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Hydroxycarbamide versus chronic transfusion for maintenance of transcranial doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWiTCH): a multicentre, open-label, phase 3, non-inferiority trial.

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TCD With Transfusions Changing to Hydroxyurea (TWiTCH): a multicentre, randomised controlled trial

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Abstract

Background—For children with sickle cell anaemia and elevated transcranial Doppler (TCD) flow velocities, regular blood transfusions effectively prevent primary stroke, but must be continued indefinitely. The efficacy of hydroxyurea in this setting is unknown.

Methods—TWiTCH was a multicentre Phase III randomised open label, non-inferiority trial comparing standard treatment (transfusions) to alternative treatment (hydroxyurea) in children with abnormal TCD velocities but no severe vasculopathy. Iron overload was managed with chelation (Standard Arm) and serial phlebotomy (Alternative Arm). The primary study endpoint was the 24-month TCD velocity calculated from a general linear mixed model, with non-inferiority margin = 15 cm/sec.

Findings—Among 121 randomised participants (61 transfusions, 60 hydroxyurea), children on transfusions maintained <30% sickle haemoglobin, while those taking hydroxyurea (mean 27 mg/kg/day) averaged 25% fetal haemoglobin. The first scheduled interim analysis demonstrated non-inferiority, and the sponsor terminated the study. Final model-based TCD velocities (mean ± standard error) on Standard versus Alternative Arm were 143 ± 1.6 and 138 ± 1.6 cm/sec, respectively, with difference (95% CI) = 4.54 (0.10, 8.98), non-inferiority $p=8.82 \times 10^{-16}$ and post-hoc superiority p=0.023. Among 29 new neurological events adjudicated centrally by masked reviewers, no strokes occurred but there were 3 transient ischaemic attacks per arm. Exit brain MRI/MRA revealed no new cerebral infarcts in either arm, but worse vasculopathy in one participant (Standard Arm). Iron burden decreased more in the Alternative Arm, with ferritin difference −1047 ng/mL (−1524, −570), p<0.001 and liver iron difference −4.3 mg Fe/gm dry weight (−6.1, −2.5), p=0.001.

Interpretation—For high-risk children with sickle cell anaemia and abnormal TCD velocities, after four years of transfusions and without severe MRA vasculopathy, hydroxyurea therapy can substitute for chronic transfusions to maintain TCD velocities and help prevent primary stroke.

INTRODUCTION

Stroke is a devastating clinical event for children with sickle cell anaemia (SCA), causing severe motor and neurocognitive sequelae. Historically, stroke occurred frequently in this patient population, with an incidence of 0.61–0.76 events per 100 patient-years and cumulative childhood incidence of 7–11%. [1] After first stroke, there is a high rate of recurrence or progression even with chronic transfusion therapy; a recent publication with median transfusion duration of 5.5 years documented 17.5% of children on transfusions developed an overt stroke while an additional 27.5% had new silent cerebral infarcts. [2] Transcranial Doppler (TCD) screening of intracranial blood flow identifies children at risk for stroke; elevated time-averaged mean velocities in the distal internal carotid or middle cerebral arteries confer an increased risk, and velocities 200 cm/sec are associated with a 40% primary stroke rate over 3 years. [3] The multicentre STOP trial demonstrated that regular blood transfusions, with the goal of maintaining <30% sickle haemoglobin (HbS), reduced the rate of first stroke by 90% in children with SCA and abnormal TCD velocities. [4]

Aiming to limit the long-term consequences of chronic transfusions, the STOP2 trial investigated whether therapy could be safely stopped in patients who normalised their TCD

velocities after 30 months of transfusions and lacked severe vascular disease. This trial was terminated early, due to reversion to abnormal TCD velocities in 14 participants and two overt strokes in children discontinuing transfusions compared to none who continued transfusions, suggesting that indefinite therapy for primary stroke prevention was required. [5] With annual TCD screening of children with SCA now strongly recommended, [6] and evidence that transfusions successfully reduce the rate of first stroke, [7] an alternative therapy that maintains TCD velocities with fewer complications than transfusions could increase acceptance and long-term adherence, and further decrease primary stroke in this high-risk population.

