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A multicenter randomised controlled trial of hydroxyurea (hydroxycarbamide) in very young children with sickle cell anaemia

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Abstract

Background—Sickle cell anaemia (SCA) is associated with significant morbidity from acute complications and organ dysfunction beginning in the first year of life. In the first multicenter randomised double-blinded trial in very young children with SCA, the impact of hydroxyurea (hydroxycarbamide) therapy on organ dysfunction, clinical complications, and laboratory findings, and its toxicity, were examined.

Methods—Eligible subjects had HbSS or Sp⁰thalassaemia, were age 9–18 months at randomisation, and were *not* selected for clinical severity. Subjects received liquid hydroxyurea, 20 mg/kg/day, or placebo for two years. Primary study endpoints were splenic function (qualitative uptake on ^{99m}Tc spleen scan) and renal function (glomerular filtration rate by ^{99m}Tc-

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Contributors

WCW prepared the first draft of the report after discussion by the writing committee. FDA, JCB, RCB, JFC, RVI, STM, SR, ZRR, BWT, MAW, WCW, REW, and LWW participated in study design. JCB, RCB, JFC, THH, RVI, STM, SR, ZRR, SAS, CDT, WCW, REW, and LWW performed patient recruitment and coordination of study site activities. JCB, RCB, JFC, BAF, THH, XH, RVI, RVK, AK, STM, CPM, SR, ZRR, SAS, BWT, CDT, MAW, WCW, REW, and LWW conducted data collection, analysis and interpretation and BAF, XH, and BWT performed data verification. JCB, RCB, JFC, BAF, JCG, THH, RVI, RVK, AK, STM, CPM, SR, ZRR, SAS, BWT, CDT, MAW, WCW, REW, and LWW performed writing and editing. JCG provided administration of the project. FDA provided oversight of neurodevelopmental data and AK, ZRR, and REW supervised the central labs.

Conflicts of Interest

None of the authors had conflicts of interest.

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DTPA clearance). Additional evaluations included: blood counts, HbF, chemistry profiles, spleen function biomarkers, urine osmolality, neurodevelopment, transcranial Doppler ultrasonography, growth, and mutagenicity. Study visits occurred every two to four weeks.

Findings—Ninety-six subjects received hydroxyurea and 97 placebo; 86% completed the study. Significant differences were not seen for the primary endpoints, but suggestive benefit was noted in quantitative measures of spleen function. Hydroxyurea significantly decreased pain and dactylitis with trends for decreased acute chest syndrome, hospitalisation and transfusion. Hydroxyurea increased haemoglobin and HbF and decreased WBC count. Toxicity was limited to mild-moderate neutropaenia.

Interpretation—Although hydroxyurea treatment did not reduce splenic and renal dysfunction assessed by primary endpoint measures, it resulted in major clinical benefit because of diminished acute complications, favorable haematologic results, and a lack of unexpected toxicities. Based on the safety and efficacy data from this trial, hydroxyurea can now be considered for all very young children with SCA.

INTRODUCTION

Sickle cell anaemia (SCA) is characterized by haemoglobin (Hb) polymerisation that results in sickle-shaped red blood cells (RBC), vaso-occlusion, haemolytic anaemia and vasculo-endothelial dysfunction, causing pain, organ injury, and early mortality. Clinical symptoms begin in the first year of life with the physiologic decline in fetal haemoglobin (HbF) concentration, but higher levels of HbF are associated with fewer pain crises¹ and improved survival.² Hydroxyurea, an antineoplastic agent that inhibits ribonucleotide reductase, increases HbF in RBC and has other potentially salutary effects, including improved nitric oxide metabolism, reduced red cell-endothelial interaction and decreased erythrocyte density.³ Fifteen years ago the double-blinded placebo-controlled Multi-Center Study of Hydroxyurea (MSH) in adults with severe SCA demonstrated that hydroxyurea substantially reduced episodes of pain and acute chest syndrome (ACS), hospitalisations and transfusions.⁴ Subsequently, multiple smaller studies have shown similar benefits and minimal toxicity in school-age children and adolescents.⁵

In 2000, the U. S. National Heart, Lung, and Blood Institute (NHLBI) awarded a competitive contract to conduct a clinical trial to test whether hydroxyurea administered to infants with SCA for two years slows or prevents organ damage. In a pilot trial (HUSOFT), very young children with SCA had tolerated a liquid hydroxyurea formulation (20 mg/kg/d) and had improved blood counts and HbF levels.⁶ After two years, splenic radionuclide uptake was absent in only 47% of children, although 80% absence was predicted, leading to the choice of splenic function as a primary endpoint for the Phase III trial. Because glomerular filtration rate (GFR) is abnormally increased early in life and may lead to progressive renal dysfunction,⁷ the effect of hydroxyurea on GFR also was considered an evaluable target and a second primary endpoint. The BABY HUG trial thus was designed to determine if hydroxyurea safely prevents early damage to the spleen and kidneys in infants with SCA. Importantly, however, comprehensive data on adverse clinical events, blood counts, and additional measures of organ function, as well as assessment of potential toxicities, were collected.⁸

METHODS

Study Design

After informed consent was obtained, potential study participants were extensively evaluated to establish eligibility and baseline status. Subjects were randomised using a 1:1 ratio at age 9 – 18 months to receive blinded hydroxyurea or placebo for two years.⁸ Design

features included an ombudsman at each site to promote understanding of the risks and demands of study participation, an unblinded “primary endpoint person” to monitor laboratory values and assist in clinical management, and a Feasibility and Safety Pilot Study to evaluate the first 40 subjects for toxicity.⁸ At study exit, initial evaluations were repeated.

Subjects

Eligible subjects had HbSS or S β^0 thalassaemia and were enrolled regardless of clinical severity. All received standard age-appropriate care for SCA, including penicillin prophylaxis, pneumococcal immunization, and parental education. Potential participants were excluded for recent transfusion; height, weight or head circumference <5th percentile; mental developmental index (MDI) <70; or abnormal transcranial Doppler ultrasound (TCD) velocity.⁸

Endpoints and Monitoring

Blinded readings of splenic uptake on ^{99m}Tc-sulfur colloid liver-spleen scans were categorized qualitatively as normal, decreased (but present), or absent. It was hypothesized that a decline in splenic uptake (from “normal” to “decreased” or “absent”, or from “decreased” to “absent”) would occur 50% less frequently in the hydroxyurea group. GFR using ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) plasma clearance was chosen as a co-primary endpoint with a 0.6 standard deviation difference anticipated⁹. Evaluation of this endpoint was discontinued in May, 2009 because the monitoring board determined that further data collection would be statistically “futile”. Secondary measures of splenic function included the quantitative ratio of nuclear decay counts in the spleen and liver, the proportion of RBC containing “pits” (small vacuoles normally removed by the spleen), and the proportion of mature RBC containing Howell-Jolly bodies (HJB).¹⁰ Secondary measures of renal function were serum creatinine and cystatin C, urinalysis, and urine osmolality after limited fasting.¹¹ Investigations of the brain, lungs, hepatobiliary system, and growth and development were included as secondary endpoints or indices of toxicity; growth curves derived from Cooperative Study of Sickle Cell Disease (CSSCD) data were used to monitor height, weight and head circumference. Neurodevelopmental evaluation (Bayley Developmental and Vineland Adaptive Behavior Scales) and neurological examinations were performed every 6–12 months.⁸ Potential for mutagenesis was measured with assays for VDJ immunoglobulin receptor rearrangement, flow cytometric quantitation of young reticulocyte micronuclei, and chromosome and chromatid breaks.^{12,13}

Study Drug

Hydroxyurea and placebo were distributed to clinical centers in encoded kits. Local pharmacists reconstituted powder with syrup and water to a concentration of 100 mg/mL¹⁴ and dispensed a 35-day supply. As in the HUSOFT trial, treatment was initiated at 20 mg/kg/day without subsequent dose escalation. Infants were monitored every two weeks for adverse events and laboratory toxicities until a tolerable dose was confirmed, then every four weeks.⁸

Adverse Clinical Events

Adverse clinical events included known complications of SCA, such as pain, dactylitis, ACS, stroke, priapism, sepsis/bacteraemia, splenic sequestration, hospitalisation, and transfusion. Serious adverse events were reviewed by an independent classification committee.

Definitions included:

Acute chest syndrome: Clinical syndrome characterized by a new pulmonary infiltrate and at least three of the following: chest pain, temperature $>38.5^{\circ}\text{C}$, tachypnea, wheezing, or cough. *Pain event:* Pain in the extremities, back, abdomen, chest, or head with no other explanation, lasting at least two hours, and requiring non-steroidal anti-inflammatory or narcotic analgesia. Events managed at home were included. *Dactylitis:* Pain and tenderness with or without swelling, limited to the hands and/or feet. *Splenic sequestration:* Increase in palpable spleen size by ≥ 2 cm below the costal margin from the last examination, accompanied by a decrease in haemoglobin of ≥ 2 g/dL or $\geq 20\%$ from steady-state values.

Statistical Analysis

All randomised treatment group comparisons were by intention-to-treat analysis. Methodology for the collection and analysis of study endpoint data has been published.⁸ Data analysis was performed using the statistical package SAS 9.2 (SAS Inc., Cary, NC). The alpha level for the two primary endpoints was divided disproportionately with 0.04 allocated to the spleen and 0.01 to the renal endpoint. A sample size of 100 subjects per group provided $>95\%$ power to detect an estimated proportion with worsening spleen function of 0.6 in the control group versus 0.3 in the hydroxyurea group, assuming a two-sided type I error rate of 4%, and to detect a 60% difference in the exit versus baseline GFR measurements in the two groups, allowing a two-sided type I error rate of 1%. A group sequential design was used to adjust for six-month interim-analysis reviews carried out by an independent Data Safety and Monitoring Board. Interim boundaries were widely set to allow for the most powerful comparison to be performed at the end of the study, should an interim boundary not be crossed during the trial. For secondary endpoints, $p \leq 0.01$ was considered significant.

Continuous variables are presented as means/standard deviations, and compared using the two-sample Student t-test. Categorical variables are presented as proportions and compared using the Pearson Chi-square or Fisher Exact test. All adverse events were treated as either time to first event or a counting process event variable and analysed using the log-rank life test or the counting process approach of Anderson and Gill.¹⁵ Efficacy analyses were adjusted for baseline measurements. The Generalized Estimating Equation (GEE) method was used for analysing correlated or serially collected data and multiple imputation was used to adjust for missing data.

Role of the Funding Source

The study was funded by the sources indicated below. The NHLBI provided an initial draft of the study design. The study sponsors did not collect, analyse or interpret data. Two employees of the NHLBI (JCG, MAW) contributed to the writing of the manuscript.

RESULTS

From October 2003 to September 2007, 193 infants with HbSS (187) or $\text{S}\beta^0$ thalassaemia (6), mean age 13.6 months (range 9–18), were randomised at 13 clinical centers (Figure 1). A temporary administrative “clinical hold” on all study activity occurred from March to June, 2006 due to the lack of a specified expiration date on one lot of treatment bottles. One hundred seventy-nine (93%) subjects who completed at least 18 months of the trial and at least one exit assessment were analysed; 167 (86%) completed the full study.

Baseline Characteristics (Table 1)

Ninety-six children were randomised to hydroxyurea and 97 to placebo, with no significant differences in age, gender, genotype, clinical severity, laboratory values, or physical findings.

Organ Function

Primary endpoints (Table 2): Qualitative spleen scans were worse at exit than at entry in 27% of those on hydroxyurea and 38% of those receiving placebo [difference= -11%, CI (-26, 5), $p=0.2$]. GFR measured by ^{99m}Tc -DTPA clearance, was not different in the two groups [difference=2 ml/min/1.73m², CI (-16, 20), $p=0.8$].

Secondary endpoints (Table 3): Quantitative measures of spleen function (HJB and pit counts, spleen:liver count ratio) suggested benefit from hydroxyurea when exit vs. entry differences of the two groups were compared. Exit vs. entry differences in the hydroxyurea group also trended toward higher urine osmolality and specific gravity and lower total kidney volume when compared with those in the placebo group. The increase in time-averaged mean maximum (TAMM) TCD velocity from baseline to exit was less in the hydroxyurea group compared with the placebo group (20 cm/sec vs. 32 cm/sec). No differences between groups were seen in the average Bayley MDI or Vineland scores for communication, daily living skills, socialisation, and motor skills, although all five subjects with MDI <70 at exit had received placebo. Growth was not impacted by hydroxyurea.

Haematology (Table 3, Figure 2): By one to three months after starting study drug, blood counts were different in the hydroxyurea and placebo groups. Haemoglobin and HbF levels were relatively stable in the hydroxyurea group between entry and exit, whereas normal age-related declines occurred in the placebo group. The hydroxyurea group had higher mean exit levels of haemoglobin (by 0.5 g/dL), HbF (by 5.3%) and MCV (by 6.0 fL) and lower WBC, neutrophil, and absolute reticulocyte counts; entry vs. exit differences between the groups were highly significant.

Adverse Events

Acute event rates for the most common complications of SCA were markedly different in the two groups (Table 4). Pain was nearly twice as frequent and dactylitis five times more common in subjects receiving placebo. ACS was threefold greater with placebo. Hospitalisations and transfusions were marginally more common with placebo; most hospitalisations occurred for reasons other than pain (usually fever). Differences between the two groups appeared by about 50 days (dactylitis), 100 days (pain) and 300 days (ACS and transfusion) after treatment initiation (Figure 3).

Sepsis or bacteraemia occurred three times in those receiving hydroxyurea and six times in the placebo group. Episodes of splenic sequestration were equal in the two groups. Gastroenteritis occurred less frequently in those receiving hydroxyurea.

Toxicity (Table 5)

The only frequent toxicity, mild-moderate neutropaenia (ANC 500–1249/mm³), occurred 107 times in 45 subjects in the hydroxyurea group and 34 times in 18 subjects in the placebo group. Recurrent or persistent neutropaenia resulted in nine long-term dose decreases (to 17.5 mg/kg/d) in the hydroxyurea group and five in the placebo group (difference not significant). More severe neutropaenia (ANC <500/mm³) was rare and not associated with invasive infection. Thrombocytopaenia and reticulocytopaenia were similar in the two groups. At study exit, chromosome and chromatid breaks were not different in the two groups when compared to baseline levels, nor were any differences seen in VDJ recombination events or micronuclei assay results.

DISCUSSION

Panel: Research in Context

Systematic Review—We searched PubMed for review articles with the search terms “sickle cell” and “hydroxyurea”. Two systematic reviews were identified: one involving adults¹⁶ and another children.¹⁷ Additionally, there was a recent Cochrane Library review of “Hydroxyurea for sickle cell disease”.¹⁸ Together, these reviews reported only two randomised controlled prospective trials of hydroxyurea in sickle cell disease. The Multi-Center Study of Hydroxyurea (MSH, see text) was a “high quality” double-blinded trial of hydroxyurea in 299 adults with *severe* sickle cell anaemia, that demonstrated significant reduction in pain, acute chest syndrome, hospitalisation, and transfusion in the treated group.⁴ In the systematic review of the paediatric literature, 26 articles were evaluated, but there was only one randomised trial (considered “moderate quality”), in which 22 evaluable children (median age eight years) with *severe* sickle cell anaemia were treated in a single-blind 12 month crossover study; hydroxyurea resulted in decreased hospitalisations.⁵ There were no randomised trials of the effect of hydroxyurea on organ function, nor any in infants.

Interpretation—Our study is the only double-blinded prospective paediatric trial. Patients were unique in two important ways: their very young age and the lack of an eligibility requirement for a severe clinical course. Our primary endpoints for splenic and renal function did not show benefit from hydroxyurea, although several secondary endpoints did. Importantly, our findings of significant reduction in pain and dactylitis and trends toward decreased acute chest syndrome, hospitalisation and transfusion closely resemble those seen in the MSH but occurred in a much different population. Benefit in haematologic results and the relative lack of toxicity were similar to findings reported in older children and adults with more severe disease. Based on the safety and efficacy data from this trial, hydroxyurea can now be considered for all children with sickle cell anaemia, starting at an early age.

Three recent systematic reviews of the use of hydroxyurea for sickle cell disease have been published.^{16–18} BABY HUG is the first randomised double-blinded trial of hydroxyurea in children with SCA (see Panel: Research in Context). It differs from all other paediatric trials (except for the pilot HUSOFT study) in the subjects’ much younger age at enrollment (mean age 13.6 months) and the absence of a prerequisite for a severe clinical course (e.g., ≥ 3 vaso-occlusive events in the previous year). No previous systematic reviews or meta-analyses of the effects of hydroxyurea on splenic or renal function in SCA have been published. In our literature review we found two retrospective studies which showed modest preservation of splenic uptake in older children^{19,20} and a small prospective study which showed no effect.²¹ Published data regarding the effects of hydroxyurea on renal function report possible effects on proteinuria/microalbuminuria.^{22,23} One study found that GFR did not increase after two years of hydroxyurea treatment in preschool-age children.²³

Hydroxyurea did not prevent reduction in splenic function assessed by qualitative spleen scan uptake. However, compared to previous reports, the decline in function on spleen scan occurred in a smaller than expected proportion in the placebo group (38%), and in an even smaller (but not significantly different) proportion (27%) of those treated with hydroxyurea. Because serum creatinine levels, often used to estimate glomerular function, are diminished in individuals with SCA, ^{99m}Tc-DTPA clearance, a more definitive assessment of GFR that does not require urine collection, was utilised. As expected, increased GFR for age was present at baseline^{7,9} and further increases were seen at exit, but hydroxyurea had no impact on glomerular hyperfiltration.

Possible explanations for the failure to demonstrate primary endpoint differences include: (i) use of a fixed dose of hydroxyurea (20 mg/kg/day), lower than the usual maximum tolerated

dose (MTD) and perhaps clinically less effective;^{3,24} (ii) the relatively short duration of the trial (perhaps with further compromise by the three month “clinical hold”), which may have been insufficient to see changes; (iii) the limited number of subjects; and (iv) suboptimal endpoint measurements, which may not have been sensitive enough to detect subtle changes in splenic and renal function. Unfortunately, at entry nearly three-fourths of subjects fell into the intermediate category of “decreased but not absent” splenic uptake on qualitative spleen scan; changes within this category could not be appreciated in assessing the primary endpoint. Of course, it is possible that hydroxyurea does not prevent organ dysfunction among infants or offers limited protection. An open-label follow-up study of this cohort that allows dose escalation is underway. Of 167 children who completed the BABY HUG trial, 91% are enrolled in follow-up, and 79% of families have chosen to give open-label hydroxyurea, usually with escalation to MTD.

Three secondary measures of splenic function (quantitative spleen: liver count ratios, pit counts and HJB) suggested benefit from hydroxyurea when adjusted for baseline values; the potential utility of baseline pit counts and HJB from the BABY HUG study has been analysed recently.¹¹ Two secondary measures of renal function (urine osmolality and specific gravity) were suggestive of an effect from hydroxyurea. The greater total kidney volume on ultrasonography in the placebo group may reflect nephromegaly due to hyperfiltration. Moreover, hydroxyurea may favorably influence CNS pathology, a major problem in older children with SCA. The average increase in TCD velocity, an established indicator of stroke risk in older children, was significantly less in those receiving hydroxyurea, probably reflecting the higher mean haemoglobin level in that group. Although the mean MDI was similar in the two groups, all five with scores <70 at exit had received placebo.

Most importantly, subjects in the hydroxyurea group had substantial clinical benefit, including lower rates of pain and dactylitis and suggestive decreases in the occurrence of acute chest syndrome, hospitalisation and transfusion, along with improved haematologic parameters. Proportionally, the decreases in pain, ACS, hospitalisation and transfusion seen in our unselected patients were remarkably similar to those demonstrated in the severely affected adults in the MSH,⁴ the trial which led to U.S. Food and Drug Administration (FDA) approval of hydroxyurea for adults with severe SCA.

The reduction in clinical events and improved haematological parameters with hydroxyurea should improve long-term prognosis. Recurrent pain and ACS and higher WBC counts were associated with early mortality in the CSSCD.¹ Haemoglobin <7 g/dL and increased WBC count during the second year of life and dactylitis before age one were associated with increased risk for severe outcomes later in life,²⁵ although these findings were not replicated in a more recent newborn cohort.²⁶ Higher haemoglobin and lower reticulocyte counts and bilirubin levels in the BABY HUG hydroxyurea group indicate reduced haemolysis, which should diminish nitric oxide depletion and its deleterious vascular effects.

The only toxicity from hydroxyurea in this infant cohort was mild-moderate neutropaenia, but there were no significant differences in severe neutropaenia or in the number of bacteraemia/sepsis events. Despite some previous concerns, hydroxyurea did not increase the frequency of splenic sequestration or have any detrimental effects on growth or neurodevelopment. Using several assays for mutagenesis, no significant differences between the hydroxyurea and placebo groups were observed. Long-term follow-up of subjects with sickle cell disease, averaging 17.5 years for those in the MSH,²⁷ eight years for an adult Greek cohort,²⁸ 12 years (to date) for the HUSOFT subjects,²⁹ and eight years for the Duke paediatric cohort,²⁴ has not revealed any unexpected toxicities from hydroxyurea. In fact,

extended use has likely improved survival in adults.^{27,28} However, because the risk of neoplasia is unknown when hydroxyurea is begun early in life, long-term follow up is vital.

A recent National Institutes of Health Consensus Conference concluded that hydroxyurea is greatly underutilised in adults with SCA.³⁰ Its laboratory and clinical benefits for children and adolescents with SCA, coupled with an excellent short-term and long-term safety profile, suggest that hydroxyurea is underutilised in young patients as well. Further follow-up of the unique BABY HUG cohort is now planned until 2016, when subjects will be 9–13 years of age, and will provide invaluable data regarding longer-term beneficial and/or toxic effects.

In conclusion, hydroxyurea was safe and resulted in a marked decrease in common but serious adverse events, especially pain and dactylitis, as well as improved laboratory parameters. Several secondary measures of spleen, kidney, and central nervous system function suggested benefit, but these results were not conclusive. We believe that the results of the BABY HUG study should have a major impact on guidelines for the management of children with sickle cell anaemia. Based on the safety and efficacy data from this trial, hydroxyurea therapy can now be considered for all very young children with SCA whether or not they have clinical symptoms. Future monitoring of this approach should be enhanced by a sickle cell registry (U.S.) and a global sickle disease network.

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Figure 1.
Trial Profile

Figure 2. Longitudinal changes in mean haematologic values in Hydroxyurea Group vs. Placebo Group

The Hydroxyurea group is indicated by red lines (—); the Placebo group is indicated by blue lines (—). Panels show mean values over time from initiation of study drug for Haemoglobin (Panel A), MCV (B), Haemoglobin F (C), Platelet Count (D), Absolute Neutrophil Count (E) and Absolute Reticulocyte Count (F). Blood counts were obtained every two to four weeks (although not all data points are shown), except for HbF, which was obtained every six months. The p-values were ≤ 0.005 for all comparisons except for platelet counts ($p=0.2$).

Figure 3. Cumulative probability of adverse events in Hydroxyurea Group vs. Placebo Group
 The Hydroxyurea group is indicated by red lines (—); the Placebo group by blue lines (—). The cumulative probability curves were generated by the time to first event for acute chest syndrome (**Panel A**), pain (**B**), dactylitis (**C**), and transfusion (**D**). The differences in the events between the treatment groups were evaluated from the log-rank life test. The p-values for cumulative probability curves for ACS, pain, dactylitis, and transfusion were 0.02, 0.002, <0.001, and 0.03, respectively.

Table 1**Baseline Characteristics of Hydroxyurea and Placebo Groups**

Baseline Variable	Hydroxyurea (N=96)	Placebo (N=97)
Age – mo. (mean ± standard deviation)	13.6±2.7	13.5±2.8
Male sex -- no. (%)	44 (46)	40 (41)
Sickle Cell Disease type -- no. (%)		
Hb SS	94 (98)	93 (96)
Hb S β^0 thalassaemia	2 (2)	4 (4)
Hospitalisations before study enrollment -- no. (%)	65 (68)	70 (73)
Dactylitis before study enrollment -- no. (%)	31 (33)	39 (41)
Pain events before study enrollment -- no. (%)	25 (27)	26 (27)
Acute chest syndrome before study enrollment -- no. (%)	3 (3)	5 (5)
Splenic sequestration before study enrollment -- no. (%)	5 (5)	10 (11)
Transfusion before study enrollment -- no. (%)	10 (11)	17 (18)
<i>Primary endpoint measurements:</i>		
Spleen Scan Uptake – no. (%)		
Normal	8 (8)	13 (13)
Present but decreased	73 (76)	72 (74)
Absent	15 (16)	12 (12)
DTPA GFR -- mL/min/1.73m ² (mean±S.D)	126±39	124±30
<i>Haematologic measurements (mean±S.D.):</i>		
Haemoglobin -- g/dL	9.0±1.3	9.2±1.3
HbF -- %	25.9±8.5	26.0±8.5
WBC -- x10 ³ /mm ³	14.3±5.3	14.3±5.9
Platelet Count -- x10 ³ /mm ³	365.5±125.2	375.0±126.7
<i>Central Nervous System measurements (mean±S.D.):</i>		
TCD TAMM Velocity -- cm/sec	124±22	120±22

Primary Endpoint Results

Table 2

	Hydroxyurea				Placebo				Difference ¹ (95% CI)	Effect Size	p-value [*]
	no.	N [†]	%	%Diff	no.	N [†]	%	%Diff			
Decreased Spleen Function at Exit (Compared with Baseline)	19	70	27		28	74	38		-11% (-26, 5)	-28%	0.21
DTPA GFR -- mL/min/1.73m ² (mean values)	67	123	146	18	66	125	146	17	2 (-16, 20)	0.04	0.84

[†]N: excludes subjects who had absent uptake at entry or who did not have exit spleen scan results.

Difference in the proportion of evaluable subjects with decreased splenic function in the Hydroxyurea group vs. the Placebo group.

^{*} p-value is from Fisher's exact test comparing distributions between the two treatment groups.

[^] N: Total number of subjects evaluated for each endpoint. N differs from number reported in Table 1, because only entry values that are paired with exit values (from the same subject) are included.

Difference in the mean increase in DTPA GFR in the Hydroxyurea group vs. the Placebo group.

[§] p-value is from a t-test statistic comparing the exit versus entry difference in means between the two treatment groups.

CI = Confidence Interval

Table 3

Secondary Endpoint Results

	Hydroxyurea			Placebo			Difference ¹ (95% CI)	p-value [*]		
	no.	N [^]	%	no.	N [^]	%				
Splenic Function										
Pitted Cell Counts ≥3.5% at Exit	41	86	48	51	82	62	-15% (-29%, 0.4%)	0.06		
Pitted Cell Counts ≥3.5% at Exit (Compared with Baseline)										
Better (number ≥3.5% →<3.5%)	9	85	11	3	82	4	7% (-8%, 22%) [#]			
Same	63		74	55		67	7% (-7%, 21%)			
Worse (number <3.5% →≥3.5%)	13		15	24		29	-14% (-26%, -2%)			
Howell Jolly Body Counts ≥300/10 ⁶ RBC at Exit	54	83	65	67	85	79	-14% (-28%, 1%)	0.06		
Howell Jolly Body Counts ≥300/10 ⁶ RBC at Exit (Compared with Baseline)										
Better (number ≥300 →<300)	1	76	1	0	82	0	1% (-14%, 17%) [#]	0.005		
Same	59		78	47		57	20% (6%, 35%)			
Worse (number <300 →≥300)	16		21	35		43	-22% (-36%, -8%)			
	N [^]	Entry	Exit	%Diff	N [^]	Entry	Exit	%Diff	Difference ² (95%CI)	p-value [§]
Howell-Jolly Bodies -- per 10 ⁶ RBC	76	663	1360	106	82	495	1470	197	-274 (-538, -10)	0.04
Pitted Cells -- %	85	4.3	5.7	32	82	4.6	8.4	84	-2.5 (-4.7, -0.2)	0.04
Spleen:Liver Ratio of Counts (400K)	84	0.36	0.28	-20	79	0.38	0.20	-46	0.10 (0.01, 0.19)	0.03
Spleen Volume -- cm ³	80	110	157	43	80	98	132	34	14 (-14, 42)	0.33
Renal Function										
Creatinine -- mg/dL	69	0.2	0.3	10	78	0.2	0.2	12	-0.005 (-0.04, 0.03)	0.77
Schwartz GFR -- mL/min/1.73m ²	69	192	218	13	73	196	229	17	-8 (-37, 21)	0.60

	Hydroxyurea			Placebo			Difference ^I (95% CI)	p-value [*]		
	no.	N [^]	%	no.	N [^]	%				
Cystatin C -- ug/mL	39	0.91	0.92	1	48	0.91	0.89	-3	0.03 (-0.05, 0.11)	0.42
Urine Osmolality -- mOsm/kg H ₂ O	81	384	494	29	84	400	454	13	57 (3, 110)	0.04
Urine pH	86	6.5	6.3	-2	82	6.6	6.3	-4	0.15 (-0.22, 0.51)	0.43
Urine Specific Gravity	86	1.010	1.012	0	82	1.012	1.011	0	0.002 (0.0004, 0.004)	0.02
Total Kidney Volume -- cm ³	80	61	91	48	79	58	97	67	-9 (-16, -2)	0.01
Central Nervous System										
TCD Velocity (TAMM)-- cm/sec	79	126	146	16	79	118	150	27	-12 (-18, -6)	<0.001
Bayley Mental MDI	85	97	97	1	80	97	94	-3	3 (-2, 8)	0.22
Bayley Motor PDI	85	97	101	5	79	97	99	2	2 (-3, 7)	0.37
Growth										
Height -- cm	87	75.9	96.2	27	82	75.7	96.2	27	-0.2 (-1.0, 0.6)	0.62
Weight -- kg	87	9.7	14.5	50	85	9.6	14.3	49	0.1 (-0.2, 0.4)	0.53
Head Circumference -- cm	85	46.9	50.3	7	82	46.8	50.3	8	-0.2 (-0.6, 0.2)	0.44
Pulmonary										
O2 Saturation -- %	86	99	99	0	86	99	98	-1	0.6 (-0.04, 1.2)	0.07
Laboratory Results										
Haemoglobin -- g/dL	79	8.9	9.1	3	79	9.2	8.6	-7	0.9 (0.5, 1.3)	<0.001
HbF -- %	80	25.6	22.4	-13	78	27.1	17.1	-37	6.7 (4.8, 8.7)	<0.001
MCV -- fL	79	80.2	92.2	15	79	80.0	86.2	8	5.7 (4.0, 7.5)	<0.001
WBC -- x10 ³ /mm ³	79	14.4	10.6	-27	79	14.3	13.9	-3	-3.4 (-6.0, -0.9)	0.008
Absolute Neutrophil Count -- x10 ³ /mm ³	69	4.9	4.5	-7	72	4.2	5.6	33	-1.7 (-2.9, -0.5)	0.005

	Hydroxyurea			Placebo			Difference ^f (95% CI)	p-value [*]		
	no.	N [^]	%	no.	N [^]	%				
Platelet Count -- x10 ³ /mm ³	78	374	351	-6	79	404	415	3	-33 (-87, 21)	0.23
Absolute Reticulocyte Count -- x10 ³ /mm ³	79	286	227	-21	79	265	277	5	-71 (-107, -35)	<0.001
Reticulocytes -- %	80	9.2	7.9	-14	79	8.2	10.0	22	-3.1 (-4.5, -1.8)	<0.001
Total Bilirubin -- mg/dL	81	1.6	1.8	16	85	1.6	2.3	46	-0.5 (-0.8, -0.1)	0.01

[^] N: Total number of subjects evaluated for each endpoint. N differs from the number reported in Table 1 because only entry values that are paired with exit values (from the same subject) are included.

^f Difference of (Exit – Entry) percentages in Hydroxyurea and Placebo groups

^{*} p-value is calculated using Fisher’s exact test under the assumption that proportions are equal in the Hydroxyurea and Placebo groups.

[#] Confidence interval is determined using the Exact test calculation from a non-central hypergeometric distribution.

² Difference of (Exit – Entry) differences in Hydroxyurea and Placebo groups

[§] p-value is calculated using Student’s t-test comparing the exit versus entry differences in means between the Hydroxyurea and Placebo groups.

TCDD = Transcranial Doppler ultrasound

TAMM = Time-averaged mean maximum (velocity)

MDI = Mental Developmental Index

PDI = Performance Developmental Index

Table 4

Adverse Events: Hydroxyurea Group vs. Placebo Group

	Hydroxyurea (N=96) (Subject-years=189)		Placebo (N=97) (Subject-years=185)		Hazard Ratio [^] (95% CI)	p-value [*]
	Events	Subjects	Events	Subjects		
Pain (all reports including pain)	177	62	375	75	0.59 (0.42 – 0.83)	0.002
Pain alone	63	37	121	55	0.54 (0.36 – 0.83)	0.004
Acute Chest Syndrome	8	7	27	18	0.36 (0.15 – 0.87)	0.02
Hospitalisation (for any cause)	232	69	324	84	0.73 (0.53 – 1.00)	0.05
Transfusion	35	20	63	33	0.55 (0.32 – 0.96)	0.03
Dactylitis	24	14	123	42	0.27 (0.15 – 0.50)	<0.001
Stroke	0	0	1	1		0.31
Priapism	4	3	2	2	1.48 (0.25 – 8.84)	0.67
Sepsis or Bacteraemia	3	2	6	5	0.40 (0.08 – 2.06)	0.26
Splenomegaly	127	32	88	36	0.87 (0.54 – 1.40)	0.56
Splenic Sequestration	12	8	12	9	0.88 (0.34 – 2.27)	0.79
Gastroenteritis	26	18	70	41	0.35 (0.20 – 0.60)	<0.001
Death	0	0	0	0		

[^] Hazard ratio and confidence interval are generated from a Cox model.^{*} p-values are generated from the log rank life test comparing the time to first event between the two treatment groups.

CI = Confidence Interval

Table 5

Toxicities: Hydroxyurea Group vs. Placebo Group

Clinical Toxicity Events	Hydroxyurea (N=96) (Subject-yrs=189)		Placebo (N=97) (Subject-yrs=185)		Hazard Ratio [^] (95% CI)	p-value [*]
	Events	Subjects	Events	Subjects		
Absolute Neutrophil Count <500/mm ³	5	5	2	2	2.5 (0.5 – 13)	0.26
Absolute Neutrophil Count 500–1250/mm ³	107	45	34	18	3.0 (1.7 – 5.1)	<0.001
Thrombocytopaenia (platelet count <80 ×10 ³ /mm ³)	12	11	8	7	1.6 (0.6 – 4.1)	0.32
Severe anaemia (Hb<7 g/dL+ARC<80×10 ³ /mm ³)	1	1	2	2	0.47 (0.04 – 5.2)	0.53
ALT (>150 U/L)	3	2	1	1	2.0 (0.2 – 22)	0.56
Bilirubin (>10 mg/dL)	0	0	1	1		0.31
Creatinine (≥2× baseline + ≥1.0 mg/dL)	0	0	0	0		
Skin and Subcutaneous Disorders	122	62	165	69	0.74 (0.5 – 1.0)	0.08

[^] Hazard ratio and confidence interval are generated from a Cox model.

^{*} p-values are generated from log rank life tests comparing the time to first event between the two treatment groups.

ARC = absolute reticulocyte count

ALT = alanine aminotransferase

CI = Confidence Interval