned Study Treatment (Gray Areas) and Follow-Up

Light gray: Feasibility and Safety Pilot Study (N=40 children are enrolled over 15 months and each maintained on assigned study treatment for 24 months)
Dark gray: Remainder of Phase III Clinical Trial cohort (N=160 additional children are enrolled over 16 months and each maintained on assigned study treatment for 24 months)

Follow-up may continue for up to 5 years or longer after the end of study treatment. 12/21/05

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 3

PATIENT ELIGIBILITY, RECRUITMENT, ORIENTATION, AND INFORMED CONSENT

3.1 INTRODUCTION

The primary objective of this study is to determine whether hydroxyurea administration can prevent organ damage commonly found in young children with sickle cell anemia. This will be accomplished by the administration of hydroxyurea or placebo to a group of very young children with sickle cell anemia (or sickle beta zero thalassemia) at an age before extensive organ damage has usually occurred. Patients will be randomly assigned to receive either hydroxyurea or placebo. Investigators and patients/parents will be blinded to treatment assignments.

Parents (or guardians) of the first forty patients enrolled were informed that they were participating in a Feasibility and Safety Pilot Study -- randomized, placebo-controlled, and double-blind -- testing oral hydroxyurea for the prevention of primary end organ damage (spleen and kidney) in children with sickle cell anemia 12 through 17 months of age at entry. The DSMB has authorized additional recruitment after the Feasibility and Safety Pilot Study without an interim wait. All families will be informed that their renewed agreement will be sought at the end of treatment for five years or up to ten years of follow-up clinical visits. Primary aims of the Feasibility and Safety Pilot Study are outlined in Section 2.3.

The end-organ damage found in sickle cell anemia is not uniform in time of onset or distribution across organs. While all organs and systems may ultimately be affected, the timing and severity of organ damage is variable. For example, damage to the spleen and brain is known to begin early in life, while that affecting the bones and eyes occurs later. An additional 160 patients from 9 months through 17 months of age will be enrolled, before the frequency of extensive organ damage is high. However, even at that young age, some children will already have early evidence of disease-specific organ damage.

Care will be taken to avoid coercion of this vulnerable population of parents of children with sickle cell anemia. Many of these parents will be young, some single heads of households, some limited in English language skills, and some may have highly constrained financial resources. Patient/Family advocates have been identified at each Clinical Center to advise families independently of any discussions with study investigators or health care providers (see Exhibit 3-1). The services of these advocates will be supported from study resources. Documentation of thorough review of the study and consent form with a Patient/Family Advocate will be required of each family enrolling a child in BABY HUG. The consent form will be appropriately worded and available in translation as needed at individual Clinical Centers. Clinical Center Principal Investigators and staff will be sensitive to the need to diminish the burdens of the study on patients and families without compromising safety; funds will be provided to families for the purpose of removing barriers to participation rather than being inducements for enrolling dependent children to provide experimental observations.

3.2 INCLUSION AND EXCLUSION CRITERIA

3.2.1 Inclusion Criteria

- Children with majority fetal and sickle (FS or SF) hemoglobin pattern confirmed centrally by electrophoresis, who are 9 through 17 months of age, and whose parents have provided written informed consent can be eligible for BABY HUG. Patient screening may begin at 7 months of age.
- For the Feasibility and Safety Pilot Study, eligible children were enrolled between 12 and 17 months (up to 18 months) of age. The age range of organ damage onset is usually between six and 23 months of age. Children less than nine months of age will be excluded from BABY HUG due to potential toxicity concerns; nine to twelve-month old children were excluded until a final safety analysis (April, 2005) was performed on the 12-17 month old children enrolled in the Feasibility and Safety Pilot Study; enrollment of 18-23 month old children will not be allowed in BABY HUG.

- Parents or guardians must provide informed consent for the Feasibility and Safety Pilot Study for the first 40 children and for the main study for the subsequent 160 children enrolled.

3.2.2 Exclusion Criteria for Pilot and Main Study

- Chronic transfusion therapy.
- Malignancy.
- Less than 5th (10th percentile for the pilot study) percentile height, weight or head circumference for age.
- Severe developmental delay (e.g., cerebral palsy or other mental retardation; Grade III/IV intraventricular hemorrhage).
- Stroke with neurological deficit.
- Surgical splenectomy.
- Participation in other clinical intervention trials.
- Probable or known diagnosis of Hemoglobin S-Hereditary Persistence of Fetal Hemoglobin.
- Known hemoglobin S-beta plus thalassemia (hemoglobin A present).
- Any condition or chronic illness, which in the opinion of the Principal Investigator makes participation unadvised or unsafe.
- Inability or unwillingness to complete baseline (pre-enrollment) studies, including blood or urine specimen collection, liver-spleen scan, abdominal sonogram, neurological examination, neuropsychological testing or transcranial Doppler ultrasound (interpretable study not required, but confirmed velocity $\geq$200 cm/sec results in ineligibility).
- Previous or current treatment with HU or another anti-sickling drug.
- The following exclusion criteria are transient; patients can be re-evaluated for eligibility: (Children must be declared eligible and randomized by 18 months of age.)
  - Hemoglobin less than 6.0 gm/dL.
  - Reticulocyte count less than 80,000/cu mm if hemoglobin is less than 9 gm/dL.

- Neutrophil count less than 2,000/cu mm.
- Platelet count less than 130,000/cu mm.
- Blood transfusion in the previous 2 months unless HbA less than 10%.
- ALT greater than twice upper limit of normal.
- Ferritin less than 10 ng/ml.
- Serum creatinine > twice upper limit of normal for age.
- Bayley standardized Mental score below 70.

3.3 RECRUITMENT

The 14 BABY HUG Clinical Centers will have an active recruitment phase designed to enroll patients into the BABY HUG study. Each Clinical Center will draw upon its own patient rosters for potential patients, as well as additional local patients who fall within its catchment area. Patients who are not normally followed at a Clinical Center will still be eligible for enrollment. Publicity for the sickle cell community and physicians concerning the BABY HUG study will occur at each Clinical Center according to local needs and will be at the discretion of the Principal Investigator with appropriate Institutional Review Board, NICHD and National Heart, Lung, and Blood Institute approval. The BABY HUG study will have a quarterly newsletter for patient families which may also be used for recruitment.

Potentially eligible children will be identified by the Clinical Center staff. It is anticipated that most eligible children will be known to the Clinical Centers through referrals after identification through newborn screening programs. All potential study patients' families will be referred to independent, Patient/Family advocates who are not otherwise involved in BABY HUG or in providing health care to these children or their families (see Section 3.7). Parents (or guardians) will receive information on BABY HUG after they have been provided education about sickle cell anemia. Chart review and initial visits will be used to exclude children who would not be eligible for BABY HUG.

For the Feasibility and Safety Pilot Study, children began to be evaluated for eligibility as early as 10 months of age in anticipation that they would qualify for the study in two months. No child was randomized prior to his/her first birthday. For the main study, children may begin to be evaluated for eligibility as early as 7 months of age, with randomization occurring no earlier than 9 months of age.

3.4 PARENT/GUARDIAN ORIENTATION

The experience with hydroxyurea will be presented, including evidence of clinical benefit in adults and apparent safety in children above one year of age.

Known risks will be described in detail along with the fact that relatively little is known about drug effects in this age group. A list of the known and possible risks (including possible malignancy or growth retardation which are not definitely known to be risks of HU) will be provided to families.

The Protocol and related issues will be discussed in detail and a copy of the consent form provided to the parents or guardians to consider at home. Parents (or guardians) will be given a list of baseline evaluations that must be completed prior to study entry (see Appendix B), and an explanation that children who do not complete the baseline evaluations cannot enroll in BABY HUG. They will then be provided with a list of procedures that will be required after they enroll their child in BABY HUG (see Appendix B). Explanations of these procedures will include the risk of radiation exposure associated with the radionuclide kidney function test and the liver-spleen scan. Finally, parents (or guardians) will meet with assigned independent Patient/Family advocates to discuss making a decision whether or not to enroll their child in BABY HUG.

Parents (or guardians) will be informed that their child's participation in BABY HUG and their child's treatment, which will be made known to them at the end of the last-enrolled child's treatment in the study (along with study conclusions), should be noted in their child's medical record. This information should be provided by the parents (or guardians) to all doctors taking care of their child. At the start of each child's long-term follow-up, the parents (or guardians) will be contacted for

permission to examine their children for up to five to ten years to learn about any long term effects the study treatment may have.

Parents (or guardians) who express interest in BABY HUG will be advised of the importance of adherence with study visits and procedures. Evidence of compliance with clinic visits and of compliance with standard care for sickle cell anemia such as prophylactic penicillin administration (see Section 8.3) will be documented in BABY HUG. Study personnel will use this information to estimate the family's likelihood of complying with study requirements.

3.5 BASELINE ASSESSMENT

The children of those parents who express interest in participation will undergo the pre-enrollment studies listed in this section. Children who are unable to complete baseline evaluations will not be enrolled in BABY HUG. After obtaining informed consent (see attachment to this chapter) from the parent or guardian, baseline studies will be performed before randomization and enrollment. Baseline assessment includes:

- Directed history and physical examination (including neurological evaluation)
- Hemoglobin electrophoresis
- Complete blood count and other baseline blood determinations
- Urinalysis, including urine concentrating ability
- Anthropometry: Height, weight and head circumference
- Neuropsychological evaluation and parent questionnaire
- Liver-spleen scan
- Abdominal sonogram (for gallstones, spleen size and kidney size)
- Transcranial doppler measurements (interpretable study not required, but confirmed velocity $\geq$200 cm/sec results in ineligibility)

Renal DTPA clearance studies will be evaluated after the first dose of medication is given (i.e., after randomization) for all children enrolling in BABY HUG.

Children whose hemoglobin electrophoresis does not document an FS or SF pattern will not be eligible for BABY HUG. Any serious medical problems unrelated to sickle cell anemia, for example, leukemia or cerebral palsy or mental retardation, identified in the course of pre-enrollment evaluations (e.g., history and physical examination, complete blood counts, neuropsychological evaluations) will result in referral for appropriate medical care and ineligibility for BABY HUG.

3.6 INFORMED CONSENT

Individual Clinical Center consent forms will be prepared based on a model informed consent approved by the Data and Safety Monitoring Board. Recruitment brochures (including the BABY HUG study quarterly newsletter) or advertisements, consent forms and the Protocol will be submitted for approval by local Institutional Review Boards. Each final consent form will be reviewed by a member of the BABY HUG Data and Safety Monitoring Board (See Section 13.3) to ensure all appropriate issues are addressed. Patient/Family advocates have been identified at each Clinical Center to advise families independently of any discussions they have with study investigators or health care providers.

The Clinical Center Principal Investigator will obtain the consent from each family. If both parents are reasonably available and responsible for the child, they will both be asked for signed, informed consent. 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.

3.7 PATIENT/FAMILY ADVOCATES

Each BABY HUG Clinical Center will develop a plan to have a third party person (Patient/Family advocate) involved in the consent process to eliminate any coercion, in particular because of the vulnerable study population. Some BABY HUG Clinical Center Institutional Review Boards employ a full time staff to function in this capacity. The Patient/Family advocate role focuses on ensuring that systems are in place and working effectively to minimize research-related

risks experienced by patients and families, addressing concerns or complaints that arise from treatment or research, and facilitating resolution of substantive issues that could have negative impacts on patient/family understanding of and participation in research (see Exhibit 3-1). The Patient/Family advocate acts as an impartial advocate to ensure that patients/families are treated fairly and equitably, are directed to appropriate resources within the institution for resolution of non-clinical, operational problems and are assisted in their communication with all Clinical Center staff. The Patient/Family advocate is responsible for appropriately collaborating with all internal and external resources required to bring closure to the issues presented. Meeting(s) with the Patient/Family advocate will be documented for each child enrolling in BABY HUG.

Exhibit 3-1

Qualifications and Responsibilities of Patient/Family Advocate

Each BABY HUG Clinical Center will develop a plan to provide a Patient/Family advocate (ombudsman) for patients and families in the BABY HUG study. The following are minimal general requirements for this position. Each BABY HUG institution must devise their own proposal within these guidelines. Each proposal then must be approved by the BABY HUG DSMB.

1. Qualifications
   a. Familiarity with clinical trials/informed consent
   b. Familiarity with bio-ethics concepts
   c. Complete NIH acceptable human subjects training
   d. Independence from BABY HUG investigators

2. Responsibilities and Activities
   a. Ensure that patients/families are treated fairly and equitably
   b. Assist families in communication of concerns, complaints, or questions that arise from participation in the study
   c. Facilitate resolution of substantive issues that impact participation in research
   d. Direct patients/families to appropriate resources within the institution
   e. Meet potential BABY HUG subjects/families prior to enrollment, to review the study and address concerns
   f. Ensure understanding of the risks and benefits of the study
   g. Ensure that the patient's family understands the randomization process and that the subject may receive a placebo
   h. Clarify patients'/family's rights and responsibilities
   i. Monitor the informed consent process
   j. Maintain availability and contact with families during the course of the trial

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 4

STUDY ENDPOINTS

4.1 INTRODUCTION

The primary objective of Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) is to determine the safety and effectiveness of hydroxyurea (HU) in the prevention of chronic end organ injury occurring among sickle cell anemia patients who are 9 through 17 months of age at the time of study entry. A Feasibility and Safety Pilot Study initiated BABY HUG recruitment with 40 patients 12 through 17 months of age to (1) determine the feasibility of the protocol design, (2) provide additional safety and toxicity data, and (3) validate the proposed methods of quantitating splenic and renal function. This initial group was studied intensively and monitored closely. The DSMB has recommended that 160 additional participants be enrolled in BABY HUG for a total of 200 patients in the full Phase III trial. The primary analysis in the full study will compare the frequency of worsening of spleen function (from normal to decreased or absent, or from decreased to absent) as measured by splenic uptake on a technetium -99m sulfur colloid liver-spleen scan at two years of follow-up after study entry in patients randomly assigned to treatment with hydroxyurea versus those assigned to placebo. Data monitoring analyses are scheduled every six months from the start of enrollment in the study.

The analysis of the primary study outcomes of the full Phase III trial will be conducted on an "intention to treat" basis, with two-sided statistical tests comparing the outcome between groups of patients defined at study entry by random assignment to the hydroxyurea and placebo groups. Blinding of outcomes in the primary analysis is protected by use of placebo, standardized methods for performance of spleen scintigraphy studies in nuclear medicine departments not otherwise involved in the BABY HUG, and blinded central reading of spleen scans by nuclear medicine specialists not otherwise involved in BABY HUG. 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). A more detailed presentation of anticipated analyses is provided in Sections 4.4 through 4.7.

4.2 PRIMARY ENDPOINTS

4.2.1 Spleen Scintigraphy

The spleen is damaged early in life in a large percentage of children with sickle cell anemia or sickle beta zero thalassemia. The consequences of splenic injury -- episodes of splenic sequestration and invasive pneumococcal infection -- are clinically significant and quantifiable. These clinical events will not form the spleen endpoint per se, but will be evaluated along with other clinical events during the course of the study.

Nearly 40 years ago it was recognized that young children with sickle cell anemia were at high risk of bacteremia with encapsulated organisms, and that the consequence of those invasive infections was often fatal septicemia. This susceptibility was attributed to splenic dysfunction, and indeed many of these young children had Howell-Jolly bodies on their peripheral blood smears; however, on clinical examination these patients also often had palpable, enlarged spleens. Thus the concept of functional asplenia, in which a palpable, enlarged spleen did not function normally, was developed or invoked to explain the clinical observation in children with sickle cell disease (Pearson et al, 1969). The standard procedure used to detect functional asplenia is the ^{99m}Tc sulfur-colloid liver-spleen scan. Liver-spleen scans are available in all participating BABY HUG institutions and their performance in each center is well standardized. Pediatric hematologists are aware that spleen function declines with age in infants and toddlers with sickle cell anemia; however, there are no good data on the cross sectional frequency or rate of loss of splenic uptake of ^{99m}Tc sulfur colloid at a given age in young patients with sickle cell anemia.

The Cooperative Study of Sickle Cell Disease (CSSCD), a National Heart, Lung, and Blood Institute (NHLBI) - sponsored observational study that has been responsible for documenting the

clinical course of sickle cell disease in the U.S., examined pitted cell counts and liver-spleen scans in a cross-sectional cohort of 8 to 13 month old patients. Pearson (Pearson et al, 1985) reported that by 8 to 13 months of age, 23% of infants with HbSS had absent and 8% had decreased splenic uptake of ^{99m}Tc sulfur-colloid on liver-spleen scan indicating functional asplenia. Decreased or absent visualization of the spleen on liver-spleen scan correlated with a pitted cell count over 3.5% in this group of young children with sickle cell disease.

Spleen function will be assessed by uptake of ^{99m}Tc sulfur colloid on liver-spleen scan before initiation of treatment and 2 years later. The results of each of the two scans will be categorized as normal, functional but abnormal, or not functional by a panel of nuclear medicine specialists blinded to treatment assignment. In HUSOFT, the intra-observer agreement on re-reading scans was over 90% (33/36 scan readings) (Rogers, unpublished observations). The difference between the HU and placebo groups in the proportion of patients worsening in spleen function over the study period will be tested.

The CSSCD further examined spleen function by serial performance of pitted cell counts. Their data indicated that by one year of age pitted cell count values over 3.5%, which are associated with absent visualization on liver-spleen scan, occurred in 28% of SCA patients, by 18 months 44%, by 24 months 58%, by 36 months 78%, and by 48 months 89% of SCA patients. They concluded that the average age of onset of a pitted cell count greater than 2% but less than 3.5%, i.e., impaired spleen function, was 13 months and could be as early as 5 months (Pearson et al, 1985). Thus, up to one-third of enrolled children could be unassessable for worsening of spleen function (will have absent spleen function at entry).

At entry into the HUSOFT pilot trial, liver-spleen scans showed absent uptake in 24% of patients at entry (mean age 15 months). After 2 years on HU (mean age 39 months) 47% of the scans were interpreted as demonstrating absent uptake (functional asplenia). Since the CSSCD pitted cell count data suggest that functional asplenia should have been present in 34% of patients

at entry and 80% at exit, HUSOFT suggests a possible spleen protection benefit compared to untreated patients (Pearson et al, 1985; Wang et al, 2001; Hankins et al, 2005).

Therapy with hydroxyurea, however, may alter the ability of the pitted cell count to accurately reflect spleen function. A longer red cell life span (which may be expected to occur when fetal hemoglobin rises and intravascular hemolysis decreases during hydroxyurea therapy) will likely increase the pitted cell counts. Elevated levels of fetal hemoglobin may increase the rate of endocytosis, and thereby also increase the pitted cell counts. Conversely, preservation of spleen function while on HU therapy could maintain low pitted cell counts. Restoration of splenic function on HU therapy, analogous to the effect observed on chronic transfusion therapy, would be expected to decrease the pitted cell counts (Sills et al, 1988; Lane et al, 1996).

The entry and exit ^{99m}Tc sulfur colloid liver-spleen scans will be individually read by a panel of three radiologists not involved in the acquisition of the images. Scans will be categorized as showing normal, decreased, or absent spleen function, and then will be compared for change. In the unlikely event of an apparent increase in splenic uptake of sulfur colloid, splenic function will be counted as stable. The proportion of patients whose paired scans demonstrate a decline in splenic function will be compared in the HU versus placebo groups. Statistical power to detect differences that are 50% (relative) or greater in the incidence of worsening of spleen function should be good to excellent, in view of the fact that over 80% of children with sickle cell anemia would be expected to lose splenic function completely by the end of the treatment period (Pearson, 1988). Scintigraphic assessment of splenic function is one of the two primary endpoints of BABY HUG.

In a recent publication (Santos et al, 2002) on splenic function in older children (none in the BABY HUG age range) treated with hydroxyurea for sickle cell anemia, semi-quantitative analyses using spleen/liver ratios were conducted. However, only two of 21 (9.5%) patients in this before-after comparison showed large degrees of improvement, and one showed a large degree of deterioration. Small differences that were interpreted as improvement could be random noise. In HUSOFT, only one of 17 (5.9%) patients (most in the BABY HUG age range) had improved spleen

function in a before-after assessment of hydroxyurea therapy. To evaluate the potential for splenic function improvement in BABY HUG, a blinded review of spleen function will be made in the BABY HUG study.

Tables 4-1 and 4-2 show the power to detect a difference between categories of a trichotomous endpoint for varying levels of treatment and placebo patient improvement rates (in increments of 0.05) that are possible given the placebo incidence rate; the calculations in Table 4-1 assume a placebo incidence rate of worsening spleen function of 80/100 patients (see Section 4.4.3) over the course of two years whereas Table 4-2 is based on 60/100 (see Section 4.4.1.5). The boldface line in each table denotes the power “break-even” point between the dichotomous endpoint (assuming 20% of the patients have no spleen function at study entry and thus cannot worsen) and the ordinal endpoint. For example, in the current study design with a dichotomous endpoint, we have 96.8% power to detect a difference between the two categories (assuming a placebo incidence rate of 0.60 and 80 patients in each group). Thus, the boldface line in Table 4-2 is drawn along the 96.8% power continuum. For all treatment/placebo improvement rate combinations to the left of the line, the dichotomous endpoint will provide greater power; for all combinations to the right of the line, the ordinal endpoint will provide greater power.

Table 4-1
Power for Ordinal Spleen Endpoint as a Function of Improvement Rates

Placebo Incidence of Worsening Spleen Function = 0.80
Treatment Incidence of Worsening Spleen Function = 0.40

Alpha =0.04
N=100 in each group

		Y = Treatment "Improvement" Rate					
		.05	.10	.15	.20	.25	.30
X = Placebo "Improvement" rate	.05	99.5	99.6	99.8	99.8	99.9	99.9
	.10	98.7	99.1	99.3	99.5	99.7	99.8
	.15	96.7	97.7	98.3	98.7	99.1	99.3

Power (%)

Where X and Y are splenic function improvement rates with the following constraints:

	Splenic Function		
	Improvement	Stable	Worsen
Placebo	X	(1-0.8) - X	0.8
Treatment	Y	(1-0.4) - Y	0.4

Table 4-2
Power for Ordinal Spleen Endpoint as a Function of Improvement Rates

Placebo Incidence of Worsening Spleen Function = 0.60
Treatment Incidence of Worsening Spleen Function = 0.30

Alpha = 0.04
N= 100 in each group

		Y = Treatment "Improvement" Rate												
		.05	.10	.15	.20	.25	.30	.35	.40	.45	.50	.55	.60	.65
X = Placebo "Improvement" Rate	.05	92.3	95.2	97.2	98.5	99.2	99.6	99.8	99.9	100	100	100	100	100
	.10	84.3	89.5	93.3	95.9	97.7	98.7	99.3	99.7	99.8	99.9	100	100	100
	.15	72.3	79.9	86.0	90.8	93.9	96.3	97.9	98.9	99.4	99.7	99.9	99.9	100
	.20	56.0	65.4	73.9	81.2	87.1	91.6	94.7	96.9	98.3	99.1	99.5	99.8	99.9
	.25	39.5	49.2	58.9	68.1	76.2	82.5	88.6	92.3	95.5	97.2	98.5	99.2	99.6
	.30	24.0	32.2	41.4	51.1	60.8	69.8	77.7	84.3	89.5	93.3	95.9	97.7	98.7
	.35	12.6	19.0	25.5	34.0	44.3	54.0	63.6	72.3	79.9	86.0	90.4	94.2	96.3

Power (%)

Where X and Y are splenic function improvement rates with the following constraints:

	Splenic Function		
	Improvement	Stable	Worse
Placebo	X	(1-0.6) - X	0.6
Treatment	Y	(1-0.3) - Y	0.3

Both tables indicate that, in general, high rates of splenic function improvement are needed before the ordinal endpoint provides greater power than the dichotomous endpoint. Both endpoint types have high power (> 99%) when the placebo improvement rate is low and the placebo incidence rate is high. However, in general, an ordinal endpoint for BABY HUG would have less power than a dichotomous endpoint unless at least 20% of patients have improved spleen function. At the end of the Feasibility and Safety Pilot Study, it was determined that the spleen endpoint will be kept dichotomous as currently described in the Protocol. The ordinal spleen endpoint will be included as a secondary outcome.

Kidney - Glomerular Filtration Rate (GFR)

This endpoint has been removed as of May 29, 2009.

4.3 SECONDARY AND SAFETY ENDPOINTS

4.3.1 Central Nervous System

In addition to potential HU toxicity or protection of brain development, CNS infarction may occur during this study period and this will affect outcome. In the CSSCD, at the time of first CNS evaluation (around 6 years of age), 5% of children with HbSS disease had experienced a clinical CVA (Armstrong et al, 1996; Moser et al, 1996). Other CSSCD findings related to stroke suggest that the first clinical CVA may occur by 2-3 years of age (Ohene-Frempong, 1991). Evaluation of neurodevelopmental functioning will help determine whether HU (a) increases developmental risk, either through an increase in the occurrence of CNS infarction or some other pathophysiologic process (e.g., vascular formation and function, metabolic activity, hypoxia; Brown et al, 1993), (b) decreases infarction risk, or c) has no effect on CNS development. These evaluations are particularly sensitive to a child's neurodevelopmental course during the first years of life. They make comparisons based on normative motor (gross motor and fine motor) and language and verbal skill development during the first 3.5 years of life, and these discriminate between children with disease progression and those with no disease progression (Gay et al, 1995).

Neurological examinations and neuropsychologic evaluations with Bayley and Vineland tests will be performed annually, and compared between treatment groups as part of early and ongoing monitoring as well as for the 2-year outcome. Head circumference measurements will be performed quarterly and compared to a normal black population (Pivnick et al, 1999).

4.3.2 Spleen

^{99m}Tc sulfur-colloid liver-spleen scans provide significant information about spleen function but, since they involve radionuclide exposure, cannot be repeated frequently. During the process of aging, the red cell membrane forms vacuoles, which appear on the surface of the cell as “pits” under Nomarski optics. Pitted or pocked red cells are removed from the circulation by splenic macrophages. Thus the percentage of red cells with pits increases as splenic function declines (Pearson et al, 1985). Pearson reported that pit counts >3.5% correlated with nonvisualization of the spleen on liver-spleen scan (Pearson et al, 1969). Thus, serial pitted cell counts can theoretically serve as a surrogate for liver-spleen scan determinations of splenic function in the most patients with sickle cell anemia. However the cumulative effect of hydroxyurea therapy on pitted cell counts is not known.

Pitted cell counts are performed as follows: One drop of EDTA anticoagulated fresh venous blood is fixed in 0.5 ml of isotonic 1% buffered glutaraldehyde. One thousand cells are counted under Nomarski differential interference contrast microscopy and scored for percentage of pitted cells by an experienced technologist. The test should be performed in a laboratory with demonstrated long term interest and expertise in order to assure that the numerical values at the end of the study can be compared to those at the start.

Entry, exit and interim pitted cell counts will be compared. Statistical models will be used to compare entry and exit values and test for trends. In this manner we may be able to validate the use of the pitted cell count, which is less invasive than the liver-spleen scan, to assess splenic function during hydroxyurea therapy. The proportion of patients in the HU treatment group compared to the placebo treated group with pitted cell counts above predetermined thresholds will

be a secondary outcome measure in BABY HUG.

4.3.3 Kidney Function and Bladder Control

Proteinuria occurs in 6.2% of children with sickle cell anemia, and the rate increases with age (Wigfall et al, 2000). Microalbuminuria is an early marker of other nephropathies, and occurs in 26.5% of children with sickle cell anemia (Darnidharka et al, 1998); however, it was not detected in children under 7 years of age in the only reported study in children. Ultrasound imaging of the kidneys often reveals increased size and echogenicity (Miller, unpublished observations). Normal kidneys increase from approximately 6 to 8 cm in length over the first 4 years of life; in infants with a single functioning kidney, hypertrophy results in a length of 10 cm by the age of 3-4 yr. (Laufer and Griscom, 1971).

Not only is renal failure a significant problem in adults with sickle cell anemia, the problem of enuresis and/or nocturia is nearly ubiquitous in children with sickle cell anemia, and is embarrassing and inconvenient. The CSSCD documented nocturia in 58% of 6 year-olds, and enuresis in 58% (75% of males). Forty-one percent of males remained enuretic at age 12 years. In comparison, 75% of normal children have nocturnal bladder control by 4 years of age (Rudolph and Kamei, 1998). Treatment of enuresis is often ineffective. Enuresis is related to loss of ability to concentrate urine. Enrolled patients' urine concentration will be evaluated by measuring urine and serum osmolality, after overnight and early AM withholding of water at baseline (prior to random treatment assignment) and at the end of study treatment (e.g., this could be incidentally accomplished when the children are NPO for abdominal sonogram studies). Urine and sera will be shipped to the Hematology and Biochemistry Core Laboratory for measurement of the urine osmolality (mOsml/kg water), and serum osmolality and creatinine (LaPorte-Wijsman and Struyker-Boudier, 1967). Specific gravity will be measured locally at the Clinical Center on routine urinanalysis.