Hydroxyurea has emerged as an important disease-modifying treatment for SCA, with proven laboratory and clinical efficacy for children and adults. [8, 9] Through fetal haemoglobin (HbF) induction and other mechanisms of action, hydroxyurea inhibits intracellular sickle haemoglobin (HbS) polymerisation and lowers the risk of vaso-occlusive complications. [10] Hydroxyurea has the potential for lowering TCD velocities and thus reducing stroke risk, [11, 12] but definitive data are lacking regarding its efficacy in cerebrovascular disease. We hypothesized that hydroxyurea was non-inferior to transfusions for maintaining TCD velocities, after discontinuation of initial transfusion therapy to prevent primary stroke. To determine the efficacy of hydroxyurea in this setting, we conducted TCD With Transfusions Changing to Hydroxyurea (TWiTCH), a multicentre, open label, controlled Phase III randomised clinical trial.

METHODS

Study design

TWiTCH was a non-inferiority trial (ClinicalTrials.gov NCT01425307), comparing Alternative Treatment (hydroxyurea) to Standard Treatment (transfusions) for maintaining TCD velocities as a surrogate for stroke risk, based on published data showing that elevated velocities confer an increased stroke risk. [3, 5] Patients from 26 paediatric programmes (Appendix) between 4–16 years of age with SCA and abnormal TCD velocities were eligible after 12 months of chronic transfusions. Documented clinical stroke, transient ischaemic attack, or severe vasculopathy were exclusions. After local IRB approval and with written informed consent, eligibility screening included original abnormal TCD verification and baseline brain MRI/MRA, TCD examination using identical non-imaging instruments (SonaraTek, Middleton, WI), liver iron concentration (LIC) by FerriScan® R2-MRI (Resonance Health, Claremont, Australia), abdominal ultrasonography and MRI, neurocognitive testing, and quality of life assessments. Participants with baseline Grade 4 or higher severe brain MRA vasculopathy, defined as moderate stenosis in >2 arterial segments or severe stenosis/occlusion in 2 segments, [13] or with inadequate TCD velocities from poor blood flow or bone windows, were considered screening failures and removed from the study. Children with normal or persistent conditional/abnormal screening TCD velocities on chronic transfusions remained eligible for randomisation. Participants completing screening were randomised 1:1 to study treatments. Randomisation was stratified by site and blocked, and an adaptive randomization scheme was employed to balance the covariates of baseline age and TCD velocity (See Supplement for further details).

Endpoints

The primary study endpoint was the maximum TCD time-averaged mean velocity on the index side, defined as the cerebral hemisphere with the higher mean arterial velocity on baseline evaluation. TCD velocities were obtained monthly in triplicate during screening and exit, and once at 12-week intervals during the 24-month treatment period. TCD examinations were performed just before transfusions or phlebotomy, and all were read centrally by observers masked to treatment assignment and prior TCD results. Secondary endpoints included TCD velocity on the non-index side, new stroke or non-stroke neurological events, new brain MRI/MRA lesions, hepatic iron overload, sickle-related events, neuropsychological status, quality of life, growth, and treatment-related complications.

Study treatments

Participants randomised to Standard Treatment continued monthly transfusions to maintain 30% HbS, with local discretion regarding transfusion type (simple, partial exchange, or erythrocytapheresis). Deferasirox was recommended for iron overload; children on chelation maintained their current dose, while those starting chelation received deferasirox at 10–40 mg/kg/day, depending on their screening LIC value. Participants randomised to Alternative Treatment initiated hydroxyurea at 20 mg/kg/day with escalation to maximum tolerated dose (MTD), defined as moderate marrow suppression of neutrophils and reticulocytes as previously described. [14] Transfusions were slowly weaned over 4–9 months to protect against stroke during hydroxyurea dose escalation to MTD, using a standardised protocol. [15] After MTD was established and transfusions were discontinued, serial phlebotomy removed 10 mL/kg (maximum 500 mL) venous blood over 30–60 minutes at each 4-week study visit, again using a standardised protocol. [15, 16] Smaller phlebotomy volumes (5 mL/kg) were removed for haemoglobin concentrations of 8.0–8.5 gm/dL, and phlebotomy was not performed when the haemoglobin concentration was <8.0 gm/dL. The treatment period was 24 months after randomisation, with a 6-month visit after completing exit studies.

Statistical analyses

TWiTCH was a randomised, open label trial based on the endpoint variable of centrally determined TCD time-averaged mean arterial velocities. The primary endpoint was the 24-month TCD velocity, calculated from a general linear mixed model using all TCD velocities captured throughout the trial (Supplement), by intention-to-treat with two planned interim analyses after 33% and 67% of participants completed exit studies. The stopping guidelines for non-inferiority used the Lan-DeMets version of the O'Brien-Fleming group sequential method. [17] A one-sided non-inferiority margin of 15 cm/sec was used in the analysis, representing the biological variation of TCD examinations. [18] Sample size estimates included enrolment of 148 children, with 20% dropout during screening to yield 118 randomised participants, followed by 15% post-randomisation dropout, so that 100 participants (50 per arm) would complete the 24-month treatment period. This study design provided at least 90% power to test the non-inferiority hypothesis assuming a difference of 5 cm/sec (alternative higher than standard) and a standard deviation of 24 cm/sec.

Comparisons of continuous variables were performed using t-tests, and comparisons of categorical variables were by chi-squared analyses. Analyses were "intention to treat" except for a planned on-protocol analysis of TCD velocities, which excluded participants who exited the study early. A sensitivity analysis adjusting for baseline age was also performed. All analyses were performed using STATA 14.0 (College Station, Texas, USA) or SAS 9.4 (Cary, North Carolina, USA).

Stroke adjudications

All new potential stroke events were evaluated with careful neurological evaluation and prompt brain MRI/MRA examinations, and then adjudicated centrally by a panel of expert reviewers. Independent and then consensus opinions were obtained from neurologists and neuroradiologists masked to study treatment. Participants with "possible" or "likely" stroke based on new neurological signs or symptoms, but without corresponding radiological changes, were scored as transient ischaemic attack. Exit brain MRI/MRA examinations allowed confirmation that no strokes had been missed by the adjudication process.

Study monitoring and safety considerations

An NHLBI-appointed Data and Safety Monitoring Board reviewed all enrolment, safety, toxicity, and efficacy data, including new stroke adjudications, adverse events, and interim analyses. The Principal Investigator was masked to all treatment-related results, and local investigators were masked to TCD results. Protocol-defined rescue transfusions were administered to participants in either treatment arm for perceived elevated stroke risk, such as failure to suppress %HbS on the Standard Arm and excessive toxicity or failure to achieve marrow suppression targets on the Alternative Arm. A protocol-defined alert algorithm identified participants whose TCD velocities varied substantially from baseline and might confer increased stroke risk (Supplement), and these children had additional evaluations and closer therapeutic monitoring.

Role of the funding source

The study sponsor was the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health. NHLBI did not participate in the following aspects of the research: (1) study design; (2) data collection, analysis, or interpretation; (3) writing the report; or (4) decision to submit the publication.

RESULTS

Screening and enrolment

A total of 159 patients consented and enrolled in TWiTCH (Figure 1), including 38 ineligible for randomisation: 19 had severe vasculopathy, 10 withdrew from the study, 7 had inadequate screening TCD, 3 failed original TCD verification, and 1 had an inadequate brain MRI/MRA (2 participants failed for 2 reasons). Therefore, 121 participants were randomised, 3 more than the protocol specified, forming the Intention-To-Treat population (61 Standard Arm, 60 Alternative Arm). Most baseline demographic, clinical, and laboratory characteristics were equivalent between treatment arms, except for higher WBC, ANC, and bilirubin in the Alternative Arm. Despite the exclusion criterion of clinically overt stroke,

over one-third of the participants in both treatment arms had evidence of prior silent cerebral infarctions (Table 1).

Study treatment

Participants assigned to Standard Treatment received primarily simple transfusions (57%), with some partial exchange transfusions (31%) or automated erythrocytapheresis (12%). The haemoglobin concentration remained steady at ~9 gm/dL (Figure 2A) with average HbS <30% throughout the study treatment period (Figure 2C). Chelation was provided to children on transfusions with hepatic iron overload, typically deferasirox starting at an average dose of 25.7 ± 6.0 mg/kg/day. On the Alternative Arm, all 60 participants started hydroxyurea at 20 mg/kg/day followed by dose escalation. MTD was achieved in 57/60 children (95%), who then discontinued transfusions per protocol; two did not reach MTD due to medication non-adherence leading to study withdrawal and one had an early adjudicated transient ischemic attack. The average time to MTD was 29 weeks (median 27, range 15–59 weeks); average hydroxyurea MTD was 26.9 ± 4.3 mg/kg/day (median 27.4, range 15.6–35.6 mg/kg/day). On hydroxyurea, the haemoglobin concentration remained stable at ~9 gm/dL, along with expected significant hematological changes in MCV, %HbF, white blood cell count, neutrophils, platelets, and reticulocytes (Figure 2). Marrow suppression targets were achieved with mean absolute neutrophil count at MTD of $3.5 \pm 1.6 \times 10^9$/L (median 3.4, range 1.5–10.1×10^9/L) and final average neutrophil count of 3.6×10^9/L (Table 2). HbF responses included a mean MTD value of 27.0 ± 5.7% (median 26.2, range 15.5–38.5%) and final average HbF of 24.4%.

Primary study endpoint

The two treatment arms were balanced for baseline TCD velocities, but the final average velocity on the Alternative Arm was slightly lower than the average velocity on the Standard Arm (Figure 3A and 3B). After full enrolment and 37% of the participants exited, the first scheduled interim analysis revealed the stopping boundary was passed and non-inferiority was demonstrated. After 50% of participants exited, repeat analyses confirmed these findings and the study was terminated by NHLBI. Remaining participants then completed all exit studies before discontinuing protocol-directed study treatment. In total, the Standard Arm included 42 who completed study treatment, 11 truncated treatment, and 8 exited early; the Alternative Arm included 41 who completed treatment, 13 truncated treatment, and 6 exited early. The final model-based TCD velocities (mean ± standard error) on the Standard Arm versus Alternative Arm were 143 ± 1.6 and 138 ± 1.6 cm/sec, respectively, with difference (95% CI) = 4.54 (0.10, 8.98), non-inferiority $p=8.82 \times 10^{-16}$ and a post-hoc superiority p=0.023. The planned per-protocol analysis excluding study participants who exited the study early showed almost identical findings, with difference = 5.06 (0.56, 9.57), non-inferiority $p=1.05 \times 10^{-15}$ and a post-hoc superiority p=0.015. An age-adjusted analysis gave almost identical findings (not shown). Figure 3C shows baseline (enrolment) TCD velocities and final (exit) velocities for each TWiTCH study participant, illustrating that the vast majority of velocities were in the normal range at both study entry and exit. No child in either treatment arm reverted from normal to abnormal TCD velocities. Additional exit laboratory values are provided in Table 2.

Stroke adjudications and new neurological events

Central stroke adjudication occurred for 29 possible new neurological events (12 on the Standard Arm, 17 on the Alternative Arm). No child had a positive adjudication for stroke, although 6 were labeled transient ischaemic attack (3 in each arm, see Supplement for more details). Exit brain MRI/MRA examinations revealed no new cerebral infarcts in either treatment arm, including all participants with negative stroke adjudications. Worse vasculopathy developed in one participant on the Standard Arm.

Iron unloading

Comparing baseline and exit values, average serum ferritin on the Standard Arm remained stable at 2206 and 2674 ng/mL, respectively, while average LIC rose slightly from 8.5 to 11.3 mg Fe/gm dry weight liver (p=0.052). A total of 19 adverse events were attributed to deferasirox chelation treatment, which occurred in 9 participants (15%) randomised to the Standard Arm. The most frequent events included elevated hepatic transaminases (11 in 5 children), gastrointestinal pain or other symptoms (5 in 4 children), elevated serum creatinine (1), elevated serum bilirubin (1), and rash (1). On the Alternative Arm, a mean of 7 ± 3 monthly overlap transfusions (median 6) were provided without chelation. Among 57 participants who then reached hydroxyurea MTD, 54 received phlebotomy. A total of 756 phlebotomy procedures (mean 13/child) were performed with an average total blood removal of 103 ± 54 mL/kg. Of these, 47 procedures (6.2%) did not remove the full scheduled volume, primarily due to loss of venous access (37), symptoms (7) or other reasons (3). An additional 77 phlebotomy procedures were appropriately canceled per protocol due to low haemoglobin concentration <8.0 g/dL, while another 81 were canceled for various reasons including planned anesthesia (16), provider preference (14), hydroxyurea toxicity (13), intercurrent illness (11), inadequate venous access (9), family request (5), or other (13). A total of 18 adverse events were attributed to phlebotomy procedures, which occurred in 14 participants (23%) randomised to the Alternative Arm. Comparing entry and exit values in the Alternative Arm, the average serum ferritin decreased from 3080 to 1276 ng/mL (p<0.0001), and average hepatic iron decreased from 11.3 to 9.5 mg Fe/gm dry weight liver (p=0.001). Comparing the differences between treatment arms, iron overload improved more in the Alternative Arm with ferritin difference −1047 ng/mL (−1524, −570), p<0.001 and liver iron difference −4.3 mg Fe/gm dry weight liver (−6.1, −2.5), p=0.001, Table 2.

Safety Monitoring

In the Standard Arm, a total of 12 rescue transfusions were administered to 7 participants, all for >45% HbS. In the Alternative Arm, a total of 15 rescue transfusions were administered, including 9 during the initial overlap period, to 8 participants with a low hydroxyurea response. One child on the Standard Arm developed TCD velocities >240 cm/sec and was exited per the TCD Alert algorithm (Supplement). Adverse events were fairly well balanced between treatment arms but serious adverse events were more common in the Alternative Arm (Supplemental Table); there were 287 sickle-related adverse events (10 serious adverse events among 6 participants) in the Standard Arm versus 279 sickle-related adverse events

(23 serious adverse events among 9 participants) in the Alternative Arm. No deaths occurred during the study treatment period.

DISCUSSION

The TWiTCH trial results document the efficacy of hydroxyurea therapy for a cohort of children with SCA at high risk for primary stroke. Specifically, children on the Alternative Arm, who received an overlap period with transfusions until achieving a stable hydroxyurea MTD, successfully maintained their average TCD velocity throughout the study treatment period. The primary study endpoint (model-based TCD velocities) on the Alternative Arm was non-inferior to the Standard Arm with continued monthly transfusions, thus avoiding the frequent and unacceptable TCD velocity increases observed in STOP2 when no substitute therapy was provided. Since TCD is a direct measurement of intracerebral blood flow and an accepted surrogate for primary stroke risk in children with SCA, these findings suggest that hydroxyurea may also be effective for stroke prevention in this setting, although stroke was not the primary study endpoint of the TWiTCH trial. Importantly, no child in either treatment arm reverted from normal to abnormal TCD velocities, and no new overt, adjudicated, or silent strokes occurred on either treatment arm.

Hydroxyurea has multiple therapeutic effects that should be beneficial for children with SCA and abnormal TCD velocities. The most important benefit is HbF induction, which lowers the intracellular %HbS, inhibits sickle polymer formation, and prolongs erythrocyte survival. TWiTCH participants had a robust treatment response to hydroxyurea, reaching an average 27% HbF at MTD. However, since inflammation and endothelial vasculopathy are features of cerebrovascular disease, the reduced leukocyte and reticulocyte counts from hydroxyurea should also be salutary (Figure 2). Other recognised benefits of hydroxyurea include macrocytosis with increased erythrocyte deformability, reduced adhesiveness, and lower blood viscosity with improved rheology. [10] Immediate anti-inflammatory effects of hydroxyurea on the vascular endothelium have also been reported. [19]

The compelling results of the TWiTCH trial with early study termination are highly relevant to the management of this high-risk subset of children with SCA. TCD screening programmes are now well-established at most paediatric sickle cell centres and indefinite chronic transfusions are recommended by NHLBI guidelines for children with abnormal TCD velocities to prevent primary stroke. [6] However, successful comprehensive TCD screening with chronic transfusions can be difficult to implement in practice, in part due to concerns about indefinite transfusion therapy. [20–22] Children with abnormal TCD velocities now may have an alternative therapeutic option after a period of transfusions, which represents an important paradigm shift for management of stroke risk in this vulnerable patient population. The ability to screen children with SCA and offer those with abnormal TCD velocities an initial transfusion programme, followed by hydroxyurea for continued stroke prophylaxis, would likely be attractive for many families and providers. Hydroxyurea could improve implementation of primary stroke screening and treatment strategies, by reducing transfusion-associated morbidity and offering potential cost-savings. Since no new infarctions developed in either treatment arm, hydroxyurea therapy may also

be relevant in the setting of silent cerebral infarctions, where transfusions have shown efficacy for preventing overt stroke and disease progression. [23]

However, before considering the discontinuation of chronic transfusion therapy and starting hydroxyurea for stroke prevention, several key caveats must be noted. First, children with severe vasculopathy [13] were excluded from TWiTCH randomisation, so these children may not be suitable candidates for hydroxyurea. However, this criterion excluded only 12% of children enrolled in TWiTCH and unlike STOP2, the TWiTCH trial included children with persistently elevated TCD velocities despite chronic transfusions, which increased the number of eligible patients and ultimately should broaden applicability. [24] Second, all children received   12 months of transfusions (average 4 years) and then continued transfusions during an overlap period with hydroxyurea. In the setting of an abnormal TCD, transfusions should always be the initial treatment and cannot be abruptly discontinued, although the optimal duration of transfusions before considering transition to hydroxyurea has not been determined. Third, hydroxyurea was escalated to MTD to achieve target goals of myelosuppression with concomitant robust HbF responses; lower dosing regimens, infrequent monitoring, and poor adherence would reduce efficacy and potentially be associated with more sickle-related clinical events, especially during the overlap period. An additional limitation of this study is the fact that many study participants were older than the peak age of stroke incidence, although still within the age range of primary infarctive stroke. [1] Finally, the duration of hydroxyurea therapy without transfusions was relatively short; longer follow-up is clearly warranted to determine whether these findings are maintained over time.

The management of transfusion-acquired iron overload is also a challenge for children on chronic transfusion therapy. Participants in the Alternative Arm, after reaching a stable hydroxyurea MTD and discontinuing transfusions, received monthly therapeutic phlebotomy to reduce their iron burden. Previously shown to be feasible and potentially beneficial in this setting, [16, 25] hydroxyurea with serial phlebotomy was superior to transfusions with chelation for managing iron overload in the TWiTCH population. With an average of 7 transfusions and 13 phlebotomy procedures, participants on hydroxyurea had significantly lower serum ferritin and LIC than those on continued transfusions with oral chelation (Table 2). Repeated phlebotomy is safe in children with SCA, and with sufficient duration can lead to full resolution of hepatic iron overload. [26, 27]

Prevention of cerebrovascular disease in children with SCA is the ultimate goal, ideally through early intervention with disease-modifying therapy. Although genetic variants may influence stroke susceptibility, [28, 29] most stroke events in children with SCA remain unexplained. Data from the BABY HUG cohort suggest that early hydroxyurea therapy may prevent TCD elevation and neurodevelopmental decline. [30] New NHLBI guidelines recommend that all infants with SCA, age 9 months or above, be offered hydroxyurea treatment, regardless of disease severity. [6] If this recommendation is followed, the incidence of cerebrovascular disease will likely decline. Until then, hydroxyurea can now be considered a substitute therapy for maintaining TCD velocities and potentially preventing primary stroke in selected high-risk children with SCA after a period of transfusions.

PUTTING RESEARCH INTO CONTEXT

Evidence before this study

A careful search of the literature, plus review of the recently published NHLBI evidence-based guidelines, identified several open-label trials of either blood transfusions or hydroxyurea for children with SCA and cerebrovascular disease, three randomised clinical trials of transfusions versus observation for children with SCA and cerebrovascular disease (two for abnormal TCD velocities, one for silent cerebral infarcts), and one randomised clinical trial comparing transfusions to hydroxyurea for children with SCA and previous overt stroke. To date, no study has prospectively compared blood transfusions to hydroxyurea for children with SCA and abnormal TCD velocities, although this is the largest group of children with SCA who currently receive chronic transfusions, and no alternative treatment to transfusions is currently available.

Added value of this study

This multicentre randomised clinical trial provides definitive evidence that hydroxyurea treatment is non-inferior for maintaining TCD velocities, compared to continued transfusions. The results allow a major paradigm shift in the long-term management of children with SCA and established cerebrovascular disease.

Implications of all the available evidence

Children with SCA and abnormal TCD velocities are currently prescribed indefinite transfusion therapy. Now after a period of transfusions, if brain MRA does not show severe vasculopathy, a substitute regimen of hydroxyurea treatment can be considered to maintain TCD velocities and prevent primary stroke in this high-risk patient population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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APPENDIX

Texas Children's Hospital, Houston, Texas – *Clinical Investigators:* Alex George, MD, PhD, Brigitta U. Mueller, MD, MHCM; *Study Coordinator:* Bogdan R. Dinu, MD; *TCD Examiner:* Leanne Gerhart

Boston Children's Hospital, Boston, Massachusetts – *Clinical Investigator:* Matthew M. Heeney, MD; *Study Coordinators:* Latoya N. Lashley, MPH, Jesse K. McKenna, MPH, Kristen E. Sullivan, MA, Meredith L. Anderson, DO(c), BS; *TCD Examiner:* Carol E. Barnewolt, MD

Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio – *Clinical Investigator:* Theodosia A. Kalfa, MD, PhD; *Study Coordinators:* Adriane R. Hausfeld, RN, BSN, CCRP, Laurie A. Vanderah, RN, BSN; *TCD Examiner:* Janet M. Adams

Children's Hospitals and Clinics of Minnesota, Minneapolis, Minnesota – *Clinical Investigator:* Stephen Nelson, MD; *Study Coordinators:* Ashley Kinsella, Nicole Hart, RN; *TCD Examiner:* Nancy Boettger

Emory/CHOA, Atlanta, Georgia – *Clinical Investigator:* R. Clark Brown, MD PhD; *Co-Investigator:* Beatrice Gee, MD; *Study Coordinators:* Robin Pitts, FNP, MN, Mitchell Turner, Martha Ann Felder, Leann Schilling, MPH, Terrell Faircloth; *TCD Examiners:* Vivian Davis, Melissa Barber

Children's Hospital of Philadelphia, Philadelphia, Pennsylvania – *Clinical Investigator:* Janet L. Kwiatkowski, MD, MSCE; *Co-Investigator:* Kim Smith-Whitley, MD; *Study*

Coordinators: John H. Hammond, Helen Stanley, Sage T Green, Hannah Ware; *TCD Examiner:* Brandi Kozak

The Hospital for Sick Children, Toronto, Ontario, Canada – *Clinical Investigator*: Isaac Odame, MB ChB, FRCPath, FRCPC; *Study Coordinators*: Rebecca Leroux, RN, BScN; *TCD Examiner*: Manuela Merelles-Pulcini RN, BScN, MSN

Children's National Medical Center, Washington, DC – *Clinical Investigator*: Lori Luchtman-Jones MD; Jennifer Webb, MD; *Co-Investigators*: Brenda Martin, MSN CPNP, and Elizabeth Yang, MD PhD; *Study Coordinator*: Chantel Jenkins MSHS; *TCD Examiner*: Kadine L. Linden, BS, RT(R), RDMS

Columbia University – *Clinical Investigator*: Margaret T. Lee, MD; *Study Coordinator*: Susana Hernandez, RN; *TCD Examiner*: Samuel Trocio, Jr., MD, RVT

Rainbow Babies & Children's Hospital, Case Western Reserve University, Cleveland, Ohio – *Clinical Investigator*: Connie Piccone, MD; *Study Coordinator*: Mary Debarr, RN, BSN; *Additional*: Anne Chapman, RN, BSN

University of South Alabama (USA), Mobile, Alabama – *Clinical Investigator*: Hamayun Imran, MD, MSc; *Study Coordinators*: Jennifer Williams, CRNP, Andretta McCovey, RN, BSN, CRA; *TCD Examiner*: Lee Vick

Medical University of South Carolina, Charleston, South Carolina – *Clinical Investigator*: Sherron M. Jackson, MD; *Study Coordinator*: Lisa Kuisel, RN, BSN; *TCD Examiners*: Toni Mullins, Shannon Harmon

Cohen Children's Medical Center of New York, New Hyde Park, New York – *Clinical Investigator*: Banu Aygun, MD, Sharon Singh, MD; *Study Coordinator*: Antonella Farrell, BSN, RN; *TCD Examiner*: Albert Levy, RVT

St. Jude Children's Research Hospital, Memphis, Tennessee – *Clinical Investigator*: Kerri Nottage, MD, MPH, Jane S. Hankins, MD, MS; *Study Coordinators*: Jennifer Larkin MS, CCRP; Karen Wodowski, MSN, BC-PNP; Eileen N. Hansbury PA-C; *TCD Examiner*: Gail L. Fortner, BSN, RN

State University of New York-Downstate Medical Center, Brooklyn, New York – *Clinical Investigator*: Scott T. Miller, MD; *Study Coordinator*: Kathy Rey, PAC; *TCD Examiner*: Kathy Rey, PAC

University of Alabama at Birmingham (UAB) Birmingham, Alabama – *Clinical Investigator*: Lee Hilliard, MD; *Study Coordinator*: Jeanine Dumas, RN, MSN; *TCD Examiner*: Jeanine Dumas, RN, MSN

University of Miami Miller School of Medicine, Miami, Florida – *Clinical Investigator*: Ofelia Alvarez, MD; *Study Coordinator*: Tally Hustace, ARNP; *TCD Examiner*: Iszet-Campo Bustillo; *Neurologist*: Robert Lopez Alberola, MD; *Psychologist*: Winsome Thompson, PhD

University of Mississippi Medical Center (UMMC), Jackson, Mississippi – *Clinical Investigator*: Melissa Rhodes, MD, Rathi Iyer, MD; *Study Coordinator*: Stephanie Pepper, RN; Glenda Thomas, RN; *TCD Examiner*: Desirae Flohr

UT Southwestern, Dallas, Texas – *Clinical Investigator*: Zora R. Rogers, MD; *Study Coordinator*: Leah Adix, CCRP; *TCD Examiner*: Brad Cook, RN

Children's Hospital of Michigan, Wayne State University School of Medicine, Detroit, