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The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG): Challenges of Study Design

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Abstract

Evidence of the laboratory benefits of hydroxyurea and its clinical efficacy in reducing acute vaso-occlusive events in adults and children with sickle cell anemia has accumulated for more than 15 years. A definitive clinical trial showing that hydroxyurea can also prevent organ damage might support widespread use of the drug at an early age. BABY HUG is a randomized, double-blind placebo-controlled trial to test whether treating young children ages 9 to 17 months at entry with a liquid preparation of hydroxyurea (20 mg/kg/day for two years) can decrease organ damage in the kidneys and spleen by at least 50%. Creation of BABY HUG entailed unique challenges and opportunities. Although protection of brain function might be considered a more compelling

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Conflict of Interest
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endpoint, preservation of spleen and renal function has clinical relevance, and significant treatment effects might be discernable within the mandated sample size of two hundred. Concerns about unanticipated severe toxicity and burdensome testing and monitoring requirements were addressed in part by an internal Feasibility and Safety Pilot Study, the successful completion of which was required prior to enrolling a larger number of children on the protocol. Concerns over recruitment of potentially vulnerable subjects were allayed by inclusion of a research subject advocate, or ombudsman. Finally, maintenance of blinding of research personnel was aided by inclusion of an unblinded primary endpoint person, charged with transmitting endpoint data and monitoring blood work locally for toxicity. (ClinicalTrials.gov number,NCT00006400)

Keywords

hydroxyurea; clinical trial; infants; sickle cell

Introduction

Acute painful events are the hallmark of sickle cell anemia [1]. Recurrent ischemia and infarction due to vaso-occlusion, acute and chronic inflammation [2], abnormal cellular adhesion [3–5], and hemolysis-associated vasculopathy [6,7] ultimately injure the spleen, kidneys, liver, heart, lungs, brain, retina, and bones. Organ damage begins in infancy [8–10] and is primarily responsible for the shortened life expectancy in persons with sickle cell disease [11].

Clinical symptoms begin in the first year of life, coincident with the physiologic decline in fetal hemoglobin [12,13]. Individuals who maintain high hemoglobin F levels have fewer painful crises [1] and improved survival [11]. Hydroxyurea has been shown to increase the amount of hemoglobin F in red blood cells [14,15]. In 1995, a double-blind placebo controlled study demonstrated that hydroxyurea use in severely symptomatic adult patients with sickle cell anemia resulted in a 44 and 45% reduction in pain and acute chest episodes, respectively, and a 30% reduction in the need for acute transfusion [16]. Subsequent open-label single-arm trials showed that hydroxyurea had adequate safety and similar hematological effects in children [17–25] and infants [26]. The results of the open-label Phase I/II HUSOFT trial in infants with sickle cell anemia also suggested that hydroxyurea diminished the loss of spleen function compared to historical controls [26], and unpublished data provided by the investigators suggested that the age-related increase in glomerular filtration rate (GFR) was lessened [27–30].

Based on recommendations of the NHLBI's Sickle Cell Advisory Committee, (http://www.nhlbi.nih.gov/meetings/scd/796min.txt) in 1999, the National Heart, Lung, and Blood Institute (NHLBI) released a competitive contract to design and conduct a 200-subject clinical trial to test whether hydroxyurea administered to infants with sickle cell anemia for two years would slow or prevent organ damage. Ten clinical centers and a Medical Coordinating Center (MCC) were selected. The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) was the result of the ensuing collaboration; however, despite a relatively straightforward concept, the BABY HUG trial faced unique challenges with regard to choices of endpoints; toxicity monitoring; study design; enrollment and consenting processes; and overall execution.

Choice of Endpoints

The BABY HUG Principal Investigators considered all organ systems as possible candidates for the primary endpoints. The incidence of quantifiable damage to most organs was not

high enough during the early years of a child with SCA to permit statistical detection of efficacy using the proposed sample size of 200, a study treatment period of 2 years, and a 50% effect size. For example, assuming a relatively high stroke rate of 1% per year [31], a five-year recruitment period, and a 0.05 alpha level, a study of 5000 subjects (2500 in each group) would be required to demonstrate protection from brain injury. An endpoint of pulmonary function was considered, but the varying methods of measurement utilized in the first four years of life would have prevented comparing data over the duration of the study [32,33]. Cardiac function generally is not compromised during early childhood with sickle cell anemia [34,35]. Quantifiable liver damage is rare in children of this age and the incidence of gallstones is also low [36,37] and of only moderate clinical consequence..

Spleen filtrative function is lost in a large proportion of children with sickle cell anemia with damage beginning at four to six months of age. Splenic dysfunction begins in infancy [9] and by five years of age 94% of children with sickle cell anemia have lost spleen function [38] as measured by quantitation of circulating pocked red blood cells. The high rate of damage to the spleen expected over the course of the study and the availability of liver-spleen scans as well as other qualitative and quantitative surrogate markers of its function [e.g., pitted erythrocyte (PIT) counts [38] and flow cytometric enumeration of Howell-Jolly bodies (HJB) [39] made spleen function an ideal candidate for the primary endpoint. While the largest study in infants and children with sickle cell disease used a pocked RBC level above 3.5% as a surrogate marker of loss of spleen function, the impact of hydroxyurea on the validity of this measure was unknown. Splenic uptake on ^{99}Tc sulfur colloid scan has been the standard assessment, with pilot data demonstrating absent uptake early in life. [9] Because there is a gradual decrease in uptake as spleen function is lost, a separate and blinded reading panel was recruited to categorize splenic uptake as normal, decreased, or absent. While recovery of spleen function has been reported in patients taking hydroxyurea, this possibility was not included in the statistical analysis since the specific aims of the clinical trial were to measure hydroxyurea's impact on progressive organ failure. Therefore a decrease in spleen filtrative function (defined as a transition in the qualitative evaluation of splenic uptake of ^{99m}technetium sulfur colloid from normal to decreased or absent, or from decreased to absent) was chosen as a primary endpoint.

Similarly, increased renal plasma flow and glomerular hyperfiltration are believed to begin within the first few years of life [28] and to result ultimately in injury to the kidneys [40,41]. While serum creatinine normally can be used to estimate glomerular function, values are unusually diminished in sickle cell patients due to increased renal tubular secretion. Although GFR estimates using the Schwartz formula to adjust for height and age are being measured in BABY HUG, it was deemed preferable to use a more definitive assessment of GFR that would not require collection of infants' urine; therefore change in quantitative GFR using ^{99m}diethylenetriaminepentaacetic acid (^{99}Tc-DTPA) clearance was chosen as a co-primary endpoint with spleen function. The alpha level for the study was divided unequally between the two tests.

Investigations of the brain, lungs, and gallbladder, and frequent assessment of growth and development were included as secondary endpoints or indexes of toxicity (Table I). Additional safety measurements related to immune competence and potential mutagenesis were also included as secondary objectives.

Toxicity Monitoring

The use of a chemotherapeutic agent in this very young and potentially vulnerable population required a careful plan for monitoring the safety and efficacy of this intervention. In particular, there were two major concerns about the proposed BABY HUG study: 1)

unpublished but widely disseminated animal data suggested that hydroxyurea could have a deleterious effect on the growth and development of the brain; and 2) the requirement for frequent safety and efficacy evaluations might interfere with parent's willingness to have their child participate in the study.

To address these concerns, a Feasibility and Safety Pilot Study was designed to assure intensive monitoring of the first 40 randomized subjects, with comprehensive data review prior to embarking on the planned total entry of 200 subjects. Of particular focus were growth and neurodevelopment, and success in subjects completing eligibility screening to provide baseline data. Infant growth curves were derived from data from the Cooperative Study of Sickle Cell Disease and used (with Study controls) to monitor height, weight and head circumference. Neurodevelopmental testing (Bailey) was done every six months. Failure to observe an unexpected or severe adverse event in the initial 20 children randomized to hydroxyurea would predict a probability of occurrence in the larger study of less than 14% (using a 95% confidence interval method). If the risk of toxicity or adverse events differed statistically between the treatment arms (based on a 95% confidence interval method), the Data and Safety Monitoring Board would discuss whether the trial should be continued. For rare and serious events (e.g., death, stroke), the Board members would discuss continuing the trial after each occurrence.

Treatment with study drug was initiated at a dose of 20 mg/kg/day; though advocated by many, aggressive dose escalation to maximal tolerated dose was not deemed acceptable in our young subjects. During the first few months of treatment, infants were monitored every two weeks for adverse events and laboratory toxicities [e.g., low hemoglobin level, platelet count, absolute neutrophil count, or reticulocyte count; high serum creatinine, bilirubin, or alanine transaminase]. If laboratory toxicity occurred, a STOP order was conveyed to the clinical center and study treatment administration was halted; sham STOP orders were systematically generated by the MCC for placebo subjects if required. If toxicity resolved within two weeks, treatment was resumed at the original dose; prolonged or recurrent toxicity resulted in decreasing the study treatment dose by 2.5 mg/kg once toxicity resolved. Once a stable tolerated dose was maintained for eight consecutive weeks, the length between follow-up visits was increased to four weeks.

Study Design

A flow diagram for BABY HUG is presented in Figure 1. Both the qualitative assessment of decline in spleen filtrative function and change in kidney GFR were comparisons between a baseline and two-year evaluations. In an effort to balance the likelihood of detecting clinically significant differences for both end organ measures the total alpha level (study-wide false positive error rate) was not divided equally. Most (0.04) of the 0.05 alpha level for this study was allocated to the categorical endpoint of spleen function whereas a smaller amount (0.01) was allocated to the continuous GFR endpoint. This allocation allows the BABY HUG investigators to identify a 50% difference in the incidence of functional asplenia (e.g., from 60 to 30%) after two years of study drug and a 0.6 standard deviation difference in GFR between the two treatment groups, with at least 90% power when corrected for treatment crossovers and subjects lost to follow up. Patients enrolled in the trial with asplenia were included in the evaluation of the GFR endpoint.

Based on data from the Cooperative Study of Sickle Cell Disease, 28% of patients were expected to have absent spleen function by age one year and 89% by age four years [38]. Based on GFR estimates from children with sickle cell anemia [29], the expected mean GFR for two to four year-olds taking placebo in this study was 162 mL/min/1.73 m^2 with a standard deviation of approximately 10 mL/min/1.73 m^2. Using these estimates, the

proposed study size of 100 children per group would be sufficient to detect a real difference of means between the hydroxyurea treated group and the placebo group of 5.0 and 5.5 mL/min/1.73 m^2 with 82% and 90% power, respectively.

Primary comparisons would be by intention-to-treat analysis. Five primary endpoint analyses were planned (four interim analyses and one final analysis); the first interim analysis would assess children enrolled in the Feasibility and Safety Pilot Study. Extreme evidence (nominal alpha = 0.0005, Z = 3.5) of treatment differences was required at an interim analysis to demonstrate the efficacy of the proposed intervention for either endpoint. The methods of Lan and DeMets [42] would be used to maintain the experiment-wise alpha level to 0.05. Appropriate allowances were made to account for interim monitoring of the two primary endpoints. Staff at each clinical center randomized subjects in block sizes ranging from two to six, such that equal numbers of patients would be assigned to hydroxyurea and placebo at the conclusion of each block. Treatment assignments were made through the use of an automated telephone response system.

Enrollment And Consenting Process

Since sickle cell anemia often occurs in a predominantly socioeconomically-challenged and underserved minority population of diverse ethnic and geographic origin, the use of a recruitment-neutral research subject advocate insured that the risks and benefits of BABY HUG were clearly presented, that no coercion occurred, and that questions from the parents and other caregivers were asked and answered fully. Such a role was recently expanded both in scope and priority by the National Center for Research Resources, which specified that all General Clinical Research Centers must have available a research subject advocate. [44]

Overall Execution

Hydroxyurea is not approved in a liquid formulation. Bulk hydroxyurea to be used in the BABY HUG study treatment formulation was obtained from Par Pharmaceutical Companies [New Jersey, USA] and Solmag [Italy]. UPM Pharmaceuticals, Inc. [Maryland, USA] agreed to manufacture both the hydroxyurea and placebo as powder, and package the powder in bottles. The bottles would then be shipped to the Pharmacy Distribution Center at the National Health and Human Services Supply Service Center [Maryland, USA] to be labeled, placed in numbered treatment kits, and distributed to clinical centers. Pharmacists would reconstitute the study treatment powder with simple syrup and water to a concentration of 100 mg/m [43] immediately before dispensing a 35-day supply.

Administration of the first dose of study treatment would coincide with the performance of the ^{99}Tc-DTPA GFR determination and collection of first-dose PK samples. To allow for the collection of data for both measures, blood samples were planned at 0, 1, 2, 4, and 8 hours post-dose initiation. PK blood samples would be collected from all children to maintain the study treatment blind and to confirm correct treatment assignment for each child.

Secondary and safety endpoint data would be collected according to the schedule shown in Table II. Data for the co-primary endpoints would be collected during the screening/baseline evaluation and after two years of study treatment. A central application server and an Internet data entry system would be used to enter and store data.

The study design permitted treatment of emergent clinical complications while keeping the local PI, nurse coordinator, and the parents blinded to laboratory data (especially the MCV and neutrophil count), which might inform them about treatment assignment. Each institution designated a primary endpoint person, not directly responsible for routine study

care but tasked with responding to clinical issues and reviewing and reporting hematology values. In addition, a pediatric hematologist associated with the MCC, assigned to monitor each child's longitudinal laboratory measurements, would interact with the primary endpoint persons to provide toxicity notifications and review trends from BABY HUG data to ensure that blinding did not interfere with the clinical care of study subjects. Primary and secondary endpoint evaluations would be assessed by independent specialist readers without knowledge of treatment assignment.

Epilogue

BABY HUG opened to accrual in October 2003. During the Feasibility and Pilot Study, the Data and Safety Monitoring Board reviewed data monthly on recruitment, adverse events, toxicities, clinic performance of protocol procedures, and growth and neurodevelopment for each child enrolled. Semi-annual reports were presented by treatment arm; Data and Safety Monitoring Board members reviewed toxicity information without adjusting for multiple comparisons. Not finding significant differences between treatment arms, medically or statistically, continued recruitment to complete BABY HUG was recommended and pursued. A number of toxicity monitoring measures were reduced in frequency (Table II) and timing for ^{99}Tc-DTPA/pharmacokinetic specimens were changed to 30, 90, and 210 minutes post-initial hydroxyurea dose to reduce the total time to complete the study visit and utilize more informative time points. Four additional centers were added in late 2005 to enhance accrual, and the last of 193 randomized infants was enrolled in September 2007 and will complete exit studies in 2009. The National Institute of Child Health and Human Development (NICHD) joined NHLBI in sponsorship of the trial in 2005 to facilitate a pharmacokinetic (PK) assessment of the study's liquid formulation of hydroxyurea for infants and children with sickle cell anemia in anticipation of an FDA submission for this new formulation and treatment indication.

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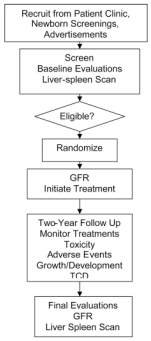

Figure 1.
BABY HUG flow diagram.

Table I

BABY HUG Laboratory Data – Laboratory Toxicity Levels

	Low Toxicity (Lower Than)	High Toxicity (Higher Than)
Hemoglobin	6.0 gm/dL **or** 20% drop from 3 month rolling average	
Platelets	80,000/mm^3	
ANC	1250/mm^3	
Reticulocytes	80,000/mm^3 **and** Hemoglobin < 7.0 gm/dL	
Creatinine		2 × baseline value and > 1.0 mg/dl
Bilirubin		10 mg/dL
ALT		150 Units/L

Table II

Frequency of Collection of BABY HUG Endpoints

Organ/System	Test	Endpoint Type	Frequency	
			Pilot Study	Current Protocol
Spleen	Liver/Spleen Scan	primary	Entry, Exit	Entry, Exit
	PIT Counts	secondary	Q 6 mos	Q 6 mos
	Ultrasound	secondary	Entry, Exit	Entry, Exit
	Howell-Jolly Bodies	safety	Not done	Entry, Exit
Kidneys	DTPA/GFR	primary	Entry, Exit	Entry, Exit
	Ultrasound, specific gravity, osmolality, urinalysis	secondary	Entry, Exit	Entry, Exit
	Cystatin C	secondary	Not done	Entry, Exit
	Creatinine	secondary	Q 4–8 wks	Q 6mos
	HPLC creatinine	safety	Entry, Exit	Entry, Exit
	BUN	safety	Q 4–8 wks	Q 6 mos
Lungs	O_2 saturation	secondary	Q 3 mos	Q 3 mos
Liver	Ultrasound	secondary	Entry, exit	Entry, exit
	Direct and indirect bilirubin	secondary	Q 4–8 wks	Q 6 mos
	ALT	safety	Q 4–8 wks	Q 6 mos
Brain	MRI/MRA	safety	Entry, exit	Not done
	Transcranial Doppler	secondary	Entry, exit	Entry, 12 mos, exit
	Neurodevelopmental, Neurological examination	safety	Q 6 mos	Q 12 mos
Growth	Height, head circumference	safety	Q 2–4 wks	Q 4 wks
	Weight	safety	Q 2–4 wks	Q 2–4 wks
Blood	HbF, F-cell	secondary	Q 6 mos	Q 6 mos
	CBC, reticulocyte count	safety	Q 2–4 wks	Q 2–4 wks
	Calcium, magnesium, ferritin	safety	Q 6 mos	Q 6 mos
Clinical Events	AEs	secondary	When occurs	When occurs
	SAEs	safety	When occurs	When occurs
Mutagenesis	VDJ, chromosome and sister chromatid breakage	safety	Entry, Exit	Entry, Exit
Immunology	Penicillin urine filter strip	safety	Q 6 mos	Not done
	Pneumococcal antibody titer, opsonophagocytic activity	safety	Entry, 24 & 25 mos of age, Exit	Entry, 24 & 25 mos of age, Exit
	MMR antibody titer	safety	13 & 24 mos of age, exit	13 & 24 mos of age, exit
	T-cell counts	safety	Entry, 24 mos of age, exit	Entry, 24 mos of age, exit
Pharmacokinetics	Hydroxyurea assay	PK	Entry, Exit	Entry, 1 mo, Exit