4.3.4 Abdominal Sonogram

Although hepatomegaly commonly occurs in infants and young children with sickle cell anemia and some patients have total indirect bilirubin levels in excess of that attributable solely to hemolysis (recently attributed to mutations of the glucuronosyl transferase gene), hepatic dysfunction, as detected by direct hyperbilirubinemia, elevation of liver specific enzymes (ALT), hypoalbuminemia, or coagulopathy, is rare; in fact if it occurs, other causes must be carefully sought. However, as with most chronic hemolytic disorders, cholelithiasis is common in sickle cell anemia. Bilirubinate pigment stones have recently been estimated to occur in 14% of children by age 10 years. However, an early study using only radiography found gallstones in 17% of children under the age of ten (Schubert, 1986; Stephens and Scott, 1980). Only about 50% of pigment stones are radio-opaque, suggesting that there may have been a much higher incidence of stones in this early series. While often stones are asymptomatic, cholecystectomy is nonetheless a common elective surgical procedure in individuals with sickle cell anemia and sometimes must be performed urgently (Vichinsky et al, 1995). Common duct obstruction is a common and morbid complication of cholelithiasis. Cholelithiasis is best diagnosed by ultrasonography (Sarnaik et al, 1980). Hydroxyurea therapy, by increasing total and fetal hemoglobin and reducing hemolysis, might reduce the incidence of gallstones.

Abdominal ultrasonography techniques will visualize the kidneys and right upper quadrant, and sonograms can be read centrally with attention to presence or absence of gallstones or sludge, the presence or absence of gall bladder thickening or duct dilation, renal volume and echogenicity, liver enlargement and spleen volume and size.

4.3.5 Pulmonary

The pulmonary system is adversely affected by SCA, in particular because of the phenomenon of the acute chest syndrome (ACS). There is evidence that the development of chronic lung disease in adults with SCA is associated with recurrent episodes of ACS (Santoli et al, 1998). Several methods have been used to assess pulmonary function in patients with SCA,

including transcutaneous pulse-oximetry. The progression of pulmonary dysfunction in SCA may be assessed with pulse oximetry, which measures hemoglobin oxygen saturation transcutaneously. Although there are some methodologic problems with its use in sickle cell anemia due to abnormal hemoglobin oxygen half-saturation (P-50) in HbSS patients, this non-invasive, inexpensive technique has been the most widely studied method in SCA. In a study of 108 children with SCA, those with histories of ACS had lower saturations (mean = 94%) than those without a history of ACS (mean = 98%) (Rackoff et al, 1993). Oxygen saturation by pulse oximetry will be measured at entry and every three months.

4.3.6 Anthropometry

Standing or sitting weight will be measured at every clinic visit (see Appendix E). Recumbent length (standing height after 18 months of age) and head circumference (see Appendix E) will be obtained quarterly. These measurements will be checked for quality with longitudinal data analysis methods and compared between treatment groups using t-tests and longitudinal data analysis.

4.3.7 Chromosome Analysis

Karyotype and chromosome breakage studies will be performed to determine whether or not hydroxyurea is clastogenic in children. Hydroxyurea has been clastogenic in onion root tips and alters mouse testicular kinetics and sperm chromosome structure, but it is not certain whether hydroxyurea is clastogenic in humans (Evenson and Jost, 1993).

4.3.8 Acquired DNA Mutations

Mutation events will be evaluated centrally in a defined DNA model to determine whether or not hydroxyurea is mutagenic. The mutagenicity of hydroxyurea is controversial. Mutation analyses will be performed for illegitimate T cell receptor (VDJ) recombinations in the Mutation Analysis Core Laboratory at St. Jude Children's Research Hospital.

4.3.9 Immune Function

Because hydroxyurea has the capacity both to suppress immune response and to alter spleen function, it is of interest to observe directly the effect of hydroxyurea on antibody formation and on immune cell populations. To this purpose, specific antibody titers and opsonization assays before and after measles-mumps-rubella (MMR) immunization and conjugate pneumococcal immunization will be compared between the treatment groups. Also, T-cell subpopulations will be evaluated by flow cytometry.

4.3.10 Clinical Events

All patients will be monitored for the occurrence and severity of clinical events. These will be identified through the use of standardized history questions administered at each clinical encounter. 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. All events will be reported utilizing standardized definitions (See Appendix F) on an Events Form. 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. Events of interest include bacteremia with known pathogens, meningitis, splenic sequestration, acute chest syndrome, stroke or transient ischemic attacks, osteomyelitis, all hospital admissions and any event for which the patient receives a transfusion. Selected events and supporting documentation collected by Clinical Center staff will be reviewed centrally by two pediatric hematologists who are not involved in the BABY HUG Clinical Center.

Pain (Platt et al, 1991) and acute chest syndrome (Castro et al, 1994) are the most frequent complications of sickle cell disease and most frequent causes of hospitalization. In addition to causing substantial morbidity, both have been associated with early death (Platt et al, 1994), and acute chest syndrome has been associated both with chronic sickle cell lung disease (Powars et al, 1988), a major cause of morbidity in adults, and also with increased risk of infarctive stroke (Ohene-Frempong et al, 1998). One of the earliest clinical manifestations of sickle cell disease,

dactylitis, is associated with adverse clinical outcomes later in childhood in the Jamaican cohort (Stevens et al, 1981), and recently early onset of dactylitis was associated with an increased risk of death, stroke, or recurrent pain and acute chest syndrome later in childhood in the Cooperative Study of Sickle Cell Disease (Miller et al, 2000).

The Multicenter Hydroxyurea Study (MSH) was stopped early due to significant reduction in these major sickle cell complications (Charache et al, 1995). Several single institution pediatric trials have suggested that similar beneficial clinical effects will be seen in children treated with hydroxyurea (Ferster et al, 1996; Jayabose et al, 1996; Maier-Redelsperger et al, 1999; Olivieri and Vichinsky, 1998; Scott et al, 1996). In the newborn cohort, comprised of infants enrolled to the CSSCD prior to six months of age, frequencies of complications in infants with Hb SS per 100 patient-years of observation were reported (Gill et al, 1995). Table 4-3 summarizes the frequencies.

Table 4-3
Complication Frequencies in Infants with Hb SS
Per 100 patient-years of observation

	CSSCD	
AGE	**PAIN EVENT**	**ACUTE CHEST**
< 6 mos	2.9	6.8
6-12 mos	9.5	16.4
1 yr	24.0	26.8
2 yr	38.3	26.3
3 yr	42.4	34.2
4 yr	49.6	25.5

In comparison, event rates among the HUSOFT children, who were treated with hydroxyurea starting at six to twenty four months of age and followed for up to two years were 17/100 patient years for pain events and 15/100 patient years for acute chest syndrome occurrences. These data suggest that hydroxyurea may have clinical efficacy in these very young children.

While the acute chest syndrome in children may be different than that seen in adults (Castro et al, 1994; Golden et al, 1998; Vichinsky et al, 1997), with children having more infectious causes,

it is clear that, at least in older children with sickle cell disease, chronic transfusion substantially reduces the frequency of this complication in the pediatric population. Both in a retrospective review by Oakland Children's Hospital (Styles and Vichinsky, 1994) and in a prospective analysis of the patients enrolled in the Stroke Prevention in Sickle Cell Anemia (STOP) (Miller et al, 2001) there were reductions in acute chest syndrome with transfusion. Hydroxyurea therapy may similarly reduce frequency of these events in BABY HUG. Since the primary concern of the BABY HUG investigators is the prevention of organ damage, the study will not be terminated for any demonstrated advantage of hydroxyurea over placebo in terms of the occurrence of clinical events. If these expectations are proven incorrect, and hydroxyurea is associated with a demonstrably increased frequency of death, stroke, acute chest syndrome or consequential splenic sequestration events, the study would be terminated.

Events to be centrally reviewed include all Serious Adverse Events (e.g., death, events that are life-threatening, events that cause or prolong hospitalization greater than 7 days, splenic sequestration crisis, acute chest syndrome, stroke, transient ischemic attacks, ICU admissions and any AE that is related to study treatment and unexpected). Other clinical occurrences will be denoted as having occurred or not occurred on clinic visit reports. There is adequate statistical power in BABY HUG to detect 50% differences between treatment groups at alpha = 0.01 if the event rates are in the range expected from the CSSCD and the study is completed with 200 patients. The BABY HUG investigators do not plan for early termination based on clinical events other than demonstrated inferiority of hydroxyurea for the outcomes death, stroke or splenic sequestration.

4.3.11 Transcranial Doppler Measurements

In children between the ages of 2 and 16 years, an abnormal TCD velocity (200 cm/sec or more) in the proximal middle cerebral artery or distal internal carotid artery has been found to predict a high risk of stroke. In the STOP study, a randomized trial in which chronic transfusion was compared with observation in patients with abnormal TCD velocities, a greater than 90% reduction

in stroke risk was associated with transfusion (Adams et al, 1998). More recently, Zimmerman and Ware (Zimmerman et al, 2002) have presented evidence that hydroxyurea treatment in school-aged children is associated with a lower TCD velocity, in part related to improved hemoglobin levels. In addition, Bowman and Adams (Bowman et al, 2002) have recently presented information indicating that TCD measurements can be performed in very young children with sickle cell disease, ages 2-21 months (mean 11 mo.); 23 of these 27 children were able to have TCD studies performed and in 22 of those 23, meaningful results were obtained (although only 7 had complete studies). The mean TCD velocity was 109 cm/sec., substantially lower than in school-aged children. Studies from the French group led by Bernaudin (Bernaudin et al, 2001) have also shown a lowering of TCD velocity in children (age 3-18 years) when treated with hydroxyurea.

These data raise the possibility that infants who are randomized to hydroxyurea in the BABY HUG trial may have a lower TCD velocity compared to the control group receiving placebo. If TCD velocity is considered a surrogate marker for stroke risk in this population, velocities may provide a continuous variable which can be used as an endpoint of hydroxyurea efficacy. There are, however, a number of unanswered questions: Are the lower velocities seen in infants with sickle cell disease likely to be modulated by hydroxyurea? Are the effects, if any, mediated by changes in hemoglobin level and other red cell properties? Does a lowering of TCD within the normal range (for example, from 150 to 130 cm/sec) actually result in a reduced stroke risk? At this time, these questions cannot be fully addressed and, therefore, TCD would not be likely to be useful for addressing the primary objective of BABY HUG: Does hydroxyurea prevent organ damage? However, the potential for TCD measurements to provide relevant data on the risk of CNS events in this population warrants inclusion of these studies as a secondary endpoint for evaluating prevention of organ damage by hydroxyurea.

4.4 STATISTICAL CONSIDERATIONS IN DESIGN AND STUDY SIZE

4.4.1 Feasibility and Safety Pilot Study

The Feasibility and Safety Pilot Study has been completed and these sections have been removed for the sake of Protocol continuity. The relevant modifications, based on information from the Feasibility and Safety Pilot Study, have been incorporated into this Protocol revision.

4.4.2 Primary Treatment Comparison (Full Phase III Trial)

Most of the studies conducted in BABY HUG will compare the effect of treatment with hydroxyurea (HU) to treatment with placebo (PLBO). This study will be conducted with one primary endpoint: worsening of spleen function from normal to decreased or absent, or from decreased to absent on uptake of technetium sulfur colloid.

The primary analysis in the full Phase III trial will be done using an intention-to-treat principle. Patients randomly assigned to a treatment will be analyzed according to the assigned treatment, irrespective of whether the treatment was actually given. The spleen endpoint will be tested at an overall alpha = 0.04. An interim monitoring plan is proposed for each of these endpoints. The interim monitoring plan will be constructed such that the experiment-wise error rate of the study is preserved at the specified overall alpha levels (0.04 for spleen). The monitoring plan will reserve most of the alpha level for the final comparison. The shape of the rejection regions will follow a Haybittle (Haybittle, 1971) type plan with consistent, wide bounds set for the interim analyses and a bound for the final analysis approximately equal to the critical value that would be used if there were no interim monitoring. Actual allocation of alpha will be done using the spending functions of Lan and DeMets (Lan and DeMets, 1983).

The BABY HUG investigators considered using a single primary endpoint for this study and developing composite scores of different endpoints. Five different organs were considered: the brain, lung, spleen, liver, and kidney. The rationale follows:

Primary endpoints based on neurological and neuro-imaging evaluations were excluded because the incidence of central nervous system injury is too infrequent (e.g., stroke less than 1

per 100 children per year) for a study of 200 very young children. Also, central nervous system outcomes will be monitored for safety as are continuous measures of brain function (e.g.,neuropsychological tests and head circumference). Differences in transcranial doppler (TCD) velocities have not yet been confirmed as clinically important below 170 cm/sec and have not been shown to be clinically useful in children less than two years old. All of these assessments are of interest as secondary endpoints.

The lung was excluded since there are no reproducibly good pulmonary function tests over this age range; spirometry is hard to obtain on infants and pulse-oximetry lacks the variability necessary to be considered as a primary endpoint (e.g., infants do not vary much from 94-98% under normal circumstances).

Liver damage that could be detected using standard measures is rare in untransfused very young children. The incidence of gallstones in very young children is expected to be low.

As described fully in Chapter 1, spleen function deteriorates rapidly in the absence of treatment, and treatment with HU may have a substantial effect on this endpoint over a two-year period. Spleen function on ^{99m}Tc sulfur colloid liver-spleen scan can be characterized as normal, decreased or absent. This evaluation of the spleen is categorical in nature. Categorical endpoints generally require greater sample sizes than continuous measures to detect clinically meaningful differences.

DTPA GFR was originally a primary endpoint for this study but its measurement was discontinued as of May 29, 2009.

The features common to power evaluations for this endpoint are the critical values used to determine the alpha level and power. We have designated these values as Z_α and Z_β respectively. ”N” is the number of children in each treatment group necessary for this study. We have presented the formulas for study size calculations, but each of these formulas can be algebraically rearranged to provide corresponding power calculations.

4.4.3 Spleen Endpoint

Worsening of spleen function will be a co-primary endpoint of this study. Spleen function will be evaluated by scintigraphy at the time of the baseline evaluation, and at the conclusion of two years of treatment. Based on these two evaluations it will be determined whether spleen function has worsened conditional upon the spleen having some level of function at the time of the baseline evaluation (i.e., we will not include in this analysis children who have absent spleen function at the beginning of the study). This test is in broad clinical use and can be performed in all BABY HUG Clinical Centers. HUSOFT demonstrated the feasibility of collecting and interpreting categorical scan readings. Liver-spleen scans will be read centrally by two independent readers blinded to treatment assignment. Disagreements will be settled by a third reader.

In children with sickle cell anemia, spleen function rapidly deteriorates. It is anticipated that up to one-third of the children entering the BABY HUG study may have absent spleen function at the time of study entry. Thus, an effective sample size lower than 100 per group will likely be available for the evaluation of this endpoint. A large majority of children will likely have abnormal spleen uptake at the time of entry into the study. Thus, worsening for this endpoint (the main study objective is to determine if HU prevents end organ damage) will be closely related to "absent spleen function" among children enrolling with functional spleens. The sample size formula for the analysis of a binary endpoint is:

$$N=\frac{(z_\alpha\sqrt{2\ \overline{pq}}+z_\beta\sqrt{(p_1q_1+p_2q_2)})^2}{(p_1-p_2)^2}.$$

In the above equation: p_1 is the proportion of children with some level of spleen function at entry who are assigned to HU who have categorically worse or absent spleen function by the end of the study; $q_1 = 1-p_1$; p_2 is the proportion of children with some level of spleen function who are assigned to PLBO who have categorically worse or absent spleen function by the end of the study; $q_2=1-p_2$;

$\bar{p}$ is the average of p_1 and p_2, $\bar{q} = 1-\bar{p}$ and N = study size (each group). The overall alpha level of this comparison will be alpha = 0.04 (two-tailed).

We will test the null hypothesis:

H_O: $p_1 = p_2$

versus the alternative:

H_A: $p_1 \neq p_2$.

There are four planned interim reviews of this endpoint by the DSMB over the course of the study. Allowing for sequential tests of the endpoint, nominal values of Z = 3.5 will be used for interim looks, the final comparison will be performed at a nominal alpha level of 0.0394 (Z_α =2.06). It is assumed that the rate of worsening function of the spleen will be at least 80% in the PLBO group. Figure 4-1 presents the power to detect 50% reductions in the spleen endpoint event rate as a function of a range of anticipated placebo event rates for categorically worse or absent spleen function by the end of the study, and of a range of possible sample sizes from 20 to 100 per group. A review of this figure shows that the proposed study will have adequate power (80% or more) to detect 50% reductions in the rate of absent spleen function in the HU group, provided that the sample size is 70 or more in each of the treatment arms. Even with 20% of the initial study population classified as having absent spleen function, the realized sample size of 160 infants (80 in each group) will have more than 90% power to detect a 50% reduction in the incidence of the endpoint as a result of the administration of HU, if the PLBO group incidence of this endpoint is 60% or more. With 30% of the initial study population classified as having absent spleen function, the realized sample size of 140 infants (70 in each group) will have more than 80% power to detect a 50% reduction in the incidence of the endpoint as a result of the administration of HU. Based on CSSCD data, by 48 months of age, at least 80% of patients assigned to placebo are expected to have absent spleen function.

The power to detect a 50% reduction in worsening of spleen function for the current study design is shown graphically in Figure 4-1 for five placebo incidence (of worsening) rates. In the presence of treatment crossover, both the placebo and treatment incidence rates are adjusted (the placebo rates decrease and the treatment rates increase), and the power to detect a difference is calculated based on these adjusted incidence rates.

Table 4.4 shows the reductions in power resulting from crossovers (children assigned to hydroxyurea who discontinue taking the treatment or children assigned to placebo who take hydroxyurea) according to four levels of treatment and placebo crossover rates (0.05, 0.10, 0.15, 0.20), two levels of placebo (0.80 and 0.60) and treatment initial incidence rates, two levels of sample size and alpha=0.04. The adjusted rates are the initial incidence of worsening rates adjusted for the specified crossover rate and are shown in the table in parentheses under the crossover rates. The sample sizes used assume either 20% or 30% of the initial study population are classified as having absent spleen function, resulting in N=80 and N=70 (in each group), respectively.

The first two sub-tables in Table 4-4 show that for a placebo incidence rate of 80% for worsening of spleen function (dichotomous outcome) and low crossover rates (i.e., 10% or less), the study has at least 97% power to detect a difference between the treatment and placebo. This level of crossover presents no threat to the integrity of BABY HUG. The last two sub-tables show that for an incidence rate of 60% for the placebo combined with low noncompliance rates, the study has at least 80% power to detect a difference. Thirty percent crossover will be a threat to study integrity. The BABY HUG investigators will do everything possible to prevent treatment crossovers.

Table 4-4
Power Tables for Treatment Crossover
Alpha = .04

		Treatment crossover rate (adjusted incidence rate)			
		0.05 (0.42)	0.10 (0.44)	0.15 (0.46)	0.20 (0.48)
Placebo crossover rate (adjusted incidence rate)	.05 (0.78)	0.997	0.994	0.987	0.976
	0.10 (0.76)	0.993	0.986	0.973	0.952
	0.15 (0.74)	0.985	0.971	0.948	0.914
	0.20 (0.72)	0.969	0.945	0.909	0.859

Placebo incidence rate = 0.80
Treatment incidence rate = 0.40
Power (100% compliance) = 1.00

N=80 in each group

		Treatment crossover rate (adjusted incidence rate)			
		0.05 (0.42)	0.10 (0.44)	0.15 (0.46)	0.20 (0.48)
Placebo crossover rate (adjusted incidence rate)	.05 (0.78)	0.993	0.986	0.975	0.956
	0.10 (0.76)	0.985	0.972	0.952	0.922
	0.15 (0.74)	0.970	0.949	0.917	0.873
	0.20 (0.72)	0.946	0.913	0.867	0.808

Placebo incidence rate = 0.80
Treatment incidence rate = 0.40
Power (100% compliance) = 0.999

N=70 in each group

		Treatment crossover rate (adjusted incidence rate)			
		0.05 (0.315)	0.10 (0.33)	0.15 (0.345)	0.20 (0.36)
Placebo crossover rate (adjusted incidence rate)	.05 (0.585)	0.924	0.890	0.846	0.793
	0.10 (0.57)	0.892	0.848	0.795	0.733
	0.15 (0.555)	0.851	0.798	0.736	0.666
	0.20 (0.54)	0.801	0.739	0.669	0.594

Placebo incidence rate = 0.60
Treatment incidence rate = 0.30
Power (100% compliance = 0.968

N=80 in each group

		Treatment crossover rate (adjusted incidence rate)			
		0.05 (0.315)	0.10 (0.33)	0.15 (0.345)	0.20 (0.36)
Placebo crossover rate (adjusted incidence rate)	.05 (0.585)	0.885	0.843	0.793	0.735
	0.10 (0.57)	0.846	0.795	0.737	0.673
	0.15 (0.555)	0.798	0.740	0.675	0.605
	0.20 (0.54)	0.743	0.678	0.608	0.535

Placebo incidence rate = 0.60
Treatment incidence rate = 0.30
Power (100% compliance) = 0.944

N=70 in each group

Analysis of GFR

This endpoint evaluation was discontinued on May 29, 2009.

4.4.4 Data Analysis

4.4.4.1 Introduction

Primary analyses for BABY HUG will focus on estimating treatment effects on the designated primary endpoint: loss of spleen function. 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.

4.4.4.2 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 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. 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.

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 provide point estimates for the regression coefficients, standard error estimates and confidence intervals. The results of these analyses are printed into computer files so that they can be directly inserted in progress reports using PROC REPORT. In some instances, the procedures in SAS will not suffice since SAS procedures usually do not include methods to incorporate information about missing data, nor do they include complex models specifically designed to relate a biological process with the risk of disease progression. We will use PROC IML and PROC NLIN to program the required models if necessary.

4.4.4.3 Missing Data - Prevention and Analysis

BABY HUG will use the methods of Probstfield (Shumaker et al, 1990; Probstfield et al, 1986) for improving adherence with follow-up. A key feature of this approach is the management of parents who show signs of dropping out of the study. Senior level clinical staff will contact these families in an effort to improve compliance with the next visit. This action conveys a sense of how important the child is to the study and can increase the parent's compliance with future follow-up visits. For those patients who are already missing visits, the Principal Investigator of a Clinical Center can contact the parents to assure that they continue with the follow-up visits. As part of the conversation, the Principal Investigator can negotiate with the parents (or guardians) on the parts of the study they are willing to complete. By prioritizing the components of the Protocol, a mutually acceptable follow-up procedure can be established for the patient.

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. We have successfully used this technique 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.

4.4.5 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 the study for evidence of beneficial and adverse treatment effects. The DSMB will review reports on study performance including recruitment, protocol violations, complications of treatment, patients lost to follow-up, submission of forms, quality of laboratory and forms data submitted, monthly during the Feasibility and Safety Pilot Study and semiannually (months 6, 12, 18, 24, 36, 42 and 48) from the start of Feasibility and Safety Pilot Study enrollment. The study will also be monitored for efficacy with respect to the two co-primary endpoints and for adverse events.

Below we present the proposed interim monitoring plan for efficacy for the BABY HUG study. This plan will call for five primary endpoint analyses (four interim analyses and one final analysis) during the course of the study. The first interim analysis will be conducted with data collected on children enrolled in the Feasibility and Safety Pilot Study. Extreme evidence (nominal alpha = 0.0005, Z = 3.5) of treatment differences will be required in the interim analyses to demonstrate the efficacy of the proposed intervention for either endpoint. The use of these extreme Z values will allow for final nominal alpha levels for each co-primary endpoint to be just under the specified alpha level for the study (nominal alpha = 0.0394, Z = 2.06 for spleen function. Table 4-7 shows the nominal alpha levels expected to be used during the course of the study.

TABLE 4-7
P-Values for Interim Analyses

Month of Study	Type of Report	p-value (Spleen)
27	Interim	0.0005
33	Interim	0.0005
39	Interim	0.0005
45	Interim	0.0005
51	Final analysis	0.0394

If appropriate, the DSMB may recommend revising the interim analysis schedule by altering the proposed number and/or dates of the interim analysis; the method of Lan and DeMets (Reboussin et al, 1992; Lan and DeMets, 1983) will be used to determine the actual “spending” of the alpha over the course of the study.

The interim analysis reports for the DSMB will include, but are not limited to comparisons by treatment group for:

1. Primary endpoint;

    Spleen – worsening of function on scintigraphy

2. Secondary endpoints;

    Clinical Events (See Appendix F)

    Laboratory Evaluation

3. Adverse events;

    Serious Adverse Events (See Table 4.8)

    Splenic Sequestration

    Abnormal Neurological Tests

    Abnormal Neuro-development Evaluation

    Abnormal CBC

    Abnormal Chemistry

4. Distributions of baseline characteristics;

    Gender

    Age at Entry

    Initial Spleen and Kidney Function

    Initial Transcranial Doppler Measurements

    Initial Neuropsychosocial Testing Level

5. Clinical Center Performance;

   Recruitment

   Completeness of Follow-up

   Submission of Forms

   Quality of Forms Data

   Distribution of Specimens to Core Laboratories

   Drop Out Rate.

Monitoring Safety and Adverse Events

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

1. Recruitment: Expected vs. Actual
2. Patients screened, eligible and randomized
3. Patient characteristics at baseline
   A. Age, race and gender
   B. Spleen function
   C. Spleen size
   D. Pitted cell counts
   E. Schwartz equation GFR estimates
   F. Urine concentrating ability
   G. CBC
   H. Presence of gallstones
   I. Blood chemistries
   J. Microalbuminuria
   K. O_2 saturation
   L. Physical examinations

M. Neurological examination and neuropsychological development

N. Height, weight, head circumference

O. Transcranial doppler (TCD) measurements

4. Blood count toxicities

5. Dose adjustments

6. Intra- and Inter-observer agreement liver-spleen scans

7. Immunological impairment

8. Safety assessments and adverse events

A. Height, weight, head circumference

B. Neurological examination and neuropsychological development

C. Unexpected and serious adverse events: update tallies, rates and individual summaries (case reports) for immediate review by DSMB Chair, Executive Secretary of DSMB and Project Officer

4.4.6 Safety Related Outcomes

Adverse event (AE) forms will be submitted to the MCC and tabulated based on the affected organ system according to standardized monitoring procedures (e.g., the National Cancer Institute Cancer Therapy Evaluation Program Adverse Event Expedited Reporting System). Table 4-8 defines adverse events and specifies classification and reporting considerations.

The timing of the AE report will depend upon the nature of the AE. If the AE is serious, Clinical Center staff will be instructed to report the AE to the MCC within 24 hours of the event. MCC staff will immediately review the material and forward it to the central review group. If the central review group finds that an event is serious, MCC staff will attach the treatment assignment to the report, and send the information to the DSMB Chair, Executive Secretary of DSMB and the NHLBI and NICHD Project Officers for review. This AE will be tabulated with the other AEs that have been reported for the study and a systematic review will be made to determine if one treatment has more

reports of serious AEs than the other treatment. The occurrence of serious AEs will be reported to the FDA promptly after the Executive Secretary of the DSMB and the Project Officer review. The Clinical Center will report the occurrence of serious AEs that occur at their institution according to the requirements of their local IRB.

Other AEs will be collected and tabulated by treatment group on a monthly basis. A critical value corresponding to $p = 0.05$ will be used for each AE evaluation (except for the events discussed earlier). The approach here will be to err on the side of detecting a trend early rather than protecting alpha. Depending upon the evidence accumulated and severity of the events, it will be the responsibility of the DSMB Chair, 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.

The MCC staff plan to send a written notification to the DSMB Chair, Executive Secretary of DSMB and the Project Officer for each severe complication and if any severe complication is found six times in one treatment group without a single occurrence in the other treatment group. If six complications have been observed, all in one of the treatment groups, the alpha level of rejecting the null hypothesis of no treatment effect on the appearance of the adverse event is less than 0.05 and suggests that the risk of this complication in one group is not equal to the risk of complication in the other group. The alpha level of rejecting the null hypothesis of no treatment effect is obtained by using a binomial approximation for the log-rank statistic.

If complications occur in both treatment groups, a 95% confidence interval for the difference between proportions for the assigned study treatments will be used to compare the occurrence of symptoms which are potential adverse effects of treatment, such as a neurological deficit. 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.

4.4.7 Pharmacokinetics of Hydroxyurea (HU)

NOTICE: Exit PK Studies are not being collected as of May 29, 2009.

Full pharmacokinetic (PK) studies of hydroxyurea will be performed on all children (both HU and placebo) at entry and exit, at the times that the DTPA measurements for GFR are made. The results of PK analyses from the Feasibility and Safety Pilot Study suggest that the time frame for sample selection should be changed from 0,1,2,4,and 8 hours to 30,90, and 210 minutes. These informative times of blood collection for the PK studies will coincide exactly with blood collection times for the DTPA measurement without any further requirements for additional blood by administering the daily dose of study treatment 30 minutes after the DTPA procedure is started. Figure 4-2 shows an overlay of the collection schedule for these two PK and DTPA studies.

Standard blood collections for the DTPA study were collected at 1,2, and 4 hours after the DTPA procedure was initiated. By lagging the administration of the study treatment, the blood collections will yield PK data points at 30, 90, and 210 minutes. Based on data from the Feasibility and Safety Pilot Study, trivial residual HU concentrations are predicted to persist from a previous dose, thus collecting a pre-dose concentration adds little information, and these three post-dose blood collections will allow estimation of the PK curve for each infant. In addition, the collection times will allow the measurements to bracket the expected peak concentration for an infant as shown in the PK concentrations curve for the first 22 infants studied in the BABY HUG Feasibility and Safety Pilot Study (Figure 4-3). The collection of entry and exit PK specimens in this fashion imparts minimal risk and discomfort to the child.

A third PK study will be done approximately one month (at the week 004 visit) after each child begins taking study treatment. Blood specimens will be collected at only two time points for this third study as it does not coincide with a DTPA procedure. The time points for collection will be 30 and 90 minutes after the daily dose of study treatment is administered. The collection of specimens

at these two time points after one month of study treatment administration will allow the study investigators to confirm that first dose HU pharmacokinetics predict HU exposure with chronic administration. The collection of these two additional data points represents a minimal risk assessment since only one extra venipuncture is required.

All of the data from the HU assays will be analyzed using population pharmacokinetic models. A one-compartment open model with first order elimination will be utilized. First order absorption, with and without lag times, will be evaluated as well as other input functions justified by the data. The data will be modeled recognizing the two stages of a non-linear hierarchical model. The first stage introduces the structural model (e.g., the one compartment open model), the population parameters, individual effects, and within-patient variation. The second stage of the model recognizes that variation between patients in pharmacokinetic parameters exists, and will attempt to determine covariates that may identify different pharmacokinetic subpopulations. The collection of these data will support the New Drug Application to the FDA for package insert specifications about the metabolism of the liquid formulation of hydroxyurea for use in infants with sickle cell disease.

Table 4-8
Definition of Adverse Events and
Classification and Reporting of 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

8. Any AE related to study treatment and unexpected.

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 given that frequent hospitalizations occur as a consequence of having sickle cell anemia without being enrolled in a clinical trial.

In addition, a centralized over-ride system (the central review group) will be carried out by individuals with knowledge about the treatment assignments. These individuals will review adverse events that are not thought to be serious in the eyes of the blinded investigators and make a decision 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.

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

Figure 4-1

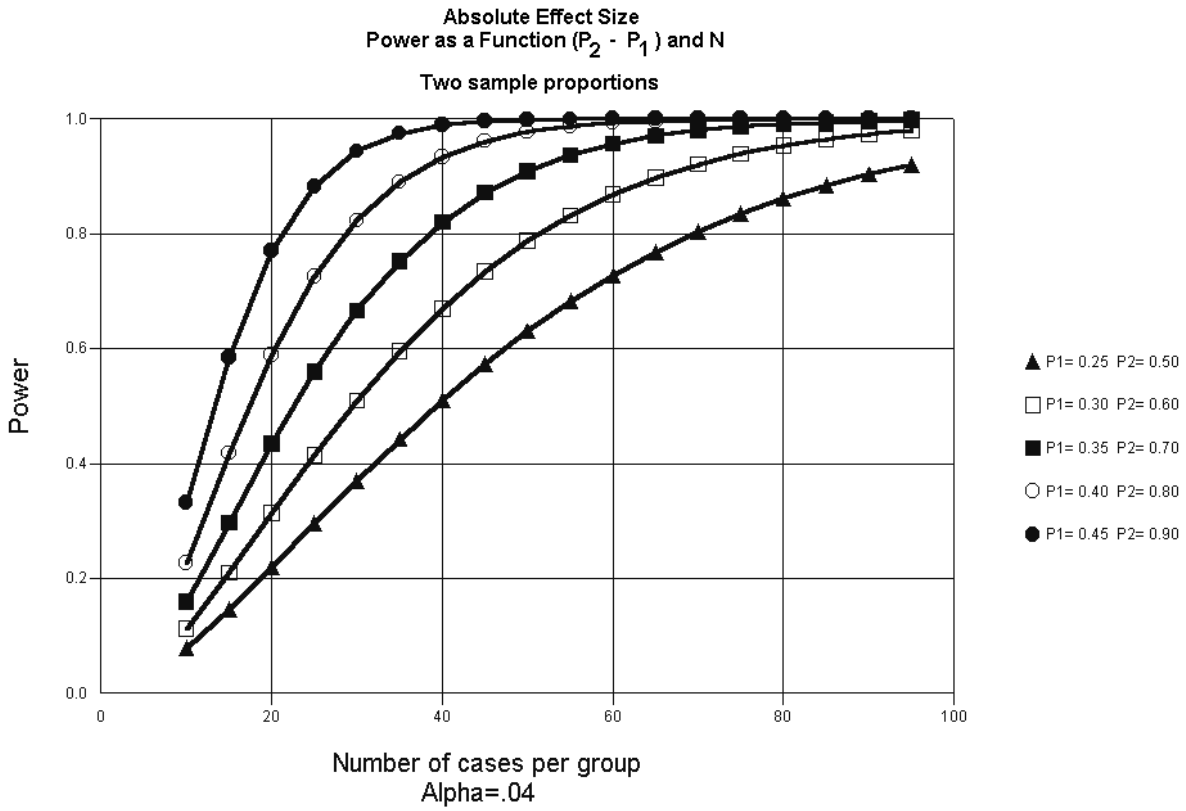
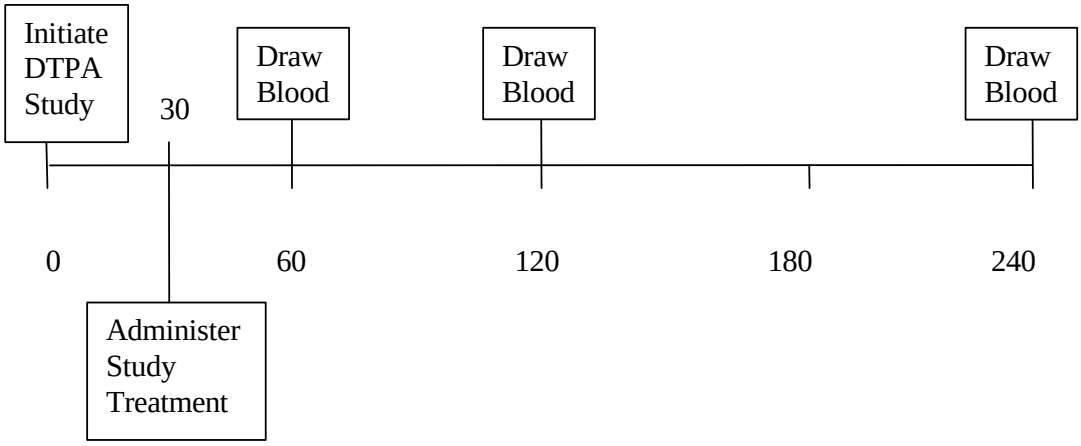

Absolute Effect Size
Power as a Function (P_2 - P_1) and N
Two sample proportions
Power
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Number of cases per group
Alpha=.04
P1= 0.25 P2= 0.50
P1= 0.30 P2= 0.60
P1= 0.35 P2= 0.70
P1= 0.40 P2= 0.80
P1= 0.45 P2= 0.90

Figure 4-2

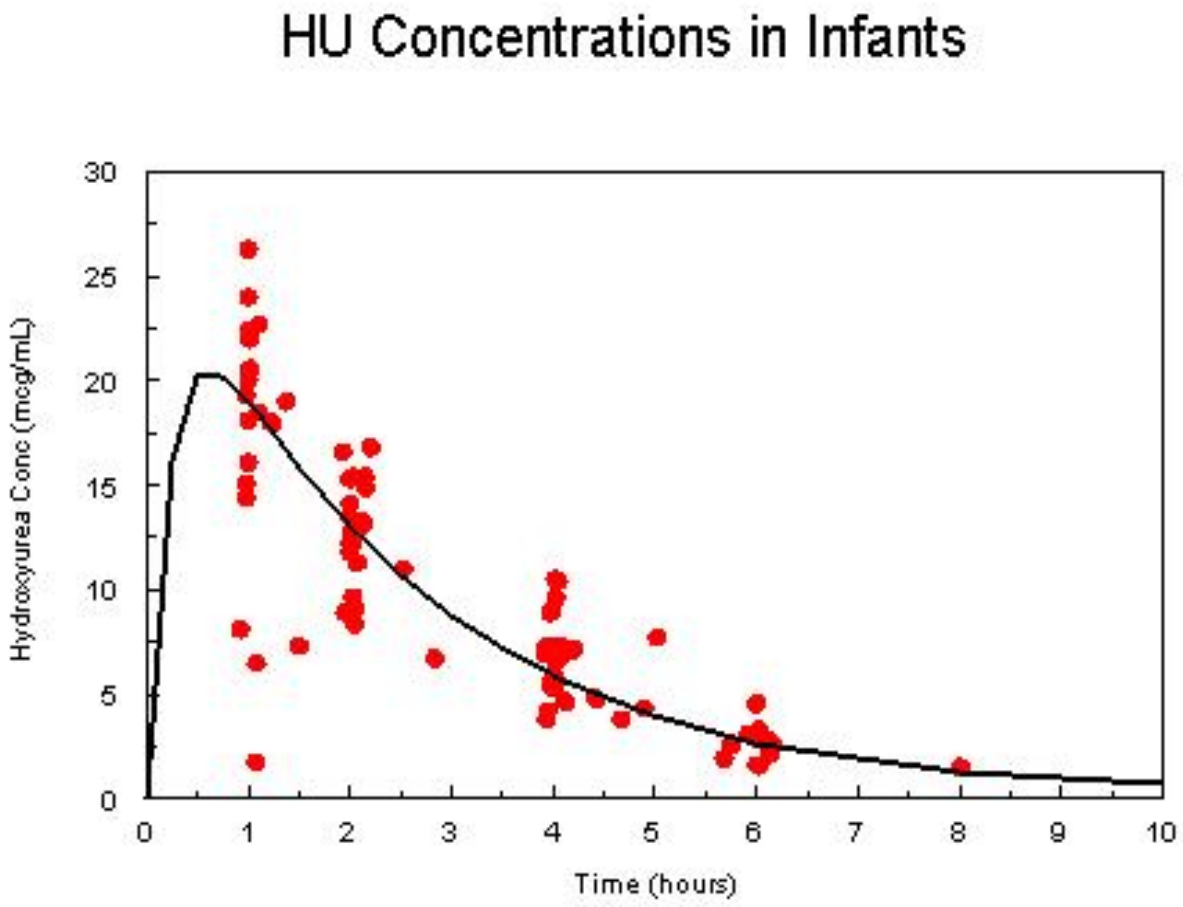
Overlay of the Entry and Exit PK Studies on the DTPA Procedure

NOTICE: Exit PK studies were discontinued as of May 29, 2009.



Time (minutes)

Figure 4-3


HU Concentrations in Infants
Hydroxyurea Conc (mcg/mL)
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
Time (hours)

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 5

RANDOMIZATION AND ENROLLMENT OF PATIENTS

5.1 ELIGIBILITY ASSESSMENT

Potential patients are identified by the Clinical Center Principal Investigators and coordinators. Patients' parents or guardians will complete orientation and provide informed consent, and patients will complete the pre-enrollment assessment period and procedures. All pre-enrollment forms must be received at the Medical Coordinating Center (MCC) where eligibility will be assessed based on data submitted.

Patients who meet all eligibility criteria in a Clinical Center enrolling BABY HUG patients will be able to enroll in the study. Patients who are not eligible may be re-evaluated as long as the enrollment period is still open and baseline evaluations can be completed before the close of the enrollment period. Exclusion criteria, which could change and allow re-evaluation for eligibility, include: age less than 9 months for the main study (12 months for the Feasibility and Safety Pilot Study); transfusion within two months of enrollment unless HbA < 10%; abnormal liver function or renal function tests; Bayley Mental Scale standardized score <70; low hemoglobin, reticulocyte or neutrophil count.

5.2 RANDOMIZATION AND TREATMENT ALLOCATION

MCC staff will prepare a unique randomization schedule for each BABY HUG Clinical Center. Each randomization schedule will be known only to a small group of people at the MCC. Individual treatment assignments will be available to selected MCC staff only on a "need-to-know" basis. Clinical Center investigators forced by urgent circumstances to unblind an individual patient's treatment will be required to follow procedures and provide documentation as set out in Section 6.6.1.

MCC staff will use an automated telephone response system (ATRS) to randomly assign treatments to patients enrolling at a Clinical Center in sequence according to the randomization schedule for that Clinical Center. Each treatment assignment will specify a kit number for a kit that contains either hydroxyurea or placebo. Treatment group allocations will be stratified according to Clinical Center to ensure balance of numbers to each treatment arm within each Clinical Center. A standard procedure for stratified, blocked randomization will be used.

5.2.1 Treatment Allocation

The MCC will identify eligible patients on a daily basis throughout the enrollment period. The MCC will provide a continual report of pending requirements for patients still in eligibility assessment. After the child's parents or guardians have provided written informed consent, the child's baseline evaluations are completed and the child has been declared eligible, the Clinical Center staff may use the MCC automated telephone response system (ATRS) to enroll a child and to obtain a study treatment kit assignment. The MCC will send the Pharmacy Distribution Center directions to maintain the supply of study treatments for this patient at the Clinical Center pharmacy. For each eligible patient the ATRS will issue to the Clinical Center a confirmation of enrollment into the study, including the BABY HUG Patient Identification Number and Alphabetic Code. Clinical Center staff will return to the MCC the form confirming the date the patient starts study treatment.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 6

STUDY TREATMENTS

6.1 OVERVIEW

For children enrolled in BABY HUG and assigned to study treatment, treatment will begin at a fixed dose of 20 mg/kg/day, and remain at this dose unless repeated or prolonged toxicity is encountered, in which case the dose will be reduced to a lower stable dose. The goal for dose adjustment is given in Table 6-1. Patients will be maintained at 20 mg/kg/day or the lower stable dose for the duration of the 2-year period on blinded study treatment.

The NHLBI has submitted an investigational new drug (IND) application to the U.S. Food and Drug Administration (FDA) for use of hydroxyurea in BABY HUG (IND #67,289). Administration and monitoring of treatment in BABY HUG will follow all accountability and reporting requirements of the FDA.

Hydroxyurea will be formulated as a liquid preparation (100 mg/ml). Parents will be advised to administer treatment at approximately the same time each day (e.g. each morning or each evening before bedtime) to assist with compliance. All study medications (hydroxyurea and placebo) will be distributed by the Pharmacy Distribution Center in bottles of powder to the Clinical Centers. Each child's treatment supply will be formulated by the appropriate Clinical Center before the patient's scheduled visit.

Before each scheduled clinic visit, the Medical Coordinating Center will recommend a dose for the upcoming 2- or 4-week therapy period, and transmit this information electronically to the Clinical Center Principal Investigator and coordinator. On the day of the clinic visit (Day 1), the child will be weighed, examined, and monitored for toxicity. At each 2- or 4-week visit, the used bottle will be collected, and the family will be given a new bottle with an appropriately marked oral syringe. The instructions will be to continue therapy using the new bottle, unless they receive a phone call

to stop administering treatment. If a treatment had been stopped in the previous 2- or 4-week period, a new bottle of study medication will be dispensed. The family will be instructed to start administering the study medication with the possibility that a phone call for a stop order may be issued within three days.

Blood specimens for routine study visits will be sent to the local Clinical Center laboratory for CBC determinations. Within one day of collecting the blood or by 9:00 a.m. of the following morning, the Primary Endpoint person will review local laboratory results for alert and toxicity values and data-enter the CBC values into the Internet Data Entry System. The MCC will also analyze the local CBC results. If the specimen is unusable (e.g. clotted), Clinical Center staff will be given an opportunity to collect a second hematology blood specimen. However, the second hematology specimen must be collected within 48 hours of the first specimen. By Day 3, the Medical Coordinating Center will send out a recommendation for stopping or continuing study medication for the next 2- or 4-week period based on review of all blood counts received, study medication prescription history, toxicities and adverse reactions. The Clinical Center physician verifies that the recommendations and prescriptions are consistent on Day 3. When indicated, Clinical Center staff will notify families to "STOP" using the study treatment (if it is currently being administered). Failure to contact families to "STOP" using study treatment can jeopardize patient safety (see section 12.9). If appropriate efforts are made to contact a family with a "STOP" order, but the family is not contacted, the Clinical Center Principal Investigator will provide the Operations Committee with a written explanation of the reason contact could not be established and a plan to avoid inability to contact this patient in the future. If a family cannot be contacted for two "STOP" orders, the patient's treatment will be interrupted until the Operations Committee assesses that it can be safely restarted (see Section 6.6.2).

Every six months at designated visits which must occur on a Monday - Thursday, blood will also be sent to the Hematology and Biochemistry Core Laboratory by overnight courier, and arrive there early on Day 2. Within six hours of receipt of the blood, the Hematology and Biochemistry

Core Laboratory will transmit hematology and biochemistry results to the Medical Coordinating Center. No toxicities will be determined from the Core Laboratory results. However, the MCC will analyze the results for alerts and notify the Clinical Center PEP of any alerts.

6.2 DOSE TITRATION

6.2.1 Hydroxyurea

The plan for dose reduction and toxicity monitoring in BABY HUG (Table 6-1) is based on data obtained from HUSOFT (Wang et al, 2001). The starting dose of hydroxyurea will be 20 mg/kg (once a day, orally). If toxicity occurs (e.g., neutropenia), treatment will be stopped and blood counts will be checked every two weeks until they return to non-toxic values. Transient toxicity will not cause a dose reduction, but prolonged or repeated toxicity will. Following a toxic blood count, treatment will be discontinued for 14 days. If counts recover, treatment will be resumed at the previous dose. If the toxicity persists, treatment will continue to be held for an additional 14 days and treatment will resume at a daily dose 2.5 mg/kg lower than the previous dose once the toxicity is resolved. If that dose does not cause toxicity for eight weeks, an attempt will be made to increase the daily dose by 2.5 mg/kg; if toxicity occurs, the lower dose will be assigned as the stable dose. If transient toxicity occurs more than twice at the same dose within a 12-week period, treatment will resume at a dose 2.5 mg/kg lower than the previous dose and continue for the duration of study. If blood counts reach the toxic range while on an established stable dose, treatment will be stopped until toxicity resolves and then treatment will resume with the previously established stable dose; repeated toxicity in a 12-week period will reduce the stable dose by 2.5 mg/kg for the remainder of the study. An outline of the dose titration algorithm is presented in Table 6-1.

6.2.2 Placebo

Patients assigned to placebo will be given identically-appearing bottles containing placebo powder and the same flavoring as the HU-containing bottles. The placebo-containing bottles will be labeled identically to the HU-containing bottles. The Medical Coordinating Center will devise

schedules of treatment stops and dose reductions and escalations for placebo patients based on the experience of bone marrow suppression, recovery, dose reduction and dose escalations seen in the open-label HUSOFT patients. Enough individual plans will be devised so that each of the children assigned to placebo in each Clinical Center will follow a different course of stops and dose changes that would just as readily occur in a child assigned to hydroxyurea. The goal of the plans is that the distribution of the final dose achieved among the placebo assigned patients will be similar to the final dose distribution among those assigned to hydroxyurea, and that the same proportion of children have treatment stops. This type of plan was implemented in the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) (Handy et al, 1996).

6.3 TREATMENT PREPARATION

An independent manufacturing facility will prepare and send bottles containing powdered hydroxyurea or placebo to the Pharmacy Distribution Center, which will forward these bottles, grouped as individual treatment kits, to each Clinical Center (Investigational) Pharmacy. Bottles for each patient will be reconstituted with water and simple syrup by each Clinical Center (Investigational) Pharmacy to formulate the medications into liquid preparations. Hydroxyurea powder and flavoring will be dissolved in water and simple syrup to achieve a final volume of 120 ml with a final concentration of 100 mg/ml. Placebo will be dissolved in an identically appearing and flavored solution. Each Clinical Center (Investigational) Pharmacy will bottle the liquid formulation in a child-proof container, label it with the subject's study acrostic/number, study ID number and instructions (including the "Investigational New Drug" warning, "BABY HUG Hydroxyurea Study for Sickle Cell Anemia", a prescription number, and the "emergency call" telephone number of the Principal Investigator). Inventory records for hydroxyurea and placebo will be kept by Pharmacy Distribution Center staff, and the Medical Coordinating Center will keep inventories of kit numbers used.

Several days before a patient's four-week visit, the Medical Coordinating Center staff will generate a prescription recommendation for the patient to be reviewed by a physician or physician consultant at the MCC with a synopsis of recent blood counts. The MCC physician will check all prescription recommendations, and the MCC will notify each Clinical Center of the official prescription. The Clinical Center (Investigational) Pharmacy will generate a label that includes the patient's dose for the next month with instructions on how to take the liquid. All verified prescriptions will be prepared by the Clinical Center (Investigational) Pharmacy, the bottles will be labeled appropriately, and the study treatment given to the family on the day of the patient's clinic visit. The Clinical Center (Investigational) Pharmacy will prepare a 35-day supply of the liquid formulation, and dispense it to each patient with the appropriate dose marked on the label and on syringes.

6.4 DEFINITIONS OF TOXICITY

The most common toxicity observed in preliminary studies has been transient and reversible bone marrow depression. Hydroxyurea has only rarely been reported to be the cause of fever, skin rash, nausea, vomiting or hair loss. Such manifestations will be investigated locally and will be reported to the Food and Drug Administration (FDA) as adverse reactions if other etiologies are not apparent.

Toxic bone marrow depression is defined as an absolute neutrophil count < 1250/cu mm, absolute reticulocyte count < 80,000/cu mm (if the hemoglobin concentration is below 7 gm/dL), platelet count < 80,000/cu mm, a hemoglobin concentration < 6.0 gm/dL, or >20% fall in hemoglobin concentration from the 3-month rolling average.

The following occurrences will also be defined as toxicity: unexplained gastrointestinal disturbance, or unexplained rash or hair loss.

Toxicity levels are used for adjustment of study treatment dose and are distinct from alert levels which are used for clinical management of the child (see section 7.2.4 and Table 7-3).

6.5 MONITORING FOR TOXICITY

Patients will be seen at the Clinical Centers and will have blood sampled every two weeks until a stable dose is reached, then every four weeks thereafter. A few infants will be seen in peripheral clinics that are visited by the clinic director every four weeks. Blood specimens for routine study visits can be collected any day of the week provided blood is not also scheduled for collection and shipment to the Hematology and Biochemistry Core Laboratory, which must occur on a Monday - Thursday. If a patient misses the clinic visit, and the visit can be rescheduled within the extended visit window, the Clinical Center staff should complete the clinic visit (and blood collection) within the extended window. If a patient misses a visit that cannot be rescheduled during the extended window, the family will be advised to complete the available study medication (interrupting daily doses when the treatment runs out) and return in two weeks.

One pediatric tube of blood (0.5 ml) in EDTA will be obtained for a blood count including hemoglobin concentration, white blood cell count, platelet count, absolute reticulocyte count (ARC) and absolute neutrophil count (ANC). The local laboratory will perform automated (manual if automated is invalidated) CBC counts and the Primary Endpoint Person (PEP) will data enter them into the BABY HUG database via the Internet Data Entry System within one day of collecting the blood or by 9:00 a.m. the following day. An additional EDTA purple-top tube (0.5 ml) for hematology and a red-top tube (1.0 ml) for serum chemistries will be collected every six months (see Table 7.1) and sent to the Hematology and Biochemistry Core Laboratory. The Hematology and Biochemistry Core Laboratory will perform blood counts and chemistries, and transmit results to the Medical Coordinating Center within six hours of receipt of the sample. No toxicities will be determined from the Core Laboratory results.

The PEP will review the local results to determine if a toxicity is present in the data. If there is a toxicity, the PEP will notify the Clinical Center staff of the need for a stop order. If there is not a toxicity, the PEP will open a previously delivered notice from the MCC that will instruct whether

the PEP is to report a sham toxicity for the visit. Once reported, all other aspects of the reporting mechanism will be done in the same manner as if the toxicity is real (e.g., a meeting with ancillary staff, etc. to perpetuate the notion that the report is in fact a real toxicity).

In addition to the PEP review of the local laboratory results, the Medical Coordinating Center will scan the in-coming local laboratory reports for "toxic" results. If the specimen is unusable (e.g. clotted) the Clinical Center staff will be given an opportunity to collect a second blood specimen. However, the second specimen must be collected within 48 hours of the first specimen. If there are no local laboratory results, the Medical Coordinating Center will notify the Clinical Center of the need for a stop order. After MCC staff issue/confirm a stop order, they will notify the Clinical Center coordinator, who will call the family and give the order to stop treatment if it has not already been done. The clinic coordinator and thus the family will be told to "stop treatment", without use of the word "toxicity." The coordinator will inquire concerning the child's health (e.g., regarding fever, lassitude, weakness). Parents (guardians) of any child whose condition is worrisome will be asked to bring in the child for an examination to rule out complications with potentially severe consequences such as parvovirus B-19 infection or splenic sequestration (see Section 8.4). Patient families will be notified of stop orders within 48 hours of the clinic visit. The child must then return for two week visits until the toxicity is resolved.

Medical Coordinating Center staff will provide the Clinical Centers with stop orders for placebo patients, in a similar manner as patients with toxicity due to HU. If the MCC physician(s) and other Medical Coordinating Center staff disagree on the toxicity evaluation, any stop orders, and/or the dose recommendation for the next therapy period, the laboratory results and treatment recommendation will be referred to the Study Chairman for immediate action.

6.6 BLINDING

In the Clinical Centers, the patients, their families, Clinical Center Principal Investigators, coordinators, and other study staff will be blinded to treatment assignments. The central event or

image reviewers will not be able to link study treatment assignments to individual event reports or other outcome data. Pharmacy Distribution Center staff and Medical Coordinating Center staff will have access to individual subject treatment assignment and current dose on a "need-to-know" basis. The Medical Coordinating Center will maintain records of each child's treatment assignment and current dose.

Plans have been made to prevent toxicity monitoring from resulting in unblinding of patients' assigned study treatments. Despite these precautions, if the Clinical Center Principal Investigator thinks he/she inadvertently has become unblinded, contact with the child and his/her family and clinical site staff must be carefully managed to avoid any comments regarding unblinding. The person who has the most patient contact, usually the coordinator, must be absolutely excluded from any contact with laboratory data. The Primary Endpoint Person (PEP), who monitors the local CBC evaluations for toxicity, will have access to informative data about treatment assignment and must not share that information with the Principal Investigator or coordinator. In addition, the child's 3-month rolling average hemoglobin concentration will be provided by the MCC to the Clinical Center PEP every month for use with standard medical practice guidelines (see Chapter 8) as needed locally.

The Clinical Center Principal Investigators have agreed to avoid seeking information that may unblind them with regard to individual treatment assignments, especially laboratory results. Clinic coordinators will conduct follow-up visits and process and maintain files of study documents. Study documents that contain potentially unblinding information or baseline characteristics on individual patients (e.g., local lab reports, liver/spleen scan results, GFR results) will be maintained by the PEP. Although not preferred, Clinical Center Principal Investigators may be the primary care providers for BABY HUG children, and will be aware of the need to maintain blinding under normal circumstances and maintain the child on study drug even during hospitalization. Discussion among

Clinical Center staff or with families regarding a child's treatment assignment is inappropriate. Clinical Center coordinators must avoid any information that may unblind them.

6.6.1 Emergency Unblinding

Every family will be given an identification card describing the child's participation in the BABY HUG study, listing emergency study telephone numbers (e.g., the Clinical Center Principal Investigator's telephone number and the Central Study Emergency Contact's telephone number). The Central Study Emergency Contact's telephone will be answered by a pediatric sickle cell anemia consultant to the Medical Coordinating Center at all times. If BABY HUG children become ill, treating physicians will be urged to call the Clinical Center Principal Investigator before altering the child's study regimen.

In an emergency, family members will be instructed to call a telephone number that will be manned by a pediatric sickle cell anemia consultant to the Medical Coordinating Center 24 hours a day. Arrangements will be made so that the child's medication can be disclosed to the Clinical Center Principal Investigator after consultation between the Clinical Center Principal Investigator and the pediatric sickle cell anemia consultant to the Medical Coordinating Center. Reasons for unblinding are limited and are based on clinical grounds. Unblinding must be initiated by the Clinical Center Principal Investigator. Reasons for unblinding include overdose of the study medication, accidental ingestion of the study medication by another person, development of infection or bleeding that could be due to reduced white blood cell or platelet counts and for which management might be changed if the nature of the study drug were known. Examples of clinical situations when information on study treatment could be useful include severe thrombocytopenia calling for a decision to use prednisone versus platelet transfusion or severe neutropenia calling for a decision on choice of antibiotics. If a child's therapy is unblinded, the Clinical Center Principal Investigator or staff member who unblinded the treatment must send a report to the Medical Coordinating Center.

Each Clinical Center will assign another non-BABY HUG hematologist and nurse practitioner to be available for acute care visits. The study coordinator will have the most contact with patients and families and will be excluded from contact with laboratory data collected during acute events. The Primary Endpoint Person will, however, have access to a patient's 3-month rolling average hemoglobin value every month, which will be provided by the MCC for use in evaluation of anemia. Unblinding will occur only after consultations between the Clinical Center PI and an external, Consultant Pediatric Study Hematologist. Unblinding events will be discussed by the Operations Committee and often will result in discontinuing study treatments. However, all patients, including those whose treatments have been unblinded, will continue to be followed in BABY HUG for safety outcomes and clinical outcomes not already declared at the time of unblinding. Since analysis will be conducted according to the principle of Intention to Treat and unblinding is expected to occur infrequently, the impact of unblinding is anticipated to be less than that of possible crossovers (see Sections 4.4.3 and 4.4.4).

6.6.2 Treatment Interruptions

There may be instances related to medical conditions (e.g., acute, intercurrent illnesses such as an infection) or other reasons (e.g., study medication is lost by the family or "STOP" orders cannot be delivered) when it may be advisable to interrupt study therapy without unblinding. Interruptions for medical conditions should be allowed only with consultation of the Clinical Center Principal Investigator. The Clinical Center Principal Investigator is responsible for notifying the Medical Coordinating Center of treatment interruptions. These notifications are important because they may in turn have an influence on dose titration.

6.7 ASSESSMENT OF COMPLIANCE

Study treatment will be measured at each regular follow-up visit. If the volume of study treatment remaining in the bottle is not consistent with the prescribed dosing plan, staff will inquire about any difficulties that may have occurred. The importance of compliance will be emphasized

for all patients. Even if patients are repeatedly considered to be non-compliant, they will continue to be followed and will continue to receive their study reimbursements (i.e., travel/telephone allowances).

6.8 MISSED VISITS AND DROP-OUTS

Each regularly scheduled clinic visit missed by a patient will be reported to the Medical Coordinating Center. Families who do not wish to continue attending clinic visits in the BABY HUG study will continue to be telephoned by the Clinical Center coordinator to ascertain the medical condition, any identifiable events and vital status. Attempts will be made to obtain semi-annual and annual evaluations for all study participants.

6.9 DURATION OF STUDY TREATMENT

The goal of the study treatment plans will be to maintain patients on the assigned study treatment until the end of the 24-month period and on the maximum non-toxic dose (up to 20 mg/kg/day) of study treatment for at least 18 months of the two-year trial period.

TABLE 6-1

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) DOSE TITRATION ALGORITHM

Goal: To treat at 20 mg/kg/day or a lower, stable dose, defined as the highest dose maintained for eight weeks without observed blood count toxicity; and to maintain the patient at stable dose thereafter.

Titration Phase (visits every 2 weeks):

1. Begin hydroxyurea at a dose of 20 mg/kg/day.
2. Continue treatment and monitor for toxicity.
3. If toxicity develops:
   a. A stop order is issued, and the patient will have repeat blood counts performed every 14 days until toxicity resolves.
   b. If the toxicity resolves at 14 days, then the study drug will resume at the pre-toxic dose. If the toxicity requires more than 14 days to resolve, then the daily dose will be lowered by 2.5 mg/kg. A patient must take a prescribed dose before a lower subsequent dose will be prescribed (i.e., toxicities that require more than 14 days to resolve will have the dose reduced 2.5 mg/kg from the last dose that was actually administered to the patient).
   c. As long as the patient does not become toxic over the subsequent eight-week period on the lower dose, the daily dose will be increased by 2.5 mg/kg not to exceed 20 mg/kg/day.
   d. If a patient becomes toxic at any given dose twice in a 12-week period, no further increases will be made. The highest dose that does not produce toxicity for an eight-week period is designated the stable dose.
4. No patient will receive more than 20 mg/kg/day.

Stable Dose Phase (visits every 4 weeks):

1. If the patient becomes toxic after the stable dose is established, a stop order is issued and the study drug is stopped. When the toxicity resolves, the study drug will be resumed at the pre-toxic stable dose. If toxicity recurs within a 12-week period, the stable dose will be lowered by 2.5 mg/kg.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 7

LABORATORIES AND SPECIMENS

7.1 INTRODUCTION

Eight Core Laboratories (a Hematology and Biochemistry Core, an Immunology Core, a Mutation Analysis/DNA Core, a Pitted Cell Core, a TCD Core, a Biomarkers Core, an HU Assay Core and a Cytogenetics Core) and an NHLBI Specimen Repository will be utilized for processing of BABY HUG study specimens, collected at the times specified in Table 7-1. In addition, the Clinical Center local hematology laboratories will process specimens collected at every study visit.

7.2 HEMATOLOGY AND BIOCHEMISTRY CORE LABORATORY

The Hematology and Biochemistry Core Laboratory will provide standardized hematology and routine blood chemistry results which will be used to monitor for alerts and for analysis of effects of HU on these parameters.

The Hematology and Biochemistry Core Laboratory will assay specimens according to the schedule in Table 7-1. If the specimen is deemed untestable, insufficient quantity, or missing, the Clinical Center should continue to re-draw for up to two more subsequent study visits. Blood samples will be collected and sent to the Hematology and Biochemistry Core Laboratory from Monday to Thursday by overnight express carrier so that they arrive within 24 hours. Blood counts and chemistry assays will be performed, reports generated, and results transmitted to the MCC within 6 hours of receipt.

7.3 LOCAL (CLINICAL CENTER) HEMATOLOGY LABORATORIES

7.3.1 Monitoring for Toxicity

The major toxicity of HU is bone marrow depression which will be detected by complete blood count (CBC with white blood cell differential). HU has rarely been reported to be the cause of clinical manifestations such as fever, rash, nausea, vomiting or hair loss. Since HU is excreted

in the urine, renal function will be monitored closely based on BUN and creatinine results. Clinical manifestations and toxicity will be investigated locally and will be reported to the FDA as adverse reactions if other etiologies are not apparent. Toxic bone marrow depression and other organ toxicities in BABY HUG are defined in Section 6.4.

The MCC staff will screen incoming local CBC reports for “toxic” results. If toxic values are noted on the local CBC reports, the MCC will issue a stop order to the Clinical Center. Clinical Center will notify the patient’s parent(s) and tell them to stop treatment without the use of the word “toxicity”. The MCC will notify the Clinical Center of placebo patients whose treatments are to stop in the same manner as HU patients. Stop orders will be used for all placebo patients whose laboratory results are “spontaneously” (i.e., without hydroxyurea treatment) at levels that would be considered “toxic” with hydroxyurea therapy. The parent(s) will be notified of the stop order within 48 hours of a clinic visit. The remaining study drug must be returned at the next clinic visit. Doses for placebo patients and HU patients will be adjusted in similar manners. The MCC staff will receive and edit incoming local CBC reports, and compare them to previous reports for the patient. MCC staff will check for discrepancies between the central computer reviews and the stop orders.

7.3.2 Blinding

In the Clinical Centers, the patients’ families, Principal Investigators, coordinators, and other study staff will be blinded to treatment assignments. On the Central Event Adjudication Panels and in the Core Laboratories, staff will not be able to link study treatment assignments to specimens, event reports or other outcome evaluations. The staff of the MCC will have access to individual patient treatment assignment codes and current dose on a “need to know” basis. The MCC will maintain records of each patient’s drug assignment and current dose.

The Clinical Center Principal Investigators will assert their intention to avoid seeking information that may unblind them with regard to individual patient’s treatment assignments, especially laboratory parameters. The Primary Endpoint Person (PEP) will monitor the local CBC evaluations for toxicity and thus have access to potentially unblinding information. The Clinical

Center coordinator will conduct follow-up patient visits and will be the primary contact person for the patient and families. She/he will maintain files of study documents and must be rigidly excluded from any contact with laboratory data, including data which may be collected on hospitalizations or outpatient visits. For these purposes it is preferred (but not required) that physicians and nurse practitioners outside BABY HUG manage the primary care and laboratory data during these events. The Clinical Center Principal Investigators and coordinators will be responsible for maintaining their blinded status and maintaining the patient on study drug during hospitalizations, if appropriate.

7.3.3 Emergency Unblinding

Each patient's family will be given an identification card describing the patient's participation in BABY HUG, listing emergency study telephone numbers (the Study Chairman, and the Clinical Center Principal Investigator's telephone numbers). If a BABY HUG patient becomes ill, parents will be instructed to show this identification card to the child's treating physician. The card will recommend to treating physicians that they contact the Clinical Center Principal Investigator before altering the patient's study regimen. The MCC will be allowed to disclose the patient's treatment assignment only in cases of emergencies, such as accidental drug ingestion or in cases of severe cytopenias associated with severe infections or bleeding where therapeutic options may be dependent on identification of the study treatment assignment.

7.3.4 Alert System

The Primary Endpoint Person (PEP) will review the local CBC results to determine if an alert is present in the data. In addition, the Hematology and Biochemistry Core Laboratory data and local CBC data will be used by the MCC to monitor electronically for values exceeding alert levels (Table 7-2). Local CBC results will override Core Laboratory results. If an alert level is detected, the Clinical Centers will use the following guidelines for responding to notification of an alert.

Every time a value exceeds the alert level:

1. The PEP will handle the alert in a straightforward manner when possible, or, more likely, contact a Hematologist/Sickle Cell Specialist who is not directly connected with the BABY HUG Trial.
2. When indicated, the PEP and the Hematologist will develop a plan for a clinical response to the alert value. (For example: If the patient were severely neutropenic and febrile, the patient would be hospitalized for antibiotic treatment; if the patient had > 20% decrease in hemoglobin level, the patient would be hospitalized and/or transfused; if the patient met criteria for a splenic sequestration, the patient would be hospitalized and/or transfused; etc.)
3. In the event that no non-BABY HUG Hematologist was available, the PEP would need to contact the BABY HUG PI and discuss a course of action. In doing so, the PEP would provide the PI only with “need to know” laboratory information. In most cases this will not create an unblinding problem because, for example: severe neutropenia may more likely be related to an acute viral infection rather than HU toxicity; worse anemia might be caused by an aplastic crisis or splenic sequestration rather than HU; splenic sequestration might or might not be related to HU, etc. In most situations, CBC values would not readily distinguish between treatment with hydroxyurea or placebo, although it is important that the MCV should not be revealed to the PI or BABY HUG Study Coordinators.
4. In the ensuing management of the child, the bulk of the decision-making should be carried out by the non-BABY HUG Hematologist and the PEP.
5. The alert values for increased hematologic blood counts would not necessarily warrant any immediate reaction by a clinician.

7.4 CYTOGENETICS CORE LABORATORY

Freshly collected blood will be shipped overnight to the Cytogenetics Core Laboratory for culture of cells to assess karyotype and perform a chromosome breakage analysis.

7.5 IMMUNOLOGY CORE LABORATORY

Blood specimens before and after critical immunizations (e.g., Measles-Mumps-Rubella, or pneumococcal polysaccharide vaccine) will be shipped by overnight courier to the Immunology Core Laboratory for evaluation of antibody and cellular immune response.

7.6 MUTATION ANALYSIS/DNA CORE LABORATORY

Blood specimens will be shipped to the Mutation Analysis Core Laboratory at St. Jude Children's Research Hospital for the assessment of VDJ and other mutations and DNA extraction. All DNA samples will subsequently be sent to the NHLBI repository.

Leftover patient serum from the Biochemistry specimen will be shipped from the Biochemistry Core Laboratory to the DNA Core Laboratory for Cystatin C testing.

7.7 PITTED CELL CORE LABORATORY

Every six months one drop of blood will be preserved in gluteraldehyde for each child enrolled in BABY HUG. These specimens will be stored and refrigerated locally, and shipped in batches to the Pitted Cell Core Laboratory.

7.8 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 at entry, at 12 months on study treatment and at the end of follow-up for each child enrolled in BABY HUG. The Principal Investigator of a child who has a "conditional" TCD will be contacted by the MCC medical consultant to decide the frequency of follow-up. Any requested follow-up TCDs will be paid with BABY HUG funds and will be performed according to BABY HUG Protocol. Standard of care is to perform routine TCD screening beginning at 2 years of age (approximately at 12 months on study treatment for BABY HUG).

7.9 BIOMARKERS CORE LABORATORY

A Biomarkers Core Laboratory supported by funds from a separately awarded grant will be responsible for central evaluation of circulating levels of selected biomarkers in blood specimens collected at study entry, 2 months after entry, six months after entry and at study exit.

7.10 HU ASSAY CORE LABORATORY

NOTICE: Exit HU assays were discontinued as of May 29, 2009. Plasma specimens from the DTPA evaluations of GFR at treatment initiation for the children enrolled in the Feasibility and Safety Pilot Study were stored and later shipped in a batch to the HU Assay Laboratory for assessment of circulating hydroxyurea levels.

Additionally, all incoming patients will have entry and exit pharmacokinetic (PK) studies (on plasma specimens from the DTPA evaluations of GFR), all patients currently enrolled in the study will have exit PK studies (on plasma specimens from the DTPA evaluations of GFR), and all incoming patients will have PK studies at approximately one month on study treatment. These plasma specimens will be frozen and stored locally and shipped in batches to the HU Assay Core Laboratory.

7.11 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) and, following appropriate IRB review, the specimens will be available for use, if consent was given at the time of their collection, without any link to the individual from whom the specimen(s) were obtained.

TABLE 7-1

LABORATORY DETERMINATIONS

STUDY	SCHEDULE	BLOOD VOLUME
Hematology (Local-L and Central-C)		0.5 ml (L), 0.5 ml (C)
Hemoglobin	q 2-4 weeks (L), q6 mos (C)	
Packed Cell Volume (PCV)	q 2-4 weeks (L), q6 mos (C)	
Red Cell Count (RBC)	q 2-4 weeks (L), q6 mos (C)	
White Cell Count (WCC)	q 2-4 weeks (L), q6 mos (C)	
Absolute Neutrophil Count (ANC)	q 2-4 weeks (L), q6 mos (C)	
Reticulocyte Absolute Count (RAC)	q 2-4 weeks (L), q6 mos (C)	
Platelet Count	q 2-4 weeks (L), q6 mos (C)	
Mean Corpuscular Volume (MCV)	q 2-4 weeks (L), q6 mos (C)	
Mean Corpuscular Hb Conc (MCHC)	q 2-4 weeks (L), q6 mos (C)	
F-cells	q 6 months (C)	
Fetal Hemoglobin Concentration	q 6 months (C)	
Blood Chemistry		1.0 ml
Electrolytes, Calcium, Phosphorous and Magnesium	q 6 months	
Urea nitrogen	q 6 months	
Serum alanine aminotransferase (ALT)	q 6 months	
Total Bilirubin	q 6 months	
Direct Bilirubin	q 6 months	
Ferritin	q 6 months	
Serum creatinine (HPLC)	q 6 months	
RBC pitted cell count	q 6 months	0.1 ml
DNA Analysis		3.0 ml
VDJ events	0, 24 months	
Genetic modifiers	0 months	
Alpha gene number	0 months	
Alleles (S/beta^0/beta$^+$/alpha)	0 months	
Other (banked sample)	0 months	
Cystatin C	0, 24 months	0.1 ml (chemistry serum remainder)
Cytogenetics	0, 24 months	4.0 ml
Immunology		
Pneumococcal antibody titers and Opsonophagocytic activity	entry, 24 months of age, 25 months of age (or after Pneumovax), and exit	3.0 ml
MMR titers	13 months of age (or after MMR), 24 months of age, and exit	1.2 ml
T-cell counts	entry, 24 months of age, and exit	0.5 ml
Biomarkers	entry, 8 weeks, 24 weeks, exit	1.5 ml

Approximate Phlebotomy Volumes:

Baseline:	13.1 ml (includes cytogenetics/mutation and T cell/biomarkers)
First Treatment:	11.0 ml (includes GFR assessment)
q2-4 weeks:	0.5 ml
q6 months:	2.0 ml
Exit (patients 1-140):	17.0 ml (collected over 1 month prior to exit)
Exit (patients 141-200):	15.0 ml (collected over 1 month prior to exit)

An additional 17.1 ml of blood is required for immunology studies, collected at intervals appropriate for the child's age, immunization status, and other blood specimen collection, and 3.0 ml for biomarker studies calculated as 1.5 ml at 8 weeks and 24 weeks following study entry.

Table 7-2
BABY HUG LABORATORY DATA
ALERT AND MONITORING LEVELS

	Alert Levels[a]		Change[b]
Study	**Low**	**High**	
WBC		50K	
Hemoglobin	6.0	13.3x2FVs	- 20%[c]
Platelet Count	80K	1M	
Neutrophil Count	1.25	30K	
Reticulocyte Count	80K[d]	--	
Creatinine	--	1.0	+100%[e]
Total bilirubin	--	10	
ALT	--	150	

NOTE: Low alerts must be less than those listed in the table; high alerts must be greater than those listed in the table.

[a] Alert levels which should be evaluated for clinical monitoring of the patient. FV = Follow-Up Visits.

[b] Loss or gain (sign indicates direction), in % change.

[c] Hemoglobin 20% decrease from three-month running average.

[d] If Hb<7.0gm/dl.

[e] Creatinine level at least twice baseline and greater than 1.0.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 8

GUIDELINES FOR STANDARD CLINICAL CARE

8.1 INTRODUCTION

The basic principles of supportive care for the infants with sickle cell anemia enrolled in the BABY HUG trial are based upon the recommendations found in several publications (Mountain States Regional Genetic Services Network, 2000; Council of Regional Networks for Genetic Services (CORN), 2000; Ohene-Frempong, 2001; US Department of Health and Human Services, Public Health Service, 1993). Supportive care should be similar whether the patient is receiving hydroxyurea or placebo. For the ease of clinicians involved with participants, all patients should be treated as if they were receiving hydroxyurea. The cooperation of all medical staff involved in the clinical care of study patients will be solicited to enhance patient adherence to the BABY HUG Protocol and avoid compromise of blinding of treatment assignments.

Common clinical events are addressed in specific sections below. At no time should the performance of the study Protocol be allowed to compromise the elements of good clinical care of BABY HUG participants.

8.2 IMMUNIZATIONS

All routine pediatric immunizations should be given as per standard clinical recommendations, including vaccination against diphtheria, pertussis, tetanus, polio, measles, mumps, hepatitis B, rubella and *Haemophilus influenzae* type b. Immunizations against hepatitis A and varicella may be given if indicated and commonly available locally. These vaccines should be provided through usual local pediatric primary care mechanisms if possible. Any and all vaccines may be withheld for medical indications (e.g., allergy or history of prior reaction) or parental preference.

All participants should receive all recommended doses of pneumococcal vaccine according to the following schedule. A history of vaccination with pneumococcal conjugate vaccine (Prevnar or PCV7), including date(s) given, must be documented by the BABY HUG Clinical Center and reported to the Medical Coordinating Center for each infant enrolled. Patients deficient in PCV7 immunizations at study entry should be brought up to date by the Clinical Center as soon as possible. Bacteremia and meningitis with *S. pneumoniae* will be monitored closely as clinical events in BABY HUG. Further, the antibody response to pneumococcal vaccination will be measured in the study. The total number of doses required is dependent upon the age of the patient when the first dose is given even if the interval between doses is longer than intended. Adequate series of vaccinations are indicated in the table below.

Pneumococcal Conjugate Vaccine Series (Prevnar or PCV7)

Age at first dose (mos)	Primary Series	Additional Dose**
2-6	3 doses, 2 months apart*	1 dose at 12-15 months
7-11	2 doses, 2 months apart*	1 dose at 12-15 months
12-23	2 doses, 2 months apart	
more than 24 months	1 dose	

Pneumococcal polysaccharide vaccine (pneumovax or PV23)

Age at first dose (mos)	Primary Series	Additional Dose**
less than or equal to 24 months	1 dose	1 dose at 60 months

* For children vaccinated before 1 year the minimum interval between doses is 4 weeks.

**Additional dose should be 8 or more weeks after the primary series has been completed.

Measles, Mumps, and Rubella vaccine (MMR): one dose of the vaccine should be documented as given between 12 and 15 months of age and then repeated between 4 and 6 years of age. The immunologic response to this vaccine will be assessed to document the ability of patients receiving either hydroxyurea or placebo to respond to these antigens. Thus, the date of this immunization must be recorded for each patient by the Clinical Center and reported to the Medical Coordinating Center. Parents will be encouraged, and assisted by the BABY HUG Clinical Center as necessary, to obtain this vaccine as early in the scheduled vaccine interval as possible.

Influenza vaccine: the annual flu vaccine is encouraged for all infants 6 months of age or older. The first year this is administered this should be given as two 0.25 ml doses, with a minimum of four weeks between doses. Each subsequent flu season, one dose is given. A single dose of 0.25 ml is given to patients from 6 to 35 months of age and 0.5 ml to patients over 36 months of age.

8.3 PROPHYLACTIC MEDICATIONS

Twice daily prophylactic penicillin will be prescribed from first medical contact. This should have already been initiated prior to enrollment in BABY HUG. The doses are from birth to 35 months of age - 125mg po BID – and over 36 months of age - 250 mg po BID. Either the liquid formulation, which must be kept refrigerated and refilled every 2 weeks, or tablets may be used (Mountain States Regional Genetic Services Network, 2000; American Academy of Pediatrics, 2000). Erythromycin estolate 250 mg po BID may be used for penicillin allergic patients (Mountain States Regional Genetic Services Network, 2000; Council of Regional Networks for Genetic Services (CORN), 2000; Ohene-Frempong, 2001; US Department of Health and Human Services, Public Health Service, 1993). Reminders about the need for this prophylactic agent should be offered at each clinical contact.

Folic Acid supplementation will not be required for patients on BABY HUG. It may be prescribed at parental or Clinical Center preference. It is very unlikely that infants enrolled on this study will be deficient in folate. The majority will either still be drinking or just have been weaned

from high folic acid containing infant formula. The widespread fortification of a variety of foods with folic acid and the generally adequate diets of young patients with sickle cell anemia should prevent folate deficiency.

Supplemental iron will be prescribed only for documented iron deficiency or bleeding or similar reasons. Adequacy of iron stores will be documented at study entry and every six months and supplemented as necessary.

8.4 PARENT EDUCATION

All parental and caregiver education routinely offered to families of patients with sickle cell anemia should be provided to BABY HUG participants. However, in order to standardize some aspects of care the following suggestions are offered for each Clinical Center to review with BABY HUG families at quarterly visits :

Fever: The temperature should be measured if the child feels hot. Families should be advised to have a thermometer at home and taught how to accurately use it. They should be instructed to seek medical attention for any temperature over 101.5^{0}F regardless of route (oral, axillary or rectal) measured. The parent or caregiver should be cautioned against use of antipyretics unless recommended by treating physicians. They are encouraged to seek medical attention for all febrile episodes. The medical response to a reported history of fever should include a complete blood count, blood culture, and an empiric dose of parenteral antibiotics (usually ceftriaxone) effective against encapsulated organisms. Admission or outpatient management will be at local option (Mountain States Regional Genetic Services Network, 2000; Council of Regional Networks for Genetic Services (CORN), 2000).

Neutropenia: A complete blood count with differential white blood cell count should be performed at the Clinical Center or site of care with each febrile event. If the absolute neutrophil count (ANC) on the local blood count is below 1,000/uL, the child should be admitted to the hospital for observation and parenteral antibiotics. BABY HUG study medication should be stopped and the Medical Coordinating Center notified. If the ANC on the local blood count exceeds 1,250/uL

prior to hospital discharge, BABY HUG study medication should be resumed at the same dose as on admission. If on hospital discharge the ANC on the local blood count is still below 1,250/uL, the patient and family should be instructed not to resume treatment until a local scheduled blood specimen shows a count of 1,250/µL or greater.

If the absolute neutrophil count on local blood count is between 1,000/uL and 1,250/uL during evaluation for a febrile event, the BABY HUG study medication should be stopped. The patient may be treated as an outpatient or admitted at local option. If admitted, BABY HUG study treatments will be stopped as above. If managed as an outpatient, the patient should remain off BABY HUG study medication until a local scheduled blood count is performed and the count is 1,250/µL or greater.

Hospitalization: The BABY HUG study medication should be continued during all clinically indicated hospitalizations unless the ANC of a BABY HUG participant is found to be below 1,250/uL. Then the study medication should be stopped and the Medical Coordinating Center notified. Study treatment should be resumed at the same dose when the ANC on the local blood count exceeds 1,250/uL. If the patient is otherwise able to be discharged to outpatient management before the ANC recovers, the patient and family should be instructed not to resume treatment until the next scheduled local blood specimen can be reviewed. The Clinical Center Principal Investigator and Nurse Coordinator will not review the local blood counts for mean corpuscular volume (MCV) or other parameters that may compromise blinding of the patient's study medication assignment.

Positive Cultures: The blood culture (plus cerebrospinal fluid -- CSF -- or urine cultures if performed) obtained at all febrile encounters will be monitored by each Clinical Center. Positive cultures from normally sterile sites will be managed according to Clinical Center preference and reported to the Medical Coordinating Center. The organism should be identified; if possible, and antibiotic sensitivities obtained. The serotype of *Streptococcus pneumoniae* isolates from sterile sites for patients on this trial should be obtained if possible.

Varicella: The family should be instructed to contact the Clinical Center for suspected varicella infection. If the child is febrile, management should be as above. If fever does not exceed 101.5^0 F, no extra CBC is required. BABY HUG study medication should be stopped for all episodes of varicella and the Medical Coordinating Center notified. BABY HUG medication should be resumed at the same dose when all lesions are crusted over. Acyclovir may be prescribed at Clinical Center discretion.

Tuberculosis: Surveillance for exposure to tuberculosis is standard in many pediatric clinic populations. If a patient receiving BABY HUG study medication is incidentally found to have a positive tuberculosis skin test (PPD), a repeat PPD and chest radiograph should be done per standard practice. If the chest radiograph and physical examination show no signs of active tuberculosis, the child should be treated as appropriate and the study medication continued once the PPD treatment is no longer a risk for continuation of the study treatment. If the chest radiograph is positive or there is evidence of active infection with tuberculosis, the child's situation should be discussed with the Operations Committee.

Transfusion Therapy: The use of blood products as therapy for a clinical event will be at the option of the Clinical Center. The red cell products selected should be matched for Rh (CcDEe) and Kell if possible. Similarly leukofiltration of all cellular blood components should be considered, if available. Chronic transfusion therapy for any indication, an option that would remove the patient from study treatment, should be discussed with the Operations Committee prior to implementation.

Steady State Hematologic Values: Many clinical decisions in the young patient with sickle cell anemia are based upon steady state hemoglobin levels. Obtaining such data on patients in BABY HUG Clinical Centers could undermine blinding of treatment assignments. The Medical Coordinating Center will provide the unblinded Primary Endpoint Person at each Clinical Center 3-month rolling averages of steady state hemoglobin measurements monthly. Steady state is defined by intervals when the patient is not having a febrile, acute chest syndrome, aplastic crisis or splenic

sequestration event. MCV and white cell or neutrophil counts will not be included with the information on hemoglobin level.

Spleen and Splenic Sequestration: Parents and caregivers should be instructed in techniques of spleen palpation at each clinical visit and asked to feel for the child's spleen daily. A newly palpable spleen or one more than 2 cm larger than previously noted should be reported immediately to the Clinical Center, and the patient should have a CBC and be examined by a physician or nurse practitioner knowledgeable about sickle cell anemia. The span of the spleen below the costal margin in the midclavicular and anterior axillary lines should be carefully measured and recorded (in centimeters) at each clinical visit.

Splenic sequestration and splenomegaly as defined in Appendix F will be recorded on the appropriate study form as outlined in the Manual of Operations.

The management of splenic enlargement (admission, close outpatient follow-up or transfusion) will be at the discretion of the Principal Investigator at each Clinical Center, but the following guidelines should be used whenever appropriate/possible. On initial evaluation:

1. Vital signs at presentation and q1-2 hours initially;
2. Careful physical examination with assessment of pallor, measurement of spleen size, presence of gallop, liver size; repeat examination q1-2 hours initially;
3. Labs including CBC, WBC differential, reticulocytes STAT. Type and crossmatch for packed red blood cells (PRBCs);
4. If hypovolemic or with cardiovascular compromise, emergent infusion of intravenous fluids (IVF) or PRBC to restore blood volume and maintain normal blood pressure. If normovolemic, start IVF at 1x maintenance.
5. Admission unless stable over 4-8 hour period; close follow-up.

On admission:

1. Monitor heart rate (HR), respiratory rate (RR), pulse oximetry q2 hours until stable, then q4h;
2. Repeat CBC studies q4-12 hours;
3. Maintain IVF at 1x maintenance;
4. If febrile obtain blood culture and begin antibiotics, e.g. cefuroxime 50 mg/kg IV every 8 hours;
5. Transfuse for evidence of hypotension, cardiovascular (CV) compromise, enlarging tender spleen with 10-15 cc/kg over 4 hours.

   If no CV compromise, transfuse if Hgb <5 gm/dL for stable splenomegaly regardless of reticulocyte count; if Hgb> 5gm/dL transfuse at Principal Investigator discretion.

   Goal of transfusion is Hgb about 8 gm/dL, (splenic unloading of trapped RBC may cause an "overshoot phenomenon");
6. Supplemental oxygen until condition is stable (or acute episode resolves);
7. Other clinical interventions, including antipyretics and analgesics, at Principal Investigator discretion.

After the initial splenic sequestration event, the child will be monitored every 2 weeks. The decision to continue PRBC transfusions and/or proceed to splenectomy, will be at the discretion of the Principal Investigator. However, the number of children undergoing splenectomy according to local indications will be tabulated in each treatment group. Chronic transfusion therapy or splenectomy, options that would remove the patient from study treatment or scintographic evaluation of the primary (spleen) endpoint, should be discussed with the Operations Committee prior to implementation.

Episodes of splenomegaly, splenic sequestration and associated measures will be tabulated and compared according to HU and placebo group assignment. If an excess of sequestration events is found in the HU group, the DSMB may consider stopping the trial.

Renal: Parents and caregivers will be educated about the importance of the kidneys in children with sickle cell disease, and reminded that the kidneys are special organs tested in the BABY HUG trial. Infants should remain adequately hydrated at all times, as dehydration is detrimental and could be injurious to the kidneys. Treatment that has potential risk to the kidneys, such as prolonged use of aminoglycosides or high-dose non-steroidal anti-inflammatory drugs, should be avoided. Frequent urinary tract infections and hematuria will be documented during the BABY HUG trial, as these findings may reflect or cause renal damage.

Painful Events (vaso-occlusive or dactylitis): Parents and caregivers will be educated about dactylitis and painful events as part of their education about sickle cell disease. They will be taught to push fluids and use ibuprofen and/or acetaminophen with codeine at home. Small supplies of both analgesics should be prescribed for home use at routine clinical visits. They and caregivers will be educated about dactylitis and painful events as part of their education about sickle cell disease. The definition of a painful event is an event lasting two hours or more without obvious cause requiring the use of one or more doses of non-steroidal or narcotic pain medication. Events treated as an outpatient (including emergency room) or requiring admission will be reported at the next Clinical Center contact and included in data entered. Events requiring admission will be reported to the Medical Coordinating Center on appropriate form, and supporting documents collected for central review.

Acute Chest Syndrome, Aplastic Crisis, Priapism: All events meeting defined criteria will be reported to the Medical Coordinating Center on Event Report Forms. Clinical management, including the need for simple or exchange transfusion will be at the option of each Clinical Center. Chronic transfusion therapy, an option that would remove the patient from study treatment, should be discussed with the Operations Committee prior to implementation.

Parents will be educated about signs of acute chest syndrome (fever, respiratory symptoms) and aplastic crisis (pallor, decreased energy) at regular clinic visits and asked to seek medical attention should they occur. Parents of male children will be taught about priapism, a prolonged painful erection of the penis, and asked to seek medical attention, if the episode persists beyond two hours. Briefer episodes of priapism will be recorded at the next clinic visit.

Neurologic Events: Families will be taught standardized definitions of TIA, stroke and other neurologic events and reminded at each clinical contact to call the Clinical Center immediately if the child is not able to move arms or legs (unrelated to pain), has facial drooping or dysarthria. Each Clinical Center will promptly evaluate such patients. The minimum evaluation must include documentation of complete neurologic examination, preferably by a neurologist. If the neurologist suspects stroke or TIA clinically, neuroimaging including MRI/MRA must be done. If a stroke is confirmed, acute management will be at the preference of the Clinical Center. Transfusion timing and technique (exchange or simple) will be at Clinical Center preference. Chronic transfusion therapy, an option that would remove the patient from study treatment, should be discussed with the Operations Committee prior to implementation.

If transcranial Doppler (TCD) screening is part of an individual Clinical Center's standard care, such testing should be offered to patients enrolled in BABY HUG as per usual practice. TCD screening is not yet universally available or standardized for children less than two years of age. If screening is performed and values are persistently abnormally elevated in children over 2 years of age, the parents should be offered chronic transfusion therapy for the child in accordance with standard practice at each Clinical Center. Patients for whom chronic transfusion therapy is considered should be discussed in advance with the Operations Committee.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 9

SPECIAL STUDIES AND READING GROUPS

9.1 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, pitted cell counts, karyotype and chromosome breakage studies, VDJ mutation studies, immunological function studies, clinical events, and serum Cystatin C levels.

9.2 PITTED CELL COUNTS

Pitted cell counts will 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 from specimens collected at baseline, six, twelve, and eighteen months, and 24 months will be compared between treatment groups. If the proportion of patients in the HU treatment group who have pitted cell count values above a predetermined level (e.g., 3.5 or 7-10%) decreases in relation to the placebo group, this finding will be interpreted as secondary outcome evidence of prevention by HU of injury to the spleen.

9.3 CYTOGENETICS (KARYOTYPE AND CHROMOSOME BREAKAGE ANALYSES)

Fresh blood specimens will be analyzed for karyotype and chromosome breakage at baseline and 24 months in a cytogenetics laboratory. White blood cells will be taken from fresh specimens shipped on wet ice, overnight to the cytogenetics laboratory, cultured for 48 hours and prepared with colchicine for morphologic study of the karyotype and chromosomes.

9.4 VDJ/DNA MUTATION STUDIES

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

and used directly in the VDJ mutation assay. Leftover DNA will be frozen at -70°C and used for future studies assuming proper IRB authorization and patient consent is in place.

The overall goal of the VDJ studies is the investigation and quantification of the mutagenic and carcinogenic risks of HU therapy for very young children with SCD enrolled in the BABY HUG study. To accomplish this goal, we will analyze peripheral blood for the presence of changes in chromosomal integrity that indicate unrepaired genetic damage. The specific aims of the VDJ mutation study are: to quantitate the frequency of "illegitimate" VDJ recombination events that occur between the T cell receptor gamma (TCR-gamma) and beta (TCR-beta) gene loci located on chromosome 7; and to compare the frequency of VDJ mutational events among patients assigned to hydroxyurea as compared with the placebo group, using serial measurements for both groups.

Quantitations of Howell-Jolly bodies will also be performed. For each of the patients, a small aliquot of RBC will be fixed in ice-cold methanol and frozen at -85°C according to a previously published protocol. This sample will be shipped frozen to Litron Laboratories, Inc. in Rochester, NY and analyzed by flow cytometry for quantitation of Howell-Jolly bodies (micronuclei) in both immature and mature erythrocytes.

9.5 LIVER-SPLEEN SCANS

Tc99m sulfur colloid liver-spleen scans will be performed according to standard techniques. Results will be assessed at the end of the study by a panel of 3 pediatric radiologists unaware of the treatment assignment of the patient. Two readers will be given copies of the entry and exit Tc99m sulfur colloid liver-spleen scans and asked to score each scan as splenic uptake "normal" (80-100% of liver uptake), decreased (splenic uptake 20-79% of liver uptake) or absent (splenic uptake 0-19% of liver uptake). In case of disagreement between the two readers, a third reader on the panel will evaluate the scan, and the final evaluation will be that of the majority. The proportion of patients in each treatment group classified by spleen function (normal, decreased, or absent) and the proportion of entry and exit scan pairs demonstrating a decline (from one category

to another) in splenic function will be compared in the HU vs placebo treated groups. Paired scans that demonstrate an improvement in uptake will be scored as not demonstrating a decline.

9.6 ABDOMINAL ULTRASOUND

A central reader with specialty expertise in pediatric ultrasound studies will advise the BABY HUG investigators on performance standards for abdominal ultrasound studies and image preparation. These images will be read centrally, blind to treatment assignment for assessment for gallstones, kidney size, spleen size and liver enlargement.

9.7 HYDROXYUREA ASSAY

Quantitative assays of hydroxyurea will be performed on selected bottles of study treatments at the end of manufacturing after reconstitution. Also, plasma levels of hydroxyurea will be assessed at the time of first dose, approximately one month after the first dose, and upon exit from the study.

9.8 IMMUNE FUNCTION STUDIES

To determine the effects of hydroxyurea treatment on antibody responses to standard vaccines administered before and during hydroxyurea therapy, baseline pneumococcal antibody level (in response to pneumococcal conjugate vaccine administered at two, four and six months of age -- prior to study entry) and after 23-valent pneumococcal polysaccharide vaccine (given at 24 months of age) will be measured. Antibody levels for measles, mumps and rubella (vaccine given at 12 - 15 months of age) before and after immunization will be measured for those enrolled prior to vaccination.

To determine the effects of hydroxyurea treatment on T cell maturation and T cell responses to a vaccine administered during hydroxyurea therapy, we will measure peripheral blood naïve (CD45RA) and memory (CD45RO) CD4 and CD8 cells at entry and 12 and 24 months thereafter.

To determine the effects of hydroxyurea treatment on serum opsonophagocytic function, we will measure opsonophagocytic activity against pneumococci at study entry, after the 24-month of age pneumococcal immunization, and at the end of treatment (24 months on study treatment).

Measurement of relative contributions of complement and antibody to opsonophagocytic activity will be accomplished by comparing the activity of fresh versus heat-treated sera.

9.9 CLINICAL EVENTS

Description of Clinical Events will be based on Clinical Event Forms submitted 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 will be based on CSSCD, HUSOFT and other published experience with pediatric sickle cell anemia and provided in a separate classification criteria document.

9.10 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 and certified by the MCG technicians. The Central Laboratory will perform standardized readings of the studies (supported by grant funding independent of the BABY HUG contracts).

9.11 CYSTATIN C

Leftover serum from the biochemistry treatment initiation and exit specimens sent to the Biochemistry Core Laboratory will be shipped to the DNA/VDJ Core Laboratory for determining Cystatin C levels.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 10

FOLLOW-UP PROCEDURES

10.1 INTRODUCTION

Patients will be followed every two weeks for at least eight weeks after study treatment initiation. Subsequent visits will be every 4 weeks unless toxicity occurs. The total follow-up is a minimum of 24 months. During the period of study treatment at each monthly visit, a medical review will be conducted to determine any adverse or acute events and adherence to study medication. Medical history and adherence forms will be completed. At each of these regularly scheduled visits, a complete blood count will be assessed locally by a physician who is not otherwise affiliated with BABY HUG, and who will keep this information confidential except as needed for urgent patient management on the day of the visit. All adverse events in a patient will be reported on the appropriate form accompanied by appropriate documentation from the medical record. Appendix A shows an ideal schedule of patient visits and data collection. A patient's actual schedule will depend on his/her toxicities.

10.2 FOLLOW-UP VISITS

The BABY HUG Clinical Center staff will perform all study visit examinations. Study patients will be evaluated in accordance with good clinical practice. The Medical Coordinating Center (MCC) will be responsible for organizing and transmitting data from clinical laboratory studies outlined in the Protocol and for endpoint adjudication. Under the direction of the MCC, a Pharmacy Distribution Center (PDC) will distribute hydroxyurea and placebo to Clinical Centers where dose adjustments will be prescribed based on information from the MCC. At the monthly visits, medical reviews will be conducted, and measurements of weight (height and head circumference every three months), ascertainment of possible adverse events, major procedures, and current therapies (including hydroxyurea outside of study treatment) will be recorded. Blood specimens for CBCs will be collected and analyzed locally. Other blood specimens will be collected and prepared for

shipment to the Core Laboratory every six months or at study entry/exit. Each patient's current address and telephone number will be updated and maintained in the Clinical Center files. Although not mandatory, each Clinical Center is encouraged to set aside a specific day of the week for BABY HUG clinic visits, preferably early in the week to allow dose adjustments before the weekend. In addition, a specified clinic day may help to increase adherence to the Protocol.

10.2.1 Real Time Complete Blood Counts

1. Each Clinical Center will designate the person (a hematologist, or nurse practitioner or physician's assistant qualified to perform this task relative to the regulations of the state health regulations, who has no other BABY HUG responsibility) who will monitor each child's blood work that is performed locally at each clinic visit.
2. The Primary Endpoint Person (PEP) will data enter the CBCs into the BABY HUG database vis the Internet Data Entry System within one day of collecting the specimen or by 9:00 a.m. of the following morning.
3. The PEP will keep the local laboratory results in a locked file with no access by BABY HUG staff.
4. The MCC will check the local lab data for violating BABY HUG toxicity and/or alert levels and will send the appropriate messages to the PEP.
5. The designee, if in his/her clinical judgment, believes a toxicity and/or alert level has been crossed, will contact the Clinical Center Principal Investigator to notify of the toxicity and/or alert and to arrange for appropriate emergency clinical care.

If the absolute neutrophil count (ANC) on the local blood count is <u>below 1250/uL or the Hgb <6.0 g/dl or the Hgb has dropped 20% or more from the three-month rolling average or the platelet count is <80,000 mm^3, study treatment should be stopped and the child should be clinically evaluated to determine if intervention is required</u>. BABY HUG study medication should be stopped and the Medical Coordinating Center notified.

If the ANC on the local blood count exceeds 1,250/uL prior to hospital discharge, BABY HUG study medication should be resumed at the same dose as on admission. If on hospital discharge the ANC on the local blood count is still below 1,250/uL, the patient and family should

be instructed not to resume treatment until a scheduled local blood specimen is above 1,250/uL and reviewed in the Medical Coordinating Center.

If the absolute neutrophil count on local blood count is between 1,000/uL and 1,250/uL during evaluation for a febrile event, the BABY HUG study medication should be stopped. The patient may be treated as an outpatient or admitted at local option. If admitted, BABY HUG study treatments will be stopped as above. If managed as an outpatient, the patient should remain off BABY HUG study medication until a scheduled blood count is performed locally and reviewed in the Medical Coordinating Center.

10.2.2 Ascertainment of Specified Events and Possible Adverse Effects in Patients

At each visit, parents will be queried regarding recent medical events or procedures. Events will be documented to ascertain the nature of the event, including special treatment(s) and their indication(s). Reportable events and hospitalizations will be recorded on event forms and followed up by the BABY HUG PEP, who will review hospital charts, medical records and office visit records. These forms will be forwarded to the MCC and maintained in a locked file by the PEP.

The event report forms and documentation will identify the occurrence of death, stroke with neurologic deficit, splenic sequestration, dactylitis, pain, acute chest syndrome, priapism, splenomegaly, biliary obstruction, hepatopathy, hepatic sequestration, pancreatitis, fever >101.5° F (38.5° C), acute renal failure, permanent renal failure, sepsis, severe neutropenia, aplastic crisis, acute osteomyelitis, transient ischemic attack and hospitalizations for sickle cell related events. If any study patient dies, efforts will be made to obtain complete post-mortem information. Discharge summaries and narratives of the fatal events will be sent with study forms to the MCC. All reportable events, whether treated on an out-patient or in-patient basis, will be reviewed by the Central Review Group (the NHLBI Project Director and the MCC Medical Consultant). Adverse treatment effects will be reported to the U.S. Food and Drug Administration (FDA).

10.2.3 BABY HUG Adverse Event Reporting

10.2.3.1 Introduction

The safety of interventions and treatments associated with this protocol will be under continual review by the MCC, NHLBI, NICHD and DSMB. Accrual, efficacy and safety data will be monitored by all four groups.

Accrual and safety data will be reviewed annually by each center's Institutional Review Board (IRB). Prior to implementation of this study, the Protocol and the proposed patient consent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to the 45 CFR 46 code of federal regulations. This committee will also approve all amendments to the Protocol or informed consent, and conduct continuing annual review so long as BABY HUG is open to accrual or follow-up of subjects.

The NHLBI Data and Safety Monitoring Board will review the Protocol at 6 month intervals. A progress report showing results according to treatment assignment will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB members will be provided with monthly reports documenting each child's growth, development and progress in BABY HUG. The DSMB may recommend early termination of the study for considerations of safety or efficacy.

Monitoring for unanticipated adverse clinical effects will be done using event forms. The Clinical Center staff will determine the degree of severity (mild to fatal). Event forms will be submitted to the Medical Coordinating Center (MCC) and tabulated based on the affected organ system. Each serious AE (SAE) will be reported to the MCC within 24 hours of the event; supporting information will be required from the Clinical Center. MCC staff and the central review group will immediately review the report to determine if the event is serious. If so, MCC staff will send the information to the NHLBI and NICHD Project Officers and the FDA for review. The occurrence of serious AEs will be reported to the Clinical Center IRBs within 24 hours of NHLBI review.

A serious adverse event is any of the following.

1. death
2. a life-threatening event
3. prolonged hospitalization (greater than 7 days)
4. splenic sequestration crisis
5. stroke, TIA
6. acute chest syndrome
7. an ICU admission
8. Any AE that is related to study treatment and unexpected

Certain 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 given that frequent hospitalizations occur as a consequence of having sickle cell anemia without being enrolled in a clinical trial. 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 in the above list.

In addition to this reporting mechanism, a centralized over-ride system will be carried out by individuals with knowledge about the treatment assignments. These individuals will review adverse events that are not thought to be serious in the eyes of the blinded investigators and make decisions 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 MedWatch Form 3500A and a subsequent report to the FDA.

Adverse events and serious adverse events will be listed individually and according to body system, designated according to severity (mild, moderate, severe, life-threatening, or fatal), and likelihood of relation to study 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).

10.2.3.2 DBDR Adverse Event Coverage

NHLBI Division of Blood Diseases and Resources (DBDR) staff will examine all adverse event reports from BABY HUG in real time and discuss with the BABY HUG Operations Committee appropriate clinical management. NHLBI - DBDR will hold emergency meetings to review adverse event reports as they occur. These DBDR staff will alternate night and week-end coverage via cell-phone so that the BABY HUG MCC will have access to a DBDR staff person at all times to discuss the management of adverse events in BABY HUG subjects.

10.2.3.3 Elevated Adverse Event Rate Detection

Events to be centrally reviewed include all serious adverse events (e.g., death or events that are life-threatening, or events that cause or prolong hospitalization), splenic sequestration crisis, acute chest syndrome, stroke, transient ischemic attacks and ICU admissions. Other clinical occurrences will be denoted as having occurred or not occurred on clinic visit reports. There is adequate statistical power in BABY HUG to detect 50% differences between treatment groups at alpha = 0.01 if the event rates are in the range expected from the Cooperative Study of Sickle Cell Disease (CSSCD) and the study is completed with 200 patients. The BABY HUG investigators do not plan for early termination based on clinical events other than demonstrated inferiority of hydroxyurea for the outcomes death, stroke or splenic sequestration.

The most important clinical events other than death and stroke are acute chest syndrome (ACS) and splenic sequestration (defined in Appendix F). Each child will be clinically evaluated repeatedly to determine if he/she has had acute chest syndrome, splenic sequestration or a serious, unexpected adverse event in the course of the study period. The proportion of very young children experiencing these adverse events will be compared according to assigned treatment. A one-sided test-based confidence interval (alpha = 0.005) will be used to determine if very young

children treated with HU have significantly higher frequencies than very young children treated with placebo. If the test-based, one-sided confidence interval does not cover zero, the Steering Committee proposes that the DSMB will consider all relevant information for the study and will recommend that the clinical trial not continue and HU not be recommended as a treatment for very young children.

10.2.3.4 Interim Reports

Adverse events used to evaluate the safety of the BABY HUG regimen will be collected to include any unfavorable and unintended signs (including abnormal laboratory findings), symptoms or diseases (i.e. incidence of stroke, renal failure, regimen related toxicities, or infectious complications), which either occur during the study, having been absent at baseline or if present at baseline, appear to worsen and are determined to be possibly, probably or definitely related to this investigational treatment.

Although the size of the Feasibility and Safety Pilot Study was chosen to allow the BABY HUG investigators to evaluate several administrative issues of the overall study design, it will be important to monitor the study in an ongoing fashion with respect to performance criteria and the occurrence of adverse events. For analyses other than those discussed above, we will protect our findings against finding spurious associations due to the large number of repeated tests of significance that will be performed. To do this, we propose to use monitoring bounds (for HU versus control comparisons) of 0.01 rather than 0.05 for the safety and adverse event evaluations listed below. We propose the following monitoring.

<u>Monitoring Safety and Adverse Events</u>

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

1. Recruitment: Expected vs. Actual
2. Patients screened, eligible and randomized
3. Patient characteristics at baseline

A. Age, race and gender

B. Spleen function

C. Spleen size

D. Pitted cell counts

E. Schwartz equation GFR estimates

F. DTPA GFR was discontinued as of May 29, 2009.

G. Urine concentrating ability

H. CBC

I. Presence of gallstones

J. Blood chemistries

K. Microalbuminuria

L. O_2 saturation

M. Physical examinations

N. Neurological examination and neuropsychological development

O. Height, weight, head circumference

P. Transcranial doppler (TCD) measurements

4. Blood count toxicities

5. Dose adjustments

6. Intra- and Inter-observer agreement liver-spleen scans

7. DTPA GFR was discontinued as of May 29, 2009.

8. Immunological Impairment

9. Safety assessments and adverse events

A. Height, weight, head circumference

B. Neurological examination and neuropsychological development

C. Unexpected and serious adverse events: update tallies, rates and individual summaries (case reports) for immediate review by the Executive Secretary of the DSMB, DSMB Chair, and NHLBI and NICHD Project Officers

Individual Patient and Group Safety Monitoring

a. In consultation with the Project Officer, the Executive Secretary of DSMB, and the DSMB chair may recommend full DSMB review or individual treatment interruptions.

b. All individuals whose treatment is interrupted or stopped will continue to be monitored.

10.2.3.5 Analysis of Death or Stroke

Death and clinically manifest stroke represent the most adverse outcomes that can occur within this study, and it will be important to determine if HU treatment results in an excess number of these types of events. These two outcomes will be evaluated separately from each other. One-sided test-based confidence intervals (alpha = 0.05) will be used to determine if very young children treated with HU have a significantly higher frequency of either outcome than very young children treated with placebo. If the 95% one-sided confidence interval for either stroke or death does not cover 0, it will be recommended that the trial not proceed and that HU not be used as a treatment for very young children.

Clinical Centers will be expected to report to the Medical Coordinating Center the occurrence of death or stroke within 24 hours of learning about the event. The Medical Coordinating Center will prepare a report immediately with the information at hand for the NHLBI and NICHD Project Officers, Executive Secretary of DSMB and the DSMB Chair. Within 10 days, the Medical Coordinating Center staff will provide an updated report for the NHLBI and NICHD Project Officers, DSMB Executive Secretary, and for the DSMB Chair. Each case will be reviewed individually with the DSMB. The NHLBI Project Officer and DSMB Executive Secretary will file reports on each death or stroke with the U.S. Food and Drug Administration (FDA) under the study IND. If the p-value for an association of death or of stroke with hydroxyurea is between 0.05 and

0.20 after any occurrence, the members of the DSMB will review all death or stroke reports in BABY HUG in aggregate and with other study data to consider whether or not there is a concern that should be addressed with a Protocol revision or study termination.

10.2.3.6 Analysis of Growth and Development

We will analyze each child's weight (monthly), height (quarterly), head circumference (quarterly) and growth velocity (monthly). We will measure their neurodevelopment (Bayley, Vineland) annually. Height, weight and head circumference growth will be analyzed using actual measurements and percentiles standardized to the CSSCD population of children with HbSS for height and weight, and to a normal black American population for head circumference (Pivnick et al, 1999). Average scores for the two treatment groups will be compared to test:

H_O: $\mu_2 > \mu_1$

versus the alternative:

H_A: $\mu_2 \leq \mu_1$,

where μ_2 is the mean of measurements or percentiles for children assigned to HU and μ_1 is the mean of measurements or percentiles for children assigned to placebo.

Each test will be a t-test performed on the estimates of height, weight and head circumference calculated for each child. It is proposed that DSMB members review individual growth and height for possible clinical indications of adverse effects of HU on height and weight if the mean in HU-treated very young children is between 1 and 2 standard deviations (SD) below the mean for placebo-treated very young children and to stop the study if the mean of HU-treated very young children is more than 2 SDs below placebo treated very young children.

For review, DSMB members will be provided with the growth curves of each child printed on paper with percentiles from the CSSCD and specifying treatment assignment, and a graph of the average growth velocity over three month intervals according to time from study entry and treatment. Growth will be analyzed with cubic models (mixed model analysis of variance to incorporate child-specific random effects, and in treatment group comparisons to account for

correlation of serial measurements) fit to each child. Growth velocity will be estimated with the first derivative with respect to time of the cubic models. Curves will be plotted as each child's percentiles for growth over time also.

Neuro-development questionnaires will be administered annually. For the evaluation of this endpoint, we will compare the one-year cognitive function between toddlers treated with HU and toddlers treated with placebo.

If a test-based, one-sided 99% confidence interval of the difference in mean Bayley score at one-year does not cover zero, it will be recommended that the trial not continue and that HU not be used as a treatment for very young children.

10.2.3.7 Analysis of Acute Chest Syndrome, Splenic Sequestration and Serious, Unexpected Adverse Events

The most important clinical events other than death and stroke are acute chest syndrome (ACS) and splenic sequestration (as defined in Appendix F). Each very young child will be clinically evaluated repeatedly to determine if he/she has had acute chest syndrome, splenic sequestration or a serious, unexpected adverse event in the course of the two-year follow-up period. The proportion of very young children experiencing these adverse events will be compared according to assigned treatment. A one-sided test-based confidence interval (alpha = 0.005) will be used to determine if very young children treated with HU have significantly higher frequencies than very young children treated with placebo. If the test-based, one-sided confidence interval does not cover zero, the Steering Committee proposes that the DSMB will consider all relevant information for the study and recommend that the clinical trial not continue and HU not be recommended as a treatment for very young children.

10.2.3.8 Alert and Monitoring Levels

The local Primary Endpoint person will review the local CBC results to determine if an alert is present in the data. In addition, the Hematology and Biochemistry Core Laboratory data and the local CBC data will be used by the MCC to monitor electronically for values exceeding alert levels.

Alert laboratory values (Table 10-1) are indicators to the MCC and the Center staff that the patient requires clinical follow-up and are distinct from toxicity values (Section 6.4). The laboratory alerts are intended as possible indicators of adverse events such as splenic sequestration, aplastic crises, renal insufficiency, etc requiring treatment interruptions and further clinical evaluations or interventions by the Center staff.

Table 10-1
BABY HUG LABORATORY DATA
ALERT AND MONITORING LEVELS

	Alert Levels[a]		
Study	**Low**	**High**	**Change[b]**
WBC		50K	
Hemoglobin	6.0	13.3x2FVs	- 20%[c]
Platelet Count	80K	1M	
Neutrophil Count	1.25	30K	
Reticulocyte Count	80K[d]	--	
Creatinine	--	1.0	100%[e]
Total bilirubin	--	10	
ALT	--	150	

NOTE: Low alerts must be less than those listed in the table; high alerts must be greater than those listed in the table.

[a] Alert levels which should be evaluated for clinical monitoring of the patient. FV = Follow-Up Visits.
[b] Loss or gain (sign indicates direction), in % change.
[c] Hemoglobin 20% decrease from three-month running average.
[d] If Hb<7.0gm/dl.
[e] Creatinine level at least twice from baseline and greater than 1.0.

If an alert level is detected, the Clinical Center will use the following guidelines for responding to notification of an alert.

1. The PEP will handle the alert in a straightforward manner when possible, or, more likely, contact a Hematologist/Sickle Cell Specialist who is not directly connected with the BABY HUG Trial.
2. When indicated, the PEP and the Hematologist will develop a plan for a clinical response to the alert value. (For example: If the patient were severely neutropenic and febrile, the patient would be hospitalized for antibiotic treatment; if the patient had > 20% decrease in hemoglobin level, the patient would be hospitalized and/or transfused; if the patient met criteria for a splenic sequestration, the patient would be hospitalized and/or transfused; etc.)
3. In the event that no non-BABY HUG Hematologist was available, the PEP would need to contact the BABY HUG PI and discuss a course of action. In doing so, the PEP would provide the PI only with “need to know” laboratory information. In most cases this would not create an unblinding problem because, for example: severe neutropenia may more likely be related to an acute viral infection rather than HU toxicity; worse anemia might be caused by an aplastic crisis or splenic sequestration rather than HU; splenic sequestration might or might not be related HU, etc. In most situations, CBC values would not readily distinguish between treatment with hydroxyurea or placebo, although it is important that the MCV should not be revealed to the PI or BABY HUG Study Coordinators.
4. In the ensuing management of the child, the bulk of the decision-making will be carried out by the non-BABY HUG Hematologist and the PEP.
5. The alert values for increased hematologic blood counts will not necessarily warrant any immediate reaction by a clinician.

10.2.4 Adverse Event Management

Spleen and Splenic Sequestration: Parents and caregivers should be instructed in techniques of spleen palpation at each clinical visit and asked to feel for the child's spleen daily. A newly palpable spleen or one more than 2 cm larger than previously noted should be reported immediately to the Clinical Center, and the patient should have a CBC and be examined by a physician or nurse practitioner knowledgeable about sickle cell anemia. In order to assure the availability of steady state hemoglobin level information, the Medical Coordinating Center will provide each Primary Endpoint Person with a three-month rolling average of each patient's hemoglobin measurements. The span of the spleen below the costal margin in the midclavicular and anterior axillary lines should be carefully measured and recorded (in centimeters) at each clinical visit.

Splenic sequestration and splenomegaly as defined in Appendix F will be recorded on the appropriate study form as outlined in the Manual of Operations.

The management of splenic enlargement (admission, close outpatient follow-up or transfusion) will be at the discretion of a non BABY HUG hematologist or unblinded physician at each Clinical Center, but the following guidelines should be used whenever appropriate/possible.

On initial evaluation:

1. Vital signs at presentation and q1-2 hours initially;
2. Careful physical examination with assessment of pallor, measurement of spleen size, presence of gallop, liver size; repeat examination q1-2 hours initially;
3. Labs including CBC, WBC differential, reticulocytes STAT. Type and crossmatch for packed red blood cells (PRBCs);
4. If hypovolemic or with cardiovascular compromise, emergent infusion of intravenous fluids (IVF) or PRBC to restore blood volume and maintain normal blood pressure. If normovolemic, start IVF at 1x maintenance.

5. Admission unless stable over 4-8 hour period; close follow-up.

On admission:

1. Monitor heart rate (HR), respiratory rate (RR), pulse oximetry q2 hours until stable, then q4h;
2. Repeat CBC studies q4-12 hours;
3. Maintain IVF at 1x maintenance;
4. If febrile obtain blood culture and begin antibiotics, e.g. cefuroxime 50 mg/kg IV every 8 hours;
5. Transfuse for evidence of hypotension, cardiovascular (CV) compromise, enlarging tender spleen with 10-15 cc/kg over 4 hours.

   If no CV compromise, transfuse if Hgb <5 gm/dL for stable splenomegaly regardless of reticulocyte count; if Hgb> 5gm/dL transfuse at Principal Investigator discretion. Goal of transfusion is Hgb about 8 gm/dL, (splenic unloading of trapped RBC may cause an "overshoot phenomenon");
6. Supplemental oxygen until condition is stable (or acute episode resolves);
7. Other clinical interventions, including antipyretics and analgesics, at Principal Investigator discretion.

After the initial splenic sequestration event, the child will be monitored every 2 weeks. The decision to continue PRBC transfusions and/or proceed to splenectomy, will be at the discretion of the Principal Investigator. However, the number of children undergoing splenectomy according to local indications will be tabulated in each treatment group. Chronic transfusion therapy or splenectomy, options that would remove the patient from study treatment or scintographic evaluation of the primary (spleen) endpoint, should be discussed with the Operations Committee prior to implementation.

Episodes of splenomegaly, splenic sequestration and associated measures will be tabulated and compared according to HU and placebo group assignment. If an excess of sequestration events is found in the HU group, the DSMB may consider stopping the trial.

Renal: Parents and caregivers will be educated about the importance of the kidney in children with sickle cell disease, and reminded that the kidney is one of the special organs tested in the BABY HUG trial. Infants should remain adequately hydrated at all times, as dehydration is detrimental and could be injurious to the kidney. Medications that have potential risk to the kidney such as prolonged use of aminoglycosides or high-dose non-steroidal anti-inflammatory drugs, should be avoided. Frequent urinary tract infections and hematuria will be documented during the BABY HUG trials, as these findings may reflect or cause renal damage.

Painful Events (vaso-occlusive or dactylitis): Parents and caregivers will be educated about dactylitis and painful events as part of their education about sickle cell disease. They will be taught to push fluids and use ibuprofen and/or acetaminophen with codeine at home. Small supplies of both analgesics should be prescribed for home use at routine clinical visits. They and caregivers will be educated about dactylitis and painful events as part of their education about sickle cell disease. The definition of a painful event is an event lasting two hours or more without obvious cause requiring the use of one or more doses of non-steroidal or narcotic pain medication. Events treated as an outpatient (including emergency room) or requiring admission will be reported at the next Clinical Center contact and included in data entered. Events requiring admission will be reported to the Medical Coordinating Center on appropriate form, and supporting documents collected for central review.

Acute Chest Syndrome, Aplastic Crisis, Priapism: All events meeting defined criteria will be reported to the Medical Coordinating Center on Event Report Forms. Clinical management, including the need for simple or exchange transfusion will be at option of each Clinical Center. Chronic transfusion therapy, an option that would remove the patient from study treatment, should be discussed with the Operations Committee prior to implementation.

Parents will be educated about signs of acute chest syndrome (fever, respiratory symptoms) and aplastic crisis (pallor, decreased energy) at regular clinic visits and asked to seek medical attention should they occur. Parents of male children will be taught about priapism, a prolonged painful erection of the penis, and asked to seek medical attention, if the episode persists beyond two hours. Briefer episodes of priapism will be recorded at the next clinic visit.

Neurologic Events: Families will be taught standardized definitions of TIA, stroke and other neurologic events and reminded at each clinical contact to call the Clinical Center immediately if the child is not able to move arms or legs (unrelated to pain), has facial drooping or dysarthria. Each Clinical Center will promptly evaluate such patients. The minimum evaluation must include documentation of complete neurologic examination, preferably by a neurologist. If the neurologist suspects stroke or TIA clinically, neuroimaging including MRI/MRA must be done. If a stroke is confirmed, acute management will be at the preference of the Clinical Center. Transfusion timing and technique (exchange or simple) will be at Clinical Center preference. Chronic transfusion therapy, an option that would remove the patient from study treatment, should be discussed with the Operations Committee prior to implementation.

If transcranial Doppler (TCD) screening is part of an individual Clinical Center's standard care, such testing should be offered to patients enrolled in BABY HUG as per usual practice. TCD screening is not yet universally available or standardized for children less than two years of age. If screening is performed and values are persistently elevated in children over 2 years of age, the parents should be offered chronic transfusion therapy for the child in accordance with standard practice at each Clinical Center. Patients for whom chronic transfusion therapy is considered should be discussed in advance with the Operations Committee.

10.2.5 Laboratory Specimen and Data Collection

Blood specimens will be collected at designated follow-up visits by the Clinical Center staff. A finger/heel stick or venipuncture may be performed for routine hematology evaluations. The blood specimens will be analyzed locally (and every six months specimens will be shipped to the Hematology and Biochemistry Core Laboratory by overnight courier). Results of blood counts and other blood studies performed locally used to monitor for hydroxyurea toxicity (bone marrow depression) will be available within 24 hours of collection. Every six months, blood will be sent for evaluation of liver function (ALT and bilirubin) and for renal function (BUN/Creatinine). The MCC will obtain blood test results from the local laboratories daily and will review the studies to determine the dose recommendation for hydroxyurea or placebo. Dosage adjustments or temporary treatment stops will be performed for patients assigned to placebo so that patients and Clinical Center staff will be blind to treatment assignments. The Pharmacy Distribution Center (PDC) will ship hydroxyurea and placebo supplies to the Clinic Center for each patient on a regular basis. No patient can receive a new drug prescription until a blood specimen has been analyzed locally. Blood specimen containers and mailers will be provided by the Hematology and Biochemistry Core Laboratory. The MCC will notify Clinical Centers within 48 hours of receipt of blood count to issue directions to stop study treatment (either hydroxyurea or placebo). It will be the responsibility of the Clinical Center to notify patients of stop orders.

10.3 PATIENT COMPLIANCE AND MANAGEMENT

The major obstacle to successfully completing follow-up procedures in BABY HUG will be ensuring adherence with the study protocol, and in particular, study visits. The major responsibility for this task rests with each Clinical Center. MCC staff will assist the Clinical Center staff with schedules for follow-up, identification of patients whose follow-up is interrupted, and notification of outstanding forms/data. If a patient misses three consecutive appointments, this patient will be considered inactive in the study, and study treatment will be temporarily stopped. The patient will become active again and resume study treatments only if the Clinical Center Principal Investigator

can explain the missed visits and provide a plan for more complete participation in the future. Extra effort will be made to increase Protocol adherence for any patient who misses four or more visits (not consecutive) in a six-month period.

Strategies to increase contact with BABY HUG patients may include:

1. providing comprehensive care for management of the complications of sickle cell anemia, including hydroxyurea therapy;
2. maintaining availability through telephone contact with the patients' parents;
3. promoting accessible, positive contact with the Patient/Family advocate at any time during the study (see Section 3.1 and Exhibit 3-1);
4. maintaining up-to-date, accessible records of BABY HUG patient enrollees and locator information including home address, telephone numbers;
5. fostering supportive relationship with BABY HUG families;
6. providing transportation reimbursement and meal vouchers ($30.00 per visit); and
7. providing assistance with telephone service ($30.00 per month) or providing pager system access for families.

If a patient leaves the study early (before 24 months of treatment) for any reason, every attempt should be made to secure the exit studies at that time. The studies in order of priority are Tc^{99m} sulfur colloid liver-spleen scan, blood specimen collection (for pitted cell count, and fetal hemoglobin determination), and abdominal ultrasound. If the patient plans to move to an area served by another BABY HUG Clinical Center, the family should be offered the opportunity to transfer care to the other Clinical Center. If they accept, the child will not need to become inactive in the study. Subsequent follow-up and evaluations will be at the new Clinical Center and reimbursement will be transferred to the new Clinical Center.

10.4 LONG-TERM FOLLOW-UP

10.4.1 Introduction

At the end of the study treatment each family will be asked to agree to return for long-term follow-up visits. If NHLBI and NICHD cannot fund this long-term follow-up, the investigators plan to follow their subjects at the respective clinical sites for at least ten years. Hydroxyurea therapy is part of the accepted management of adults, adolescents and school age children with severe courses from their sickle cell disease. Two limited studies observed no additional toxicity from the use of hydroxyurea by children from 6 months to 5 years of age for periods up to 4 years. However, large numbers of persons with sickle cell disease of any age have not been followed for long periods of time (decades) after initiation of hydroxyurea therapy.

Although a survival advantage has been suggested for severely involved adult patients who took hydroxyurea in the MSH, it is unclear if the same benefit will occur in children, particularly those prescribed the medication before the onset of a severe clinical course. Further, children must continue to grow and develop toward adulthood. Problems from early use of hydroxyurea might only become evident years after the completion of study treatment in BABY HUG. Thus, very young children with sickle cell anemia must be carefully assessed longitudinally for all potential toxicities, including oncogenic potential of this drug, to accurately determine the risks of hydroxyurea use.

If benefit is demonstrated from early hydroxyurea therapy, it will be important to determine how long that benefit continues. Thus, clinical ascertainment and structured evaluation should be a part of the long term follow-up of this unique group of patients. Clinical care after the two-year study treatment might or might not include use of hydroxyurea regardless of initial random treatment assignment. An additional five to ten years of follow-up will provide important information to physicians and parents of very young children with sickle cell anemia, with the goal to follow these subjects for 5 to 10 years after the end of their participation in the randomized clinical trial if their parents and the subjects are willing.

10.4.2 Follow-Up Data Collection

Data will be collected for all BABY HUG patients whose families agree to continue in follow-up. A separate protocol and consent form will be developed for long-term follow-up. Consent will be obtained after the end of treatment (the full, planned two-year course or earlier termination) in BABY HUG. If NHLBI and NICHD cannot fund this long-term follow-up, the investigators plan to follow their subjects at the respective clinical sites for at least ten years. All families would be encouraged to participate in this portion of the study. Participating families will remain in follow-up even if they decline specific follow-up evaluations.

10.4.3 Follow-Up Procedures

Standardized data collection forms will be submitted by the BABY HUG follow-up coordinators at the times specified using the BABY HUG Internet data collection system. Even if families decline to participate in specific follow-up testing or move from the area, the coordinator will attempt to contact the family at intervals indicated to obtain information on clinical events (major events and unusual events), growth, height, weight and head circumference, vital status and use of hydroxyurea. With the family's consent, this will include information from other health care providers.

10.5 DEBRIEFING CONTACTS

After final BABY HUG treatment data have been collected and final reports on treatment 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 their individual treatments, the primary results of BABY HUG and the recommendations of the investigators. At the end of long-term follow-up, the families will be informed of the results of long-term follow-up and any reconsideration of the recommendations of the investigators.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 11

CLOSE-OUT PROCEDURES

11.1 OVERVIEW

Assuming no early stop of the study for reason of either efficacy or safety, at 24 months of randomized study treatment for each patient, individual patients will have study treatment discontinued and their families will be asked to agree to return for visits for the duration of BABY HUG to allow collection of information on each child's growth, health and well-being up to five years. After the last patient enrolled has completed 24 months of randomized treatment, all patients' families (i.e., Feasibility and Safety Pilot Study and all other patients' families) will be given information on their children's assigned treatments and on the findings of the study, as will be determined by the Steering Committee, the Data and Safety Monitoring Board, the National Heart, Lung, and Blood Institute and the NICHD.

11.2 DURATION OF RANDOMIZED, BLINDED STUDY TREATMENT

Patients will be followed on randomly assigned study treatment for 24 months. At 24 months, patients' randomized, blinded study treatment will be discontinued. Patients will continue being monitored for major medical events (see Section 10.2.1) through their regular pediatric hematology care provider, who is expected to be in the BABY HUG Clinical Center, affiliated with the BABY HUG Clinical Center, or by previous agreement the child's regular pediatrician. If the patient changes primary provider, Clinical Center staff will maintain contact with the family and if necessary through the family with the new primary provider. The discontinuation of study treatment at 24 months on study and the need to maintain contact up to the common termination will be explained to the parents at the time that informed consent is obtained at study entry. The explanation will be repeated in the course of the study and at the individual patient's 24-month visit. Contact with the family will be maintained to assure that monitoring for medical events will continue, and that the family can return for final close-out when study results will be made available.

Patients' families and physicians will not be unblinded at the 24-month end of treatment visit. Neither blinded study treatment nor open-label hydroxyurea will be provided by BABY HUG for enrolled patients after 24 months. An assessment of the use of hydroxyurea for sickle cell anemia in this age group will await final study results and a review of those results by the BABY HUG Data and Safety Monitoring Board, Steering Committee, the NICHD and the National Heart, Lung, and Blood Institute (NHLBI). Labelling for use of hydroxyurea in this age group will depend on the determination of the US Food and Drug Administration (FDA) if a supplemental New Drug Application (NDA) is submitted.

11.3 DEBRIEFING CONTACTS

There will be two debriefing contacts. After final BABY HUG treatment data have been collected and final reports on treatment 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 their individual treatments, the primary results of BABY HUG and the recommendations of the investigators. In a second debriefing contact at the end of long-term follow-up, the families will be informed of the results of long-term follow-up and any reconsideration of the recommendation of the investigators.

11.4 FINAL STUDY DATA AND DISSEMINATION OF RESULTS

Data processing and analysis of final study data will proceed on a "time-of-the-essence" basis. The Data and Safety Monitoring Board will review the final data (see Section 4.4.4) including the specified analysis for efficacy and safety at a planned final meeting. A final, consensus recommendation from the DSMB, Steering Committee, NICHD and NHLBI will be shared first with the study patients' families (at which time they will also be informed of their assigned treatments). This recommendation will be made public as soon as possible thereafter.

Submission of final BABY HUG data to the FDA will proceed under the terms of the Investigational New Drug (IND) application filed by the NHLBI at the start of BABY HUG. In the event that HU is found to be safe and efficacious for infants and very young children with sickle cell

anemia, study investigators, the NICHD and the NHLBI will support a submission to the FDA for labeling for this new indication. In the event HU is not found to be safe and efficacious, established procedures for submission of the study data in a final IND report to the FDA will be followed in an orderly fashion.

The data closure upon which the determination of HU's safety and efficacy, if any, is made will form the basis of the final consensus recommendation and informing patients' families of the results. A final data closure, based on cleaned and complete data reporting, will be available for the submission to the FDA, 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 National Heart, Lung, and Blood Institute (NHLBI). Storage of frozen and preserved specimens will be maintained according to the requirements of NHLBI 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 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) PROTOCOL

CHAPTER 12

CONDUCT OF THE TRIAL

12.1 OVERVIEW

BABY HUG is a randomized, double blind clinical trial. Study procedures will be designed and implemented so as to optimize data capture and assure the highest level of quality for the scientific goals of the study while minimizing inconvenience to patients and inefficiencies in the performance of the study. Appropriate mechanisms will be implemented to maintain the study on schedule, provide tools and aids to Clinical Centers and other participating units in the performance of their activities on behalf of the study and ultimately arrive at a study data set that will provide the answers to the study primary objectives and key questions. Patient safety is the overriding concern, while maintaining the integrity of the study, and consideration of the family's efforts in their child's participation. Appropriate checks and monitoring systems will be implemented so that no study patient or his/her family experience any untoward outcome or unnecessary difficulty.

12.2 TIMELINE

The study timeline is presented in Appendix D. Planning and study design, development of the Protocol and Manual of Operations are scheduled for September 2000 through August 2003, including review of the Protocol and model Informed Consent Form by the Data and Safety Monitoring Board and the participating Institutional Review Boards (IRB). Meetings of the Steering Committee and Data and Safety Monitoring Board are scheduled throughout the first year to meet these goals.

Recruitment and enrollment for the Feasibility and Safety Pilot Study began in the fall of 2003. In January 2005, the DSMB authorized immediate continuation of recruitment following the Feasibility and Safety Pilot Study with a goal of 200 patients total. Follow-up will continue through 2008. Patients will be taken off study treatment as they complete two years of randomized

treatment, but will continue to be followed through 2008. Final study data will be collected and cleaned for the next two months, and main study results will be prepared as rapidly as possible for review first with the DSMB, then with the investigators, then with the patients, and then with the public.

12.3 PLANNING AND STUDY DESIGN

The Steering Committee will meet regularly during the first six months of the planning and design period (first year) to write the Protocol and Consent Form. These will be presented to the NHLBI-appointed Data and Safety Monitoring Board. Following suggestions for revisions, the Protocol and Consent Forms will be submitted to individual IRBs for approval. Model consent forms will in many instances need to be modified to include local IRB requirements. Individual institutions' informed consent forms will be reviewed by the Chairman of the DSMB to assure that they conform to the model consent form and they do not allow policies or practices which may be incompatible with BABY HUG.

12.4 TRAINING, CERTIFICATION AND START-UP

After the Protocol and Model Informed Consent Form are approved by the DSMB and the Protocol and individual institutional consent forms are approved by the local IRBs, the study will be ready to begin data collection. A Manual of Operations will be written to give specific performance directions. Training of Clinical Center staff in procedures of data collection and study procedures will be conducted by Medical Coordinating Center staff (MCC). Training will include:

- completion of study forms
- data entry over the Internet
- maintenance of study patient data files
- completion of data cleaning procedures including response to edit queries
- collection, labeling and processing of central laboratory specimens
- patient orientation and information

- obtaining informed consent
- conducting eligibility assessment
- the use of the Automated Telephone Randomization System (ATRS)
- retrieving initial and continuing prescription recommendations as transmitted over the Internet and coordinated with the Clinical Center (Investigational) Pharmacy to dispense study treatments to the patients
- conduct of clinic visits including assessment of patient compliance.

Clinical Center staff who will complete study forms or otherwise provide study data will be certified after successful completion of the training in these areas. Each will receive a unique certification number that will be entered on every study form completed, along with a signature and date of data collection. This certification number identifies the individual who is responsible for the accuracy and completeness of the data collected.

Clinical staff who will be conducting special evaluations will be trained in BABY HUG data collection procedures. Standardized methods for data collection for special evaluations (e.g., neurological examinations or neuropsychological evaluations) have been set by specialists designated by the Steering Committee. Clinical staff will be STE certified and given study certification numbers that will identify the evaluations for which they are responsible.

Members of adjudication panels have been given detailed instructions on the adjudication process and have completed several sample cases designed to test the adjudication process. The MCC prepared these cases in collaboration with designated specialists (e.g., for radionuclide scanning).

Clinical Center Principal Investigators and coordinators must attend at least one training session and successfully complete the certification process, which will include satisfactory completion of practice procedures and data collection with patients. Training is conducted and certification is issued by the Medical Coordinating Center staff after review with the Study Chairman, Vice-Chairman and Project Officer.

12.5 DATA EDITING AND MANAGEMENT

12.5.1 Introduction

The Medical Coordinating Center serves BABY HUG as the repository of all forms, documents and minutes. Clinical Centers will use the Medical Coordinating Center Internet Data Entry System to send to the Medical Coordinating Center data in electronic format from the original of each BABY HUG form, which will be completed and kept in a manner acceptable for regulatory purposes in Clinical Center files. All BABY HUG data collection forms and transmittal lists for blood specimens and other materials (e.g., images) shipped in the course of BABY HUG data collection will be sent to the Medical Coordinating Center via Internet Data Entry. Medical Coordinating Center staff will monitor the arrival of data and transmittal lists to identify form and procedure delinquencies based on appointment schedules and anticipated study forms. Medical Coordinating Center staff will monitor Core Laboratory specimen receipt dates for specimen delinquencies based on appointment schedules, expected specimen collection, and reports of specimens received in the Core Laboratories.

12.5.2 Receipt and Inventory

Medical Coordinating Center staff will receive, log in and store all Internet Data Entry transmissions. Clinical Centers should send specimens directly to the Core Laboratories with Internet Data Entry of transmittal lists to the Medical Coordinating Center. Data from the Clinical Centers will be automatically linked to the date of receipt. Transmittal lists are compared with data received from the Clinical Center and data transmitted from the Core Laboratories. Any discrepancies between crucial patient identifiers (e.g., name code, ID number or date of study entry/follow-up visit) that Medical Coordinating Center staff find will be brought to the Clinical Center's attention immediately by e-mail.

12.5.3 Expected Receipt of Forms

The expected dates for receipt at the Medical Coordinating Center of the BABY HUG patients' form data will be: one week after each visit for eligibility screening data; one week after their study entry visit for Treatment Initiation Forms; two days after clinic visit for Core Laboratory Reports and Missing Specimen Lists; two weeks for Follow-Up Visit Forms or Missed Visit Forms; two weeks for Clinical Event Report Forms; and one week for Imaging Study Forms. Data not sent to the Medical Coordinating Center or specimens not sent to the Core Laboratory within two weeks of the expected date will be denoted as delinquent.

Guidance to assist the questioner in obtaining the information (QxQs) for each of the forms is posted on the Website for ease of use by the Clinical Center staff. Practice with data entry and discussion of the fine points of data entry for neuropsychological evaluations have made clear to the Clinical Center staff the extent of effort necessary for intelligent, single entry of the BABY HUG data. The Internet Data Entry System has been developed to accommodate timely data entry and preliminary edit at the time of data entry so that the time critical information necessary to keep the BABY HUG patients on study treatments and adequately monitored for safety is available. Print back capability for Clinical Center verification and sign off as index document is provided by the Internet Data Entry system. The system allows for data entry at a rate of 3600 characters per hour (most people key at this rate easily). At this rate, 200 binary (or single field) variables can be entered in three minutes. The regular follow-up visit form (Form 31), has 131 keystrokes to the form and takes approximately 1.5 minutes to key. The items entered from a form are registered in the central data base immediately after entering the data. Critical forms such as the Form 31 must be entered on a more timely basis than other forms. In the event of a Clinical Center burden management problem the MCC recommends that a two-tiered system for data entry be established. Critical forms such as the Form 31 will be entered first and lower priority forms will be entered when more time is available for the coordinator (or when another designated data entry person is available) to enter the lower priority forms. To cover for the possibility that BABY HUG Clinical

Center staff are unable to transmit time critical information because of equipment failure (e. g. , the server at the Medical Coordinating Center or the server at the Clinical Center is down), a dedicated fax line in the BABY HUG Coordinating area has been established. This fax line is used for BABY HUG transmissions only. Clinical Center staff will fax their completed, time critical forms to the Medical Coordinating Center where they will be processed electronically, or if need be, manually to maintain the processing of time critical information. Information that is not time critical (e.g., annual neurology exams) can be keyed by Clinical Center staff when full functionality is restored. BABY HUG Clinical Centers that participate in the Children's Oncology Group (COG) already have experience with Internet data entry and note that this backup plan offers a level of security and assurance that is not available in other studies that delay Clinical Center data transmissions until all systems are functioning and data processing staff are available following any interruptions. In the event of national emergencies (e.g., concerted attack on the Internet, communication systems or air transportation), contingency plans will be written so that whatever communications and specimen transportation systems that can be used will be. In the short term if fax lines are working, all critical information may be sent by fax to the Medical Coordinating Center for data entry. Disruptions of specimen transportation may require short term stop-orders interrupting individual study habits for brief periods until central safety monitoring can be reestablished. Long term disruptions will have to be addressed with reconsideration of design. Long term failure of Internet data entry will also be addressed with negotiated design changes such as installation of distributed systems in the Clinical Centers or central data entry of forms transmitted to the Medical Coordinating Center by the fastest available method (e.g., fax or courier). In the event that certain other aspects of data entry are found to be highly burdensome to the coordinators, they will be discussed on the coordinators' bi-monthly conference calls and their concerns presented to the Operations Committee and as appropriate to the Steering Committee and addressed by the Medical Coordinating Center and other members of the Steering Committee.

12.6 MONITORING PROGRESS AND PERFORMANCE

a. Clinical Centers

Clinical Centers will be monitored individually and in the aggregate for unexplained lag in recruitment, missed visits, missed blood specimen collection, missed scheduled procedures, missing expected forms, agreement between treatment dose recommended and dose dispensed, study treatment not accounted for, medical events required to be reported, and routine adverse events. Weekly lists of expected visits, blood specimens, and scheduled procedures, will be posted on the web site for each enrolled patient. Forms and procedures expected but not reported within three days and treatment status not reported within one day will be noted on the website and added to a cumulative delinquency report. Some delinquencies may be corrected at a later date. Other delinquent information may not be recoverable after the critical time period has elapsed. Unrecoverable delinquent data will be counted in summary reports as Protocol deviations for the time-critical aspect. For instance, if a stop order is issued but is not reported as having been implemented for three days, the database will show the day it was reported implemented, but the time-lag will remain on record as a minor Protocol deviation. Certain events such as adverse events and stop orders not implemented will not be allowed to continue unresolved. The Study Chairman will be notified within 48 hours of a stop order not implemented or serious adverse event(s).

1. Eligibility

Status regarding eligibility criteria will be entered into the database for all consented patients. Their progress will be posted daily on the web site until they either are disqualified or are fully qualified. If they have a reversible eligibility disqualification, this will be noted. Aggregate reports will be generated weekly throughout recruitment showing, according to Clinical Center, the numbers of patients who are eligible, those disqualified and why, those qualified, those randomized and enrolled, and those in follow-up. Steering Committee members will monitor these accruing

reports and make recommendations to improve performance where needed or recommend changes in procedures.

2. Follow-Up Clinic Visits

At every follow-up visit, the form will require a report of specific events (see Chapter 4) and any unusual conditions or occurrences (write-in). The Clinical Center investigator will be required to review this and provide an assessment of whether an adverse event has occurred. Since this is an IND monitored study, the usual definitions of adverse event may be implemented, such as the occurrence of death or a life-threatening condition or an event that causes or prolongs hospitalization.

3. Study Treatments

Monitoring study treatments will be one of the most important activities of the study. Study forms will require complete accounting of study treatments to verify that each patient received his/her correct bottle with the correct dose, and that treatment was administered daily. If scheduled, blood specimens must be collected for a patient to continue taking prescribed study treatment. If blood results (CBCs) do not arrive from the local laboratory at the Medical Coordinating Center when expected, a stop-treatment order will be issued immediately and automatically. At every study visit treatments are expected to be returned, and they will be assessed for compliance by measurement of residual treatment returned. Treatment errors will be reported to the Study Chairman immediately and will be counted cumulatively, if they represent major Protocol violations. Study policies regarding remedial action on repeated treatment regimen violations will be implemented.

4. Monitoring the Clinical Centers

Medical Coordinating Center staff will produce recruitment reports weekly during the recruitment phase from the data entered from forms submitted for each entered patient. A sufficiently low recruitment performance will be responded to by a site visit from the Study Chairman or Vice-Chairman, Medical Coordinating Center staff, and NHLBI and NICHD staff. Failure to improve performance after such a site visit may result in an end to support for recruitment in a Clinical Center.

On a bi-weekly basis, the Operations Committee (Study Chairman, Vice-Chairman, Medical Coordinating Center staff, Coordinator representatives, Steering Committee representatives and NHLBI and NICHD staff) meet by conference call to review recruitment goals and Protocol violations reported for each Clinical Center. Protocol exceptions for eligible patients will be considered by the Operations Committee and may be granted as long as study integrity is not compromised. If the committee arrives at a recommendation to grant an exception to the Protocol, the local IRB will be notified and the exception will be conducted in accord with the local IRB's Standard Operating Procedures. Clinical Centers will be notified of minor violations with suggestions for remedial action. Major violations will result in a site visit by the Study Chairman or Vice-Chairman, Medical Coordinating Center staff and an NHLBI and NICHD staff member.

At each scheduled Steering Committee meeting, a report of progress toward accomplishment of study goals will be presented, both for BABY HUG as a whole and for individual Clinical Centers. These reports will include certification status, number of eligible patients identified, number of patients enrolled of those identified, completeness of scheduled visit data collection, completeness of specimen collection, completeness of clinical event reporting, adherence to Protocol and results of actions taken to improve compliance.

5. Site Visits

Clinical Centers will be visited during the study to assure quality of data collection. Medical Coordinating Center staff will generate computer printouts of form data for comparison to Clinical Center form copies and to actual patient charts.

If a Clinical Center is under consideration for being discontinued from treatment of patients in BABY HUG, the Study Chairman or Vice-Chairman, Medical Coordinating Center staff and NHLBI and NICHD Project Officers will visit the Clinical Center and provide a site visit report to the Data and Safety Monitoring Board (DSMB) for recommendation on final action.

Clinical Centers with the greatest difficulty in meeting proposed goals for recruitment may also be site visited, and recommendations for improvement made, with a report to the DSMB.

b. Central Facilities

The MCC will monitor routinely receipt of expected reports from the Core Laboratories and treatments shipped from the Pharmacy Distribution Center. Any expected blood result that is not received will be tracked by MCC staff by telephone with the Clinical Center and the Core Laboratory. Because many courier shipments can be traced on the Internet, there should be a rapid resolution to any questions about shipments lost in transit.

Quality assurance programs will be established to monitor the results from the Core Laboratories. In addition, the Core Laboratories will be chosen for their high level internal quality assurance programs. Hematology and Biochemistry Core Laboratory values will be monitored both for range and for consistency with the child's laboratory measurement history. Values that are deemed "alert" values will be discussed immediately between MCC staff and Hematology and Biochemistry Core Laboratory staff. If the value is confirmed, the alert process will be implemented. Table 7-3 shows the lab alert values.

Treatments dispensed by the Clinical Center (Investigational) Pharmacies will be checked for agreement with the treatment kit number assigned to the child, as well as the recommended prescription. The Clinical Center Principal Investigator will be notified immediately of any

discrepancies. Confirmed discrepancies will be tallied cumulatively. Pharmacies accruing unacceptable numbers of discrepancies will be reported to the Study Chairman for immediate action.

c. Endpoint Evaluation Committees

Members of endpoint evaluation committees will receive appropriate guidelines for the purpose of performing study assessments. They will first adjudicate sample cases. During the conduct of the study, various methods of quality assurance will be implemented including masked duplicate case reviews, both within-reviewer (reader) and between-reviewers (readers). Results of these reviews will be tabulated according to Clinical Center and reviewer. Between and within reviewer agreement rates will be available to the DSMB. The schedule of the reviews or readings of adverse event reports and images will be monitored and reported routinely to the Steering Committee. Reviewers or readers who fall behind in their assigned study work will be contacted by the Study Chairman and MCC Principal Investigator to assess the situation and propose a plan of action. Repeated failure to perform may result in replacement of a reviewer (reader).

d. Medical Coordinating Center

1. Randomization

The randomization schedule will be devised to assure the appropriate blocking and balance of treatment groups for clinically important clinical patient characteristics and within each Clinical Center. Two schedules will be devised for use: one for Clinical Center staff to practice using the ATRS, and one that will be the final study randomization schedule. The final schedule will also be analyzed to assure that balance is achieved by age and Clinical Centers, and that there are no unusual sequential runs of treatment assignments within any Clinical Center.

2. Laboratory Results, Prescription Recommendations and Record of Treatments

MCC staff will develop computer programs to take into account each patient's baseline laboratory values, new laboratory data and recent laboratory values. These programs will identify extreme outliers for possible safety alerts (see Table 7-3), and will be used to assess whether changes from baseline or recent results are unusual. Outlier results will be verified and reviewed if necessary with the Study Chairman. Extreme values that constitute an adverse event will be managed according to specified procedures. Abnormal values that do not exceed levels will be used in formulating treatment recommendations. The system will follow the dose adjustment Protocol (see Chapter 6) to make the appropriate dose recommendation for the next 2- or 4-week period. Dose recommendations will be reviewed along with the attending laboratory results by the designated medical staff at the MCC. Confirmed treatment recommendations will be forwarded to the Clinical Center electronically (e-mail or Website).

All occurrences of misreported laboratory results will be cumulated and reported, as well as all alert values, and routine dose changes.

MCC staff will review accruing information about reported toxicities and their resolution and consequent dose adjustments to see if the frequency of adjustments is reasonable or if patients are being stopped too frequently. Tabulations of how much of the time patients are not maintained on assigned treatment will also be reviewed. Placebo stops and adjustments will be compared for similarity to the hydroxyurea group to help maintain the blind.

3. Data entry and database quality assurance

Because data entry will be from the remote sites, data quality assurance will derive from site visits that will include complete audits of the data against the medical record. Periodically copies of original forms may be requested from Clinical Centers for independent data entry and comparison in the Medical Coordinating Center to check the quality of Clinical Center data entry.

12.7 ROUTINE REPORTING

Routine reports for the Steering Committee will be prepared on: eligibility and enrollment, by Clinical Center and overall; recruitment expected versus actual; study forms and specimens, expected and received; unusable results, alert values, values requiring verification, values modified, values deleted, and stop orders issued on the basis of unavailable results; treatment adjustments, dispensing, return of and compliance with study treatments; special studies expected and performed or not performed; endpoints evaluation; committee performance (e.g., case reports and images reviewed and cases pending); and adverse events by Clinical Center and overall.

12.8 SAFETY AND PATIENT MONITORING

a. Central Review of Laboratory Results

A Medical Coordinating Center physician will confirm all toxicities and alert values and review individual cases and tabulations of toxicities and alert values.

b. Adverse Events

Any adverse event, regardless of attribution to study treatment or sickle cell disease will be reported to the Medical Coordinating Center. Any event meeting the definition of a serious adverse event will be reported to the DSMB Chair, Executive Secretary of DSMB, the NHLBI and NICHD Project Officers and to the FDA. The Clinical Centers will report all occurrences of serious adverse events that occur at their institution according to their local IRB requirements.

c. Data and Safety Monitoring Reports and Meetings

The DSMB will meet regularly to review cumulative study data. In addition to the routine performance reports, they will review data according to the assigned groups. Interim review of the primary and secondary endpoint data with statistical testing will be presented according to the interim monitoring plan (see Section 4.4.5). All cases of adverse events and laboratory alert values will be reviewed in detail. The DSMB will make recommendations to the NHLBI and NICHD regarding recruitment goals, study performance, and patient safety.

d. Unblinding

In the event of an emergency that the Clinical Center Principal Investigator or that a local treating physician considers knowledge of the BABY HUG patient's treatment assignment is necessary for determining patient management, the Principal Investigator or treating physician will telephone the BABY HUG Study Chairman to discuss the need for unblinding. The Study Chairman will be able to use the Automated Telephone Response System (ATRS) to obtain unblinding information 24 hours/day as necessary.

12.9 PROTOCOL VIOLATIONS

Staff of Clinical Centers, Core Laboratories, Endpoint Evaluation Committee members, Medical Coordinating Center personnel and any other personnel who implement the study Protocol, manage patients on behalf of the study, report, manage or analyze study data share in responsibility for patient safety and the scientific integrity of BABY HUG. With the assistance of oversight provided by the NHLBI and NICHD through the Data and Safety Monitoring Board and the local Institutional Review Boards (IRBs), all matters of Protocol implementation and data reporting will subject to review. Procedures for monitoring of scientific integrity and quality assurance in multicenter trials have been promulgated by the Society for Clinical Trials (Knatterud et al, 1998). Violations of the Protocol will be investigated promptly to maintain patient safety.

The steps to prevent Major Protocol Violations or reporting of incorrect data are: training and certification, site visits, audits, statistical checks, edit checks, and careful follow-up of any abnormalities that may result in clinic-wide treatment stop and suspension of randomization.

Data collection by non-certified personnel will be prevented by training and certification (including education that misuse of certification number is a scientific integrity issue), detected by review of study forms all of which require signature, certification number and date, and if found, acted upon by clinic-wide audit; deletion of data found erroneous; and requirement of assurances and a plan from the Clinical Center Principal Investigator to keep the problem(s) from recurring.

Failure to provide a Patient/Family advocate will be prevented by training and certification, detected by review of initial and follow-up Patient/Family advocate forms, site visits, and data audit, and acted upon with clinic-wide treatment stop and suspension of randomization.

Requesting enrollment (randomization) for a child who does not meet eligibility criteria will be prevented by training and certification, detected on checking eligibility data collection forms, and acted upon with suspension of randomized study treatment and randomization.

Requesting enrollment (randomization) for a child on whom informed consent has not been obtained, or whom the Patient/Family advocate has not found the family in agreement will be prevented by training and certification, detected on site visit, and acted upon with suspension of randomized study treatment and randomization.

Failure to act to implement a treatment stop order within 72 hours will be prevented by training and certification, detected by review of study treatment data collection forms and Patient/Family advocate follow-up and acted upon with suspension of randomized study treatment and randomization.

Failure to report a serious adverse event (Section 10.2.3) within 24 hours of learning of event will be prevented by training and certification, detected on review of study treatment data collection forms and Patient/Family advocate follow-up, and acted upon with suspension of randomized study treatment and randomization.

12.10 IRB APPROVAL

Clinical Centers will submit the BABY HUG Protocol and the Informed Consent Form as modified for use to the Local Institutional Review Boards (IRBs) for initial review before any patient can be screened for BABY HUG. Clinical Centers will submit local updates to the IRB for annual review, Protocol amendments and study-wide reports such as DSMB recommendations and any other information that may be requested by the IRB. Clinical Centers will comply with local IRB requirements regarding reporting serious adverse events, whether or not related to study treatment. Clinical Centers will provide the Medical Coordinating Center with documentation of approval from

the IRB. The approved local consent form will be sent to the NHLBI and submitted to the DSMB Chair for review to assure that it conforms to the requirements for protection of human subjects.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 13

ORGANIZATIONAL STRUCTURE AND PARTICIPATING UNITS

13.1 INTRODUCTION

The Pediatric Hydroxyurea Clinical Trial (BABY HUG) will be conducted in 14 Clinical Centers, a Medical Coordinating Center and central units. The Clinical Center staff will be trained in accordance with the procedures set out in the study Manual of Operations. The objective is to standardize all study procedures carried out in the Clinical Centers and at the operational central units.

Study monitoring will be carried out by the Data and Safety Monitoring Board (DSMB), Steering Committee and Operations Committee. Monitoring will include adherence to protocol, achievement of recruitment goals, patient safety and efficacy of treatment.

Outcome reviewers in the specialties of neurology, neuroradiology, neuropsychology, nuclear medicine and pediatric hematology will evaluate imaging studies and reports of possible outcome events as members of an Endpoints Evaluation Committee to ascertain selected components of the primary and secondary study endpoints. The Steering Committee will review and approve or disapprove Operations Committee recommendations on proposals for secondary analyses and ancillary studies.

An organizational chart for BABY HUG is presented in Exhibit 13-1.

13.2 PARTICIPATING UNITS

13.2.1 Operations Committee

The Operations Committee will comprise the Study Chairman, the Vice-Chairman, the Principal Investigator of the Medical Coordinating Center, the NHLBI and NICHD Project Officers, two Clinical Center Principal Investigators (rotating every six months), the Coordinator Chair and two Clinical Center Coordinators (by election) and, ex officio, the directors of the Pharmacy

Distribution Center and of the Core Laboratories, and the Medical Coordinating Center Deputy Director.

The Operations Committee will maintain close ties with the Clinical Centers. The Operations Committee will provide technical and scientific guidance in developing the Protocol and Manual of Operations, study forms, Clinical Center procedures, quality control systems, study treatment titration and distribution procedures, and laboratory specimens preparation and processing. The Operations Committee will implement study procedures to address major or minor protocol violations. The Operations Committee will help prepare agendas and contents of reports for Data and Safety Monitoring Board and Steering Committee meetings.

In addition, the Operations Committee receives and reviews all scientific proposals for use of study data, including ancillary studies. Their considerations in evaluating proposals will include scientific merit, feasibility and resource availability, including statistical, computing and technical support. No ancillary study will be approved which interferes with the conduct of the overall study or is not approved by the Steering Committee and Data and Safety Monitoring Board.

13.2.2 Clinical Centers

The collaborating centers are funded by contracts from the NHLBI. At a minimum, each will have a Principal Investigator and a coordinator. Exhibit 13-2 lists the Clinical Centers identified at the start-up of the study.

A final recruitment report specifying the number of patients enrolled by each certified Clinical Center will be distributed after the end of enrollment.

13.2.3 Study Coordinator Committee

One BABY HUG study coordinator (Coordinator Chair) will be selected to have responsibility for organizing all the BABY HUG study coordinators into the Study Coordinators Committee - SCC. This person's responsibility will include:

1. foster enthusiasm for the BABY HUG project;
2. act as a liaison between the Steering Committee and the SCC;

3. coordinate regular SCC conference calls; and

4. organize SCC meeting agenda and SCC project reports.

The SCC's responsibility will include:

1. development of coordinator writing projects;

2. attending Steering Committee meetings; and

3. participating in SCC conference calls to

   a. report enrollment progress,
   b. collaborate on enrollment successes/problems,
   c. discuss adherence strategies,
   d. team build, and
   e. develop writing plans.

13.2.4 Core Laboratories

The Core Laboratories have responsibility for receiving blood samples from the Clinical Centers and performing specimen analyses as required for monitoring effects of hydroxyurea. Effects on blood counts will be used to titrate study drug dosages. In addition, the Core Laboratories will perform other analyses such as analyses for fetal hemoglobin and chromosome breakage.

13.2.5 Pharmacy Distribution Center and Investigational Pharmacies

The study treatments (hydroxyurea and placebo) for BABY HUG will be distributed to Clinical Center Investigational Pharmacies by the Pharmacy Distribution Center. Clinical Center Investigational Pharmacies will maintain records of all patient prescriptions and dosages dispensed for each patient visit.

13.2.6 National Heart, Lung, and Blood Institute

The National Heart, Lung, and Blood Institute (NHLBI) staff -- Office of Blood Diseases Program (Division of Blood Disease and Resources) and Office of Biostatistics Research (Division of Epidemiology and Clinical Applications) will participate with study investigators and key study personnel in all phases of the study. A member of the Blood Diseases Program (Division of Blood Diseases and Resources) will serve as a voting member on the Steering Committee, and other

study committees as appropriate. NHLBI staff on the Steering Committee will participate throughout the phases of Protocol development, recruitment, follow-up, data analysis and interpretation.

The NHLBI staff will address issues concerning recruitment, treatment, follow-up, quality control, and adherence to Protocol to assist the study investigators in assessing potential problems affecting the study and potential changes in the Protocol. They will provide direction in the management of the contracts which fund the study, and assistance in developing solutions to major problems such as insufficient participant enrollment. A Data and Safety Monitoring Board has been appointed by the NHLBI to provide overall monitoring of the study.

13.2.7 Medical Coordinating Center

The Medical Coordinating Center staff will include the Principal Investigator/Medical Coordinating Center Director, Project Manager/Deputy Director, statistician(s), computer programmer(s) and coordinator(s). Medical Coordinating Center staff for BABY HUG will provide expertise in the areas of study design, quality control, data processing and data analysis. Medical Coordinating Center staff will provide biostatistical and epidemiological advice for the overall conduct of BABY HUG; collaborate with the BABY HUG investigators in all phases of the study including planning, participant recruitment and follow-up, development and maintenance of a data management system for BABY HUG, preparing required statistical analyses; generate Core Laboratory work lists, report forms, blood specimen transmittal lists, and progress reports; and, assist in the preparation of manuscripts for publication. Medical Coordinating Center staff will undertake the primary responsibility for the collection, processing, storage and analysis of the study data, as well as cooperating with the Operations Committee to ascertain that the provisions of the Protocol are carried out by each Clinical Center.

13.2.8 National Institute of Child Health and Human Development

A Memo of Understanding between the NHLBI and NICHD will allow the NICHD to perform pharmacokinetic (PK) studies under the Best Pharmaceuticals for Children Act (BPCA) to support a submission to the FDA for labeling of hydroxyurea for infants and very young children with sickle cell disease. Premier Research Group will be the NICHD's coordinating center and will assist in the design of PK studies and perform data quality control.

13.3 STUDY ADMINISTRATION

13.3.1 Study Chairman and Vice-Chairman

The Study Chairman and Vice-Chairman have been elected by the Steering Committee. The Study Chairman is Chairman of the Operations Committee and Steering Committee. The Study Chairman is responsible for overall conduct of the study and adherence to the study time table (see Appendix D). The Vice-Chairman acts in place of the Study Chairman in case of the Study Chairman's unavailability. Consultants to the Medical Coordinating Center will be available 24 hours a day for emergency unblinding of assigned study medication.

13.3.2 Steering Committee

The Study Chairman will preside over the Steering Committee which will consist of the Principal Investigators from each Clinical Center and the Medical Coordinating Center, the NHLBI and NICHD Project Officers and (ex officio) directors of the central units. This committee will be responsible for overseeing the writing of main papers as directed by the DSMB and as approved by the NHLBI.

13.3.3 Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) has been appointed by the NHLBI. DSMB voting members include experts in sickle cell anemia, the clinical use of hydroxyurea, biostatistics and bioethics, who are not connected with the study, and ex officio (non-voting) members -- the Study Chairman and the Medical Coordinating Center Principal Investigator -- and representatives of the NHLBI and NICHD who will attend meetings to present information and receive recommenda-

tions. The DSMB reviewed the initial study Protocol and will approve all changes made to it during the course of the study, review Data and Safety Monitoring Reports, and make recommendations on major Protocol changes and/or early release of study results. The Operations Committee will report any unexpected or unusual findings to the DSMB which may be convened ad hoc for a special review of BABY HUG any time circumstances so warrant. The DSMB will meet at least yearly, to review the annual BABY HUG report. It will review safety as the trial progresses, will evaluate treatment efficacy at pre-specified interim time points for possible early termination of the study, and will review any proposals to discontinue treatment of patients in a Clinical Center because of non-adherence to the Protocol.

DSMB meetings begin with an Executive Session at which summary notes are taken by a representative of the NHLBI. Other BABY HUG Steering Committee members do not participate in the Executive Session. The Study Chairman, Vice-Chairman, Medical Coordinating Center Principal Investigator and invited BABY HUG investigators join the DSMB for other parts of the agenda until the presentation of study outcome data. The BABY HUG Clinical Center investigators are excused for the study outcome presentation and discussion. Medical Coordinating Center staff take summary notes of the DSMB meeting from the end of the Executive Session through the presentation of study outcomes and discussion. At the end of the presentation of study outcomes and discussion, the Medical Coordinating Center staff are excused for the DSMB to meet in a second Executive Session. The NHLBI representative is responsible for recording summary notes of the second Executive Session and the recommendations of the DSMB. At the end of the second Executive Session, the BABY HUG investigators rejoin the DSMB for a preliminary review of DSMB recommendations. The NHLBI Executive Secretary of DSMB provides the summary notes and recommendations of the DSMB, in an expeditious and timely manner, to the Medical Coordinating Center. The Medical Coordinating Center communicates these recommendations to the BABY HUG Steering Committee. At the next DSMB meeting, the DSMB votes to accept (or revise) the summary notes recording transactions of the meeting and recommendations.

13.3.4 Endpoints Evaluation

Forms, images and records received from the Clinical Centers will be reviewed on a regular basis by committees consisting of experienced clinicians who are familiar with the area of special study evaluations (i.e., liver-spleen scans, MRI, neuropsychological tests and clinical events) and with the spectrum of illness in sickle cell anemia and who have no other connection with this study. They will receive materials for review from and return classifications of reports or other information to the Medical Coordinating Center for incorporation into the study database.

Exhibit 13-1

Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)

ORGANIZATIONAL CHART

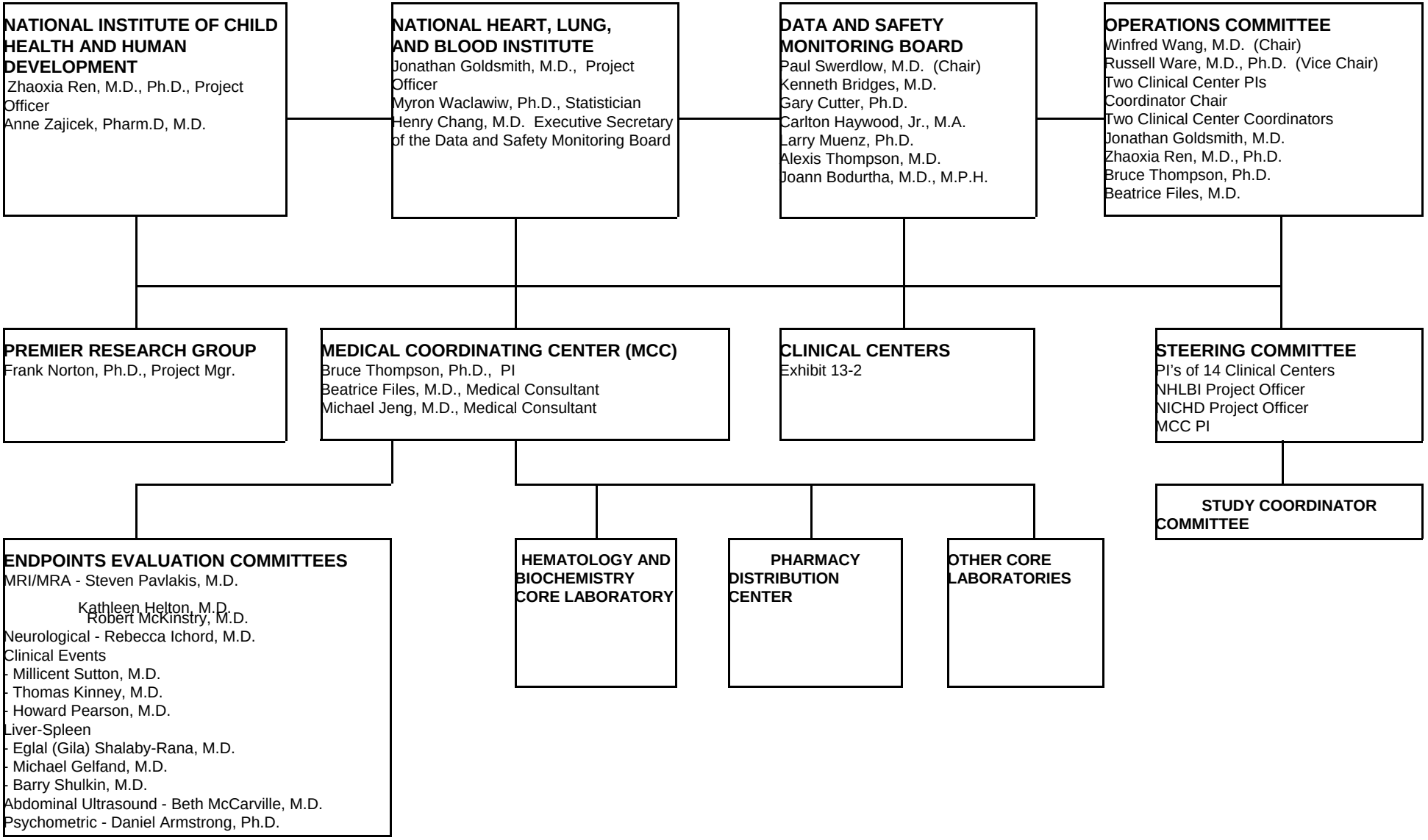


Exhibit 13-2

PARTICIPATING CLINICAL CENTERS

CLINICAL CENTERS

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

Duke University Medical Center, Courtney Thornburg, 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, Ram Kalpatthi, 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, Julio Barredo, 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, Frank Shafer, M.D. (Interim) [Norma Lerner, M.D. (Starting mid-September 2009)] - 12
(Philadelphia, PA)

Emory University School of Medicine/CHOA, R. Clark Brown, 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

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

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG) PROTOCOL

CHAPTER 14

POLICY MATTERS

14.1 INTRODUCTION

Procedural guidelines are established to ensure that all investigators adhere to the protocol, to facilitate optimum use of data generated by the study, and to ensure optimal use of the resources of the Central Units and Medical Coordinating Center (for quality control in the study see Section 10.4).

14.2 QUALITY ASSURANCE

Members of the Steering Committee will create a list of major and minor protocol violations. Major violations are those which endanger patients, such as repetitive failure to obtain scheduled blood counts or failure to discontinue therapy promptly when so advised. Minor violations are those which impede the progress of the study, such as not filing reports in timely fashion (form delinquencies) and excessive delays in supplying materials (e.g, scans, other images or event reports) for central review. Also, the Steering Committee will consider exceptions to the Protocol and advise the Operations Committee of its decision.

After the first major violation, a clinic will be asked to submit a proposal outlining how recurrence will be prevented. After a second major violation, clinics will not be allowed to recruit more patients, but will be able to follow those already recruited. After three major violations the Clinical Center may no longer be supplied with study drug. Prior to suspension of study treatments at a Clinical Center, the Study Chairman or Vice-Chairman, Medical Coordinating Center Principal Investigator and NHLBI and NICHD Project Officers will visit the Clinical Center and provide a site visit report to the Data and Safety Monitoring Board (DSMB) for recommendation on final action. Clinical Centers with the greatest difficulty in meeting their proposed goals for recruitment will also be site visited, and recommendations for improvement made to them, with a report to the DSMB.

(Clinical Centers which are not having problems with performance will also be visited at least once during the study, to assure quality of data produced). The Data and Safety Monitoring Board (DSMB) will be made aware of all major violations, and will consider discontinuing study treatment at the Clinical Center after a third violation.

The Medical Coordinating Center will document minor violations in performance reports, as well as notifying the Clinical Centers of them. Repeated minor violations which are not corrected will result in reports to the Data and Safety Monitoring Board (DSMB), the NICHD and the National Heart, Lung, and Blood Institute (NHLBI).

14.3 CHANGES IN PRINCIPAL INVESTIGATORS

Over the seven year course of the trial, it is expected that changes in Principal Investigators (PIs) will occur in some of the Clinical Centers. These changes may be necessitated by movement of the Principal Investigators to another institution, illness, retirement, or change in responsibility within the same institution. When a change in PI occurs, the viability of the Clinical Center as a BABY HUG participant could become problematic. In this situation, retention of the established/experienced nurse coordinator may help ensure that the Clinical Center can continue to function effectively. When such a change occurs, it is understood that the contractual arrangement between the NHLBI and the Clinical Center will be reviewed and possibly altered. However, because of the profound influence that such a change may have on the remaining Clinical Centers in the conduct of BABY HUG, the members of the Steering Committee and/or the Operations Committee should have the opportunity to discuss and provide input into the decisions that are made.

As noted in Section 14.2, problems in the performance of a Clinical Center will be discussed by the Operations Committee. The Clinical Centers and/or their representatives, the Medical Coordinating Center, and the NHLBI and NICHD Project Offices should all participate in any decisions which involve turnover of Principal Investigators and/or Clinical Centers.

14.4 TYPES OF BABY HUG RESEARCH

BABY HUG research and the resulting presentation and publications may be grouped into the following study categories.

1. Endpoint studies;
2. Data bank studies;
3. Ancillary studies.

The Steering Committee will exercise responsibility for all endpoint, data bank, and ancillary studies, and for all publications and presentations evolving from BABY HUG research, through the Scientific Affairs and Publications Committee. BABY HUG investigators have agreed that all BABY HUG research is collaborative in nature. No investigator will publish BABY HUG data from any one Clinical Center or group of Clinical Centers without the written approval of the Publication Committee, the NICHD and the NHLBI.

Investigators at all BABY HUG sites, including the Medical Coordinating Center and the NHLBI and NICHD Program Offices, have equal status with regard to developing protocols, participating in such studies as are approved and collaborating in the development and publication of research papers based on BABY HUG material.

The procedures in this section for endpoint, data bank, and ancillary studies, and for publication of BABY HUG research results are similar to those used in other cooperative clinical trials. These procedures are intended to protect the interests of all investigators and patients in the trial, namely, to assure that study data conform to the requirements of study design, are accurately presented, authorship is appropriately acknowledged, and the text of all publications is well written with proper attention to the protection of patient privacy. All BABY HUG presentations are subject to review and approval by the NHLBI and NICHD.

14.4.1 Endpoint Studies

An endpoint study is a study pertaining to the fundamental goals of the project (e.g., the evaluation of the efficacy of hydroxyurea in the prevention of chronic end organ damage) or which

involves data, such as treatment assignment, differences in hospitalization by treatment assignment, or mortality rates, which cannot be released prior to the end of the study. These studies will summarize the findings of BABY HUG, based on the entire study population, and will be written at the conclusion of follow-up or data collection.

14.4.2 Data Bank Studies

A data bank study is a study which uses data routinely collected on patients when they are screened for or enrolled in BABY HUG and analyzes these data to answer some scientific question. Data used in this research are not directly related to the fundamental goals of the study.

14.4.3 Ancillary Studies

An ancillary study is a study which uses supplementary data collected on patients who are screened for or enrolled in BABY HUG, over and above the data collection required by the BABY HUG Protocol. Such studies are usually restricted to consideration of a specific test technique or involve only supplemental data collected on some or all BABY HUG patients.

Approval and participation in ancillary studies are considered by the Steering Committee, the NICHD and National Heart, Lung, and Blood Institute (NHLBI) with the advice of independent review committees (the Data and Safety Monitoring Board or the Protocol Review Committee). Proposals for ancillary studies are submitted to the Medical Coordinating Center which distributes them to the Steering Committee for scientific review and Clinical Center Principal Investigator consideration with regard to feasibility and interest in participation in the ancillary study in each Clinical Center. Steering Committee members reply to a ballot distributed by the Medical Coordinating Center indicating their approval or disapproval of the ancillary study, the priority they would accord the ancillary study and whether or not their Clinical Center would participate in the ancillary study. Approval requires a majority vote.

14.5 CLINICAL CENTER ACCESS TO BABY HUG DATA FILES AT THE END OF THE STUDY

At the end of the study, Medical Coordinating Center staff will produce a well documented data tape containing a refined (and reduced) set of the BABY HUG data for the purpose of analysis

by the BABY HUG investigators and eventual release to the public domain in accordance with NHLBI policy. Clinical Center directors may analyze the data on this data tape in their own centers, but prior to submission of articles for publication must submit the analyses proposed for publication to the Medical Coordinating Center, where they will be reviewed and computations replicated. Clinical Center directors who perform their own analyses are responsible for obtaining all support necessary for the data bank or ancillary study outside of regular study resources. The Medical Coordinating Center will be the center of study analysis activities as long as the BABY HUG investigators continue in their collaborative efforts.

14.6 PUBLICATION

The design and the main results manuscripts will list all Principal Investigators and all members of the manuscript writing committees in the authorship. The authors of other publications stemming from the study will be those who actually write the document (the writing committee), plus the group as a whole ("Doe J, Roe K, and the Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) investigators), with all investigators and coordinators listed in the appendix at the end of the paper or reference made to a publication listing all investigators. Study manuscripts may only be submitted for publication for main endpoint studies and approved data bank and ancillary studies. All manuscripts related to study patients must be reviewed and approved by the Publication Committee. The Publication Committee will implement day-to-day policy on publications and authorship in accordance with the directions of the Steering Committee.

14.7 CONFLICT-OF-INTEREST

BABY HUG investigators and their immediate family will not buy, sell, or hold stock options in any of the companies* providing medication (or making competing products) under study from the time the recruitment of patients for the trial begins until funding for the study in the investigator's unit ends and the results are made public; or from the time the recruitment of patients for the trial

*Bristol-Myers Squibb, Par Pharmaceuticals

begins until the investigator's active and personal involvement in the study or the involvement of the institution conducting the study (or both) ends.

Each investigator will agree not to serve as a paid consultant to the companies during these same periods. The guidelines will also apply to the investigator's spouse and dependents. The Medical Coordinating Center will hold and update annually conflict-of-interest statements from each investigator.

Certain other activities are not viewed as constituting prohibited conflicts-of-interest but must be reported annually to the Medical Coordinating Center: the participation of investigators in education activities supported by the companies (permitted only if no honorarium is paid to the investigator); the participation of investigators in other research projects supported by the companies; and, occasional scientific consulting to the companies on issues not related to the products in the trial and for which there is no financial payment or other compensation. The BABY HUG conflict-of-interest policy will incorporate the NHLBI and U.S. Food and Drug Administration (FDA) policies on conflict-of-interest for investigators.

The BABY HUG investigators will not accept any restraint on freedom of publication.

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Appendix A

Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
Medical Coordinating Center

Detailed Schedule of Visits and Total Amount of Blood

Introduction

Over the course of the two-year BABY HUG Clinical Trial, patients will each have a total volume of approximately 77.6 ml of blood collected for evaluations, including 17.1 ml for immunology studies. The largest volume will be obtained in the pre-randomization evaluation period and the first 4 weeks on study treatment, during which time approximately 29.6 ml will be collected. Efforts are made to work with the smallest volumes of specimens possible. For example, Schwartz GFR specimens will be aliquotted from specimens already prepared for other evaluations. Using a conservative estimate of a 12 month old infant entering the study with an initial weight of 8 kg, this amount will constitute approximately 3.7 ml/kg over an 8-week period. This amount is within the guidelines for maximum volume of phlebotomy (3-7 ml/kg per 8 week period), which have been utilized by the St. Jude Children's Research Hospital and Duke University Medical Center Institutional Review Boards (IRBs). The maximum amount obtained at a single blood draw will be approximately 8 ml; this will not exceed the guideline of 1 ml/kg (Duke University Medical Center IRB) in an 8 kg infant and will not produce significant cardiovascular stress. Older children with sickle cell disease have tolerated phlebotomy of 5-10 ml/kg every 2 to 4 weeks (Ware et al., 1999). During the other time periods in the course of the BABY HUG Clinical Trial, blood sampling volumes will be considerably less.

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

Note: This is an ideal schedule with no toxicities and stable dose reached after eight weeks.

	BLOOD SAMPLE COLLECTION (All volumes in ml)								URINE	SPECIAL STUDIES								
WEEKS	Heme & HbF (Local & Central)	Chem†	Electro phor.	Pitted cells	Cyto-genet	VDJ/HJB/ DNA	O2 Satur.	Cystatin C^	Urinalysis/ Urine Concentr (ml)	Liver-spleen scan	Anthropo metry H-height W-weight C-head cir	Abdominal Sono	Neuropsych/ NQ	~~DTPA* Clearance (ml)~~	GFR Schwartz (ml)	HU Assay	Immun ology‡ (ml)	TCD
Screening	1.0*	1.0	1.0	0.1	4.0	3.0	Y		5.0	Y	H, W, C	Y	Y				3.5	Y
Treatment Initiation	1.0*	1.0						Y			W			~~9.0~~	Y	Y		
Titration Schedule - Visits every two weeks until stable dose reached.																		
2	0.5										W							
4	0.5										W					4.0		
6	0.5										W						1.2	
8	0.5										W							
Stable Dose Schedule - Schedule shifts to every four weeks from here until Exit unless otherwise noted																		
12 - 3 MO	0.5						Y				H, W, C							
16	0.5										W							
20	0.5										W							
24 - 6 MO	1.0*	1.0		0.1			Y				H, W, C				Y			
28	0.5										W							
32	0.5										W							
36	0.5										W							
40 - 9 MO	0.5						Y				H, W, C							
44	0.5										W							
48	0.5										W						3.0	
52 -12MO	1.0*	1.0		0.1			Y				H, W, C		Y		Y		1.2	Y
56	0.5										W						0.5	
60	0.5										W						3.0	
64 -15MO	0.5						Y				H, W, C							

	BLOOD SAMPLE COLLECTION (All volumes in ml)								URINE	SPECIAL STUDIES								
WEEKS	Heme & HbF (Local & Central)	Chem†	Electro phor.	Pitted cells	Cyto-genet	VDJ/HJB/ DNA	O2 Satur.	Cystatin C^	Urinalysis/ Urine Concentr (ml)	Liver-spleen scan	Anthropo metry H-height W-weight C-head cir	Abdominal Sono	Neuropsych/ NQ	~~DTPA* Clearance (ml)~~	GFR Schwartz (ml)	HU Assay	Immun ology‡ (ml)	TCD
68	0.5										W							
72	0.5										W							
76-18MO	1.0*	1.0		0.1			Y				H, W, C				Y			
80	0.5										W							
84	0.5										W							
88	0.5										W							
92-21MO	0.5						Y				H, W, C							
96	0.5										W							
100	0.5										W							
104-24MO	1.0*	1.0		0.1	4.0	3.0	Y	Y	5.0	Y	H, W, C	Y	Y	~~9.0~~	Y	Y	4.7	Y

*HbF will be assessed on aliquots from blood collected at these times. Baseline includes two measurements, one during qualifying visits and one at the start of study treatment.

†Ferritin, electrolyte and magnesium levels will be measured every six months from an aliquot of the chemistry specimen.

^Cystatin C will be assessed on leftover serum collected for biochemistry.

‡Immunology studies will require 22.0% of total blood volume collected (77.6 ml) over 2 years.

*DTPA discontinued on May 29, 2009.

Appendix B

Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
Medical Coordinating Center

List of Tests and Diagnostic Procedures
(for Parent Information)

APPENDIX B. Tests and Diagnostic Procedures (For Parent Information)

- The doctor and nurse will examine your child and ask you questions about his/her health.

- Blood specimen collection usually with a lancet to the skin but for some tests with a needle in a vein: the blood and genetic material (DNA) will be obtained.

- Urine concentrating ability and urinalysis

  You will be asked to give your child nothing by mouth overnight. Urine collection will be done by applying a bag and watching the child for several hours.

- Your child will be measured for height, weight and head size.

- Oxygen saturation will be measured with a wrap around baby's finger or toe for 1-2 minutes.

- Neurological Evaluation

  A pediatric neurologist will examine your child and ask him/her to perform various tasks, as appropriate to age.

- Psychodevelopmental evaluation (Bayley and Vineland)

  A specialty nurse working under the supervision of a licensed psychologist will ask your child to do a number of age appropriate tasks. Also, you, the parents will be asked a series of questions about your child in an interview.

- Liver-spleen scan

  Your child will be given a dose of radioactive tracer by vein. He/she will lay down on a table beneath a camera that takes detailed pictures of the abdomen to determine the location and size of vital organs.

- Abdominal sonogram

  An ultrasound machine will be passed over your child's stomach area and pictures of liver, spleen, and kidneys will be made.

- Transcranial doppler

  An ultrasound machine will be passed over your child's neck and head, and calculations of blood flow speed in the blood vessels that lead to the brain will be made.

Appendix C

Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
Medical Coordinating Center

Central Laboratories and Facilities

Central Laboratories and Facilities

Hematology and Biochemistry Core Laboratory
Medical College of Georgia Research Institute
Abdullah Kutlar, M.D.

Cytogenetics Core Laboratory
Medical College of Georgia Research Institute
K.L. Satya-Prakash, Ph.D.

Pitted Cell Count Core Laboratory
Children's Medical Center of Dallas
Zora Rogers, M.D.

DNA/VDJ Mutations Core Laboratory (and Cystatin C testing)
St. Jude Children's Research Hospital
Russell Ware, M.D., Ph.D.

TCD Core Laboratory
Medical College of Georgia
Robert Adams, M.D.

Immunology Core Laboratory
The Johns Hopkins University School of Medicine
James Casella, M.D., and Howard Lederman, M.D.

Biomarkers Core Laboratory
Thomas Jefferson University Medical Center
Marie Stuart, M.D.

Pharmacy Distribution Center (PDC)
HHS Supply Service Center
Michael Soler

HU Assay Core Laboratory
MDS Pharma

Appendix D

Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
Medical Coordinating Center

Study Timeline

Appendix D

STUDY TIMELINE

Phase I: Planning

2000	Sep	Steering Committee Meeting 1 - Protocol development
	Oct	Steering Committee Meeting 2 - Protocol development
	Dec	Steering Committee Meeting 3 - Protocol development
2001	Jan	Steering Committee Meeting 4 - Protocol development
	Apr	Data and Safety Monitoring Board (DSMB) Meeting 1
		DSMB Conference Call 1
		Protocol revision recommendations to Steering Committee
	Jun	DSMB Meeting 2
		Protocol revision recommendations to Steering Committee
	Jul	Steering Committee Meeting 5 - Further protocol revisions as necessary
	Aug	Special Review Panel
		Steering Committee Meeting 6 - Final Protocol recommendations
	Sep	Forms and data collection instruments development begins
		Manual of Operations development begins
	Oct	Protocol submitted to Clinical Center IRBs for review
	Dec	Steering Committee and Nurse Coordinators Meeting - Forms Review
2002	Jan	Analysis Plan submitted to NHLBI
	Mar	Forms and data collection instruments completed and posted to the Web Site
	May	Revised Analysis Plan submitted for DSMB Review
	July	Data collection design and database design completed
		ATRS System developed and tested
		Steering Committee Meeting and Training Session
		DSMB Conference Call requesting Protocol Review Committee to convene
	Sep	Steering Committee Luncheon Meeting
	Nov	Protocol Review Committee Conference Call
	Dec	Steering Committee Luncheon Meeting
2003	Mar	Investigational New Drug (IND) application submitted to US FDA
		Central Units subcontracts negotiated
		IRB reviews back from Clinical Centers

Phase II: Recruitment and Follow-Up

2003	May	Training Session
	Aug	Steering Committee Meeting
2004	Jun	DSMB Meeting 3
	Sep	Steering Committee Meeting
2005	Jan	DSMB Meeting 4
	Apr	DSMB Meeting 5 (Analyses of Feasibility and Safety Pilot Study final)
	Apr	Steering Committee Meeting
	Jun	DSMB Meeting 6
	Sep	Steering Committee Meeting
	Nov	DSMB Meeting 7
2006	Apr	Steering Committee Meeting
	Jun	DSMB Meeting 8
	Sep	Steering Committee Meeting
	Nov	DSMB Meeting 9

2007	Apr	Steering Committee Meeting
	Jun	DSMB Meeting 10
	Sep	Steering Committee Meeting
	Nov	DSMB Meeting 11
2008	Apr	Steering Committee Meeting
	Jun	DSMB Meeting 12
	Sep	Steering Committee Meeting
	Nov	DSMB Meeting 13

Appendix E

Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
Medical Coordinating Center

ANTHROPOMETRIC MEASUREMENT PROCEDURES

Appendix E

Anthropometric Measurement Procedures

BACKGROUND

Since BABY HUG is a longitudinal study and growth velocity data will be collected, it is important that all measures be made as accurately as possible. Accuracy and standardized procedures are also necessary in multicenter studies to make sure that data from one center are comparable to data from other centers. In BABY HUG data will be gathered on date of birth, birth weight, and gestational age. Gestational age is estimated from of the number of weeks between the mother's record (recollection) of the onset of her last menstrual period and the child's birth. In clinic anthropometric measures of weight, length, and head circumference will also be taken. Weight should be recorded in kilograms to the nearest 0.1 kg (100 grams). Length and head circumference should be recorded to the nearest 0.1 cm.

GENERAL PROCEDURES

Anthropometric measurements should be taken by BABY HUG personnel specifically trained and certified to perform these measurements. If possible, the same examiner(s) should measure participants throughout the study period. Whenever possible, measurements should be taken by a team of two observers. One observer takes the measurements while the other observer records. The observer taking the measurements calls out the results to the recorder. The recorder repeats the results and then calls out the name of the next measurement. The observer keeps the measuring instrument in place until the recorder repeats the number. The recorder checks the examinee's position during the procedure. All of the measurements – weight, length, and head circumference – are made once before repeating them a second time in the same sequence by the same observer. A third measurement is made by the same observer only if the second measurement differs from the first by the specified amounts.

It is important that the measurements be made in pleasant, warm, and quiet surroundings. If blood specimens are taken, they should be drawn after anthropometric data have been obtained. Each center will use the same apparatus for all measurements.

Weight

The weight measurement is critical to assessing growth velocity. Thus, weight must be measured accurately. Following is a description of instruments and methods used for weighing infants.

There is general agreement that weight should be measured using a beam scale with movable weights or a well calibrated electronic scale, and that a pan scale is needed for measurements made during infancy.

Weight is best measured with the subject nude, which is practical during infancy. At older ages, nude measurements may not be possible. If nude measurements are not feasible, standardized light clothing, for example, a disposable paper gown, should be worn in preference to "light indoor clothing."

There are diurnal variations in weight of about 1 kg in children and 2 kg in adults. Therefore, making a note of the time of day at which measurements are made is desirable. Usually it is not practical to measure at a fixed time, but a narrow range may be achievable.

Materials and Methods

Pan Balance

- A pan-type balance (scale) accurate to ± 10 grams (preferably to ±1 g) is needed for children under 12 months old. It may be a beam balance or a modern electronic recording balance.
- The balance should be checked for accuracy just prior to use by adjustment of zero weight on the balance beam and weekly by verifying standard (National Bureau of Standards - NBS) weights of 2 and 4 kg. At least yearly, the balance should be

inspected and adjusted if necessary by a qualified representative of the manufacturer.

- The child is weighed nude. Because a pan balance may be uncomfortable, a pad can be placed on the pan. The pad is weighed, and this weight is recorded and subtracted from the total weight (infant + pad). Alternatively, the balance is adjusted to zero with the pad in place. Whichever procedure is used, it should be practiced consistently throughout the study.
- The child must be relatively quiet and still during the weighing.
- The child is weighed to at least the nearest 100 grams.
- The first weight is recorded on the BABY HUG Anthropometry Form. The child is then weighed again, and this measurement is also recorded. If the two measurements (made to the nearest 0.1 kg) differ by more than 200 grams (0.2 kg), a third weighing is made and recorded. Individuals making the weighings should sign the data form and record their certification numbers.

Standing Scale

A subject able to stand without support is weighed using a leveled platform scale with a beam and moveable weights (see Appendix E Figure 1). The beam on the scale must be graduated so that it can be read from both sides and the scale positioned so that the measurer can stand behind the beam, facing the subject, and can move the beam weights without reaching around the subject. The movable tare is arranged so that a screwdriver is needed to shift it. The subject stands still over the center of the platform with the body weight evenly distributed between both feet. At older ages, children object to being weighed in the nude, and light indoor clothing such as a disposable paper gown can be worn but not shoes, long trousers, or a sweater. Standardized clothing, for example, a disposable paper gown should be the same at every weighing. The weight of this clothing is not subtracted from the observed weight when the recommended reference data are used. Weight is recorded to the nearest 100 g. Weight is

measured and recorded twice. If the two measurements disagree by more than 100 g, a third measurement is made and recorded (see Appendix E Figure 1).

Length

Linear growth of children is assessed by measuring length. Recumbent length should be used up to 18 months of age and standing height after 18 months of age. Of the anthropometric data, recumbent length may be the most difficult to obtain accurately. Examiners should practice measuring infants and verify their ability to measure recumbent length accurately.

Materials and Methods

- A recumbent length measuring board with fixed headboard and movable footboard that are perpendicular to a table surface is required. The measuring scale must be along the length of the board. The scale should be in centimeters with 0.1 cm divisions.
- The board should be regularly checked that the scale is in place and that the footboard is functioning properly with minimum play around a horizontal or vertical axis.
- Two measurers are needed to obtain satisfactory measurements. One measurer holds the child's head in a plane that allows the child to look upward in a line joining the left tragus and the lowest point of the inferior margin of the left orbit: line of sight (13). Slight traction is applied to bring the top of the child's head to the headboard. The other measurer holds the child's feet with toes pointed upward and brings the footboard gently against the child's feet. Press knees to straighten legs, i.e., they should be fully extended to avoid <u>under</u>-estimating length. Once the child is in place, the measurer holding the head may observe that the head is no longer touching the headboard. The footboard should not be brought forward. Instead, the head should be brought gently but firmly back to the headboard. This is appropriate,

because the child should be stretched slightly to give an accurate measurement (see Appendix E Figure 2).

- The child's recumbent length is measured to the nearest 0.1 cm.
- The recumbent length measurement in cm and the data are recorded on the appropriate data form.
- The measurement should be repeated and value recorded. If the two measurements do not agree within 0.5 cm, the recumbent length should be measured a third time. The two measurers may change positions for the third measurement.

Head Circumference

Materials and Methods

- Head circumference is measured with a flexible narrow non-stretchable tape. Steel and fiberglass tapes are satisfactory. Disposable paper tapes can also be used.
- If the same tape is used repeatedly, it should be checked periodically against a steel centimeter reference tape.
- Head circumferences are measured in young children while lying still. In measuring the older child, it is preferable for the child to be on the mother's lap.
- The tape is placed firmly around the head above the supraorbital ridges (right above the brows). While holding the tape in place with the index or middle finger of one hand, the tape is passed over the occipital prominence (bump) at the back of the head with the other hand and the maximum circumference is noted.
- The tape should be pulled snugly around the head compressing the hair (see Appendix E Figure 3). The tape must be kept in the same plane on left and right sides of the head. The measurement should be recorded to the nearest 0.1 cm.
- If the child has a hairstyle that may interfere with the tape's proper position and cannot be easily undone (e.g., "Corn rows"), the best circumference measures

possible should be taken and the presence of such a hair style should be noted on the Anthropometry Form.

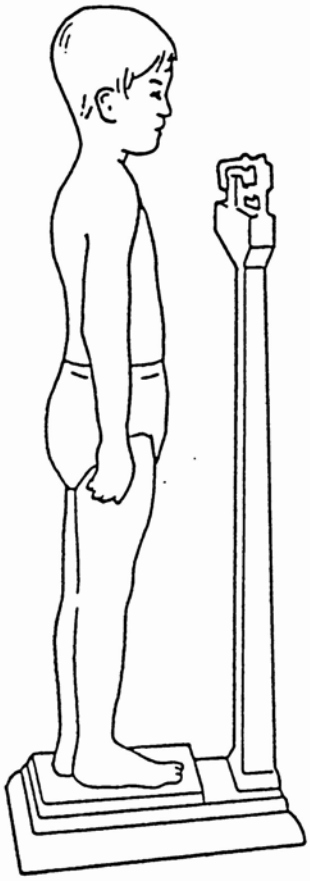
TRAINING AND CERTIFICATION

Problems with different observers over time occur and thus it is important to keep the number of observers to a minimum and ensure continued training and certification over time. Inter-observer variability and errors related to lack of skills can introduce major errors in the data. A goal will be to have an individual certified as a trainer at each Clinical Center so that individuals subsequently joining the staff can be trained on site, certified, and recertified. Training and certification will be on site at all BABY HUG Clinical Centers and carried out by the master trainer.

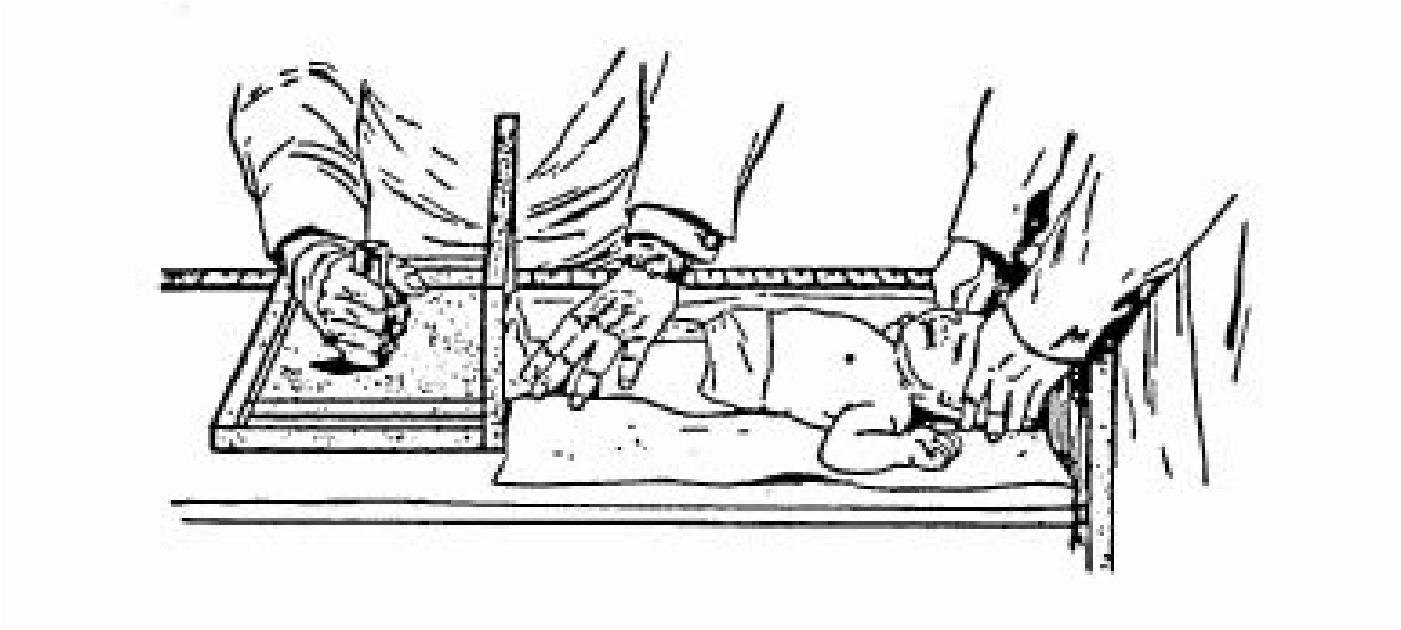
The certification procedures trains the measurers at each site in the techniques and process of taking the actual measurements covered in this manual. During this procedure recording methods, equipment calibration and potential sources of error will be demonstrated.

Recertification will be required every year after the original certification. This will be carried out by each center's designated trainer or by the Study's master trainer. The designated trainer will be certified as having demonstrable measurement skills and knowledge of the methodology, including recording, for avoidance of errors.

Appendix E Figure 1



Appendix E Figure 2



Appendix E Figure 3

From Pediatric Anthropometry.
W Moore and A Roche, 1987

Appendix F

Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
Medical Coordinating Center

Clinical Event Definitions

Appendix F: Clinical Event Definitions

Introduction

In BABY HUG, 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. Characteristically 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.

Arthritis (septic): See Infection (other).

Aseptic Necrosis: Presentation in the age range of patients in BABY HUG would be unusual. Patients encounter pain in their hip or shoulder, often of a different character than their usual crisis pain. The pain persists indefinitely, long after the expected duration of crisis pain. Early radiographic findings are subepiphyseal lucency and widening of the joint space. Late changes include flattening of the epiphysis and sclerosis with fragmentation.

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.

Cholangitis: See Right Upper Quadrant Syndrome

Cholecystitis: See Right Upper Quadrant Syndrome

Cholelithiasis: See Right Upper Quadrant Syndrome

CVA: See Cerebrovascular Accident

Dactylitis: Pain and tenderness with or without swelling in hands and/or feet. Also known as Hand-foot syndrome.

Fever without Focus: Elevation of temperature greater than 38.5°C/101.5°F (regardless of route, oral, axillary or rectal) not associated with a positive culture from any source or with any other special event. Will be characterized as an event managed with blood culture (no growth) and empiric parenteral antibiotics or by parental history (no blood culture, no empirical parenteral antibiotics).

Gastroenteritis: See Infection, Gastroenteritis

Hand-Foot Syndrome: See Dactylitis.

Hematuria: Blood in the urine (greater than 5 red blood cells per high power field), usually suggested by history, confirmed by urinalysis.

Hepatitis: See Right Upper Quadrant Syndrome

Infection (other): Inflammation, caused by a pathogenic agent, which may or may not be accompanied by a fever. Sepsis, meningitis, osteomyelitis, hepatitis and urinary tract infections are ***NOT INCLUDED*** in this category since they are categorized elsewhere. If encountered, the type of infection, site, and organism if known should be specified.

1. **Abscess/Cellulitis:** Infection of skin or deeper tissues.
2. **Gastroenteritis:** Inflammation of the stomach and intestinal tract. Signs include nausea, vomiting and diarrhea lasting at least 8 hours.
3. **Lymphadenitis/Lymphangitis:** Infection in regional lymph nodes or channels draining the primary site of infection.
4. **Mastoiditis:** Infection of the mastoid bone.
5. **Orbital Cellulitis:** Infection of the orbit and tissues posterior to the eye.

6. **Otitis Media:** Infection of the middle ear associated with ear pain and erythema of eardrum, bulging or decreased mobility of eardrum with loss of landmarks.
7. **Periorbital or Preseptal Cellulitis:** Infection of the eyelids causing erythema and swelling.
8. **Pharyngitis:** Pain in the pharynx associated with redness of pharyngeal and tonsillar mucosa with or without exudates. Indicate if streptococcal culture positive.
9. **Septic Arthritis:** Bacterial infection of a joint. The causative agent should be listed if known.
10. **Upper Respiratory Infections (URI):** An imprecise term for almost any kind of infectious process involving the nasal passages, pharynx and bronchi. Often called a "cold."
11. **Varicella Infection**: Clinical syndrome of skin lesions, fever associated with varicella zoster virus infection.

Liver Sequestration: See Right Upper Quadrant Event 5. Liver Sequestration.

Mastoiditis: See Infection (other).

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.

Orbital Cellulitis: See infection (other)

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

Other Event Not Specified: Includes any event that is not included in the list of the events specified on this form.

Painful events: Pain in the extremities, back, abdomen, chest or head for which no other explanation can be found and which is not classified as one of the other special events. The pain shall have lasted for at least 2 hours and for which medication either narcotic or non steroidal, antiflammatory agent is taken. In a young child, pain or tenderness on palpation will be considered appropriate evidence of event.

Pancreatitis: See Right Upper Quadrant Event 6. Pancreatitis.

Pneumonia: See Chest Syndrome.

Priapism: A painful erection of the penis lasting for more than 2 hours.

Proteinuria: Presence of 1+ protein or more on urinalysis.

Renal Complications (not specified): A renal condition not categorized as hematuria, urinary tract infection, proteinuria or renal insufficiency. The type of complication should be sought and specified.

Renal Insufficiency: At least a two-fold increase in serum creatinine to a level greater than or equal to 1.0 mg/dl.

Right Upper Quadrant (RUQ) Event: Defined as any two of the following; pain only in the right upper quadrant of the abdomen, twofold increase in total bilirubin over baseline to a level exceeding the upper limit of normal, two centimeter increase in liver size over baseline or twofold increase in ALT over baseline to a level exceeding the upper limit of normal.

1. Cholangitis: Inflammation of the bile ducts. Diagnosis is usually made by abdominal ultrasound.
2. Cholecystitis: Inflammatory condition of the gallbladder causing RUQ pain that may or may not be associated with gallstones. Diagnosis is usually made by abdominal ultrasound.
3. Cholelithiasis: Formation or presence of calculi or bile stones in the gallbladder or common bile duct, with minimal or no symptoms.
4. Hepatitis: An inflammation of the liver. The causative agent if known, should be specified.
5. Liver Sequestration/Intrahepatic Sequestration: Jaundice and pain in the liver not due to gallstones. May include intrahepatic sickling crisis.
6. Pancreatitis: Inflammation of the pancreas. May be related to medication, infection or gallstone blockage of the Ampulla of Vater.

Seizure: A paroxysmal disorder of cerebral function characterized by sudden, transient attack of altered consciousness, motor activity or sensory phenomena. The type of seizure (e.g., petit mal, psychomotor, grand mal) and etiology if known should be specified.

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.

Splenomegaly: without acute sequestration: Spleen is palpable in abdomen with stable hemoglobin (within 1 gm/dl of baseline), and platelet counts > 100,000/cu mm.

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.

Upper Respiratory Infection (URI): See Infection (other).

Urinary Tract Infection: A clinical event which may or may not be associated with fever and symptoms which is associated with a positive urine culture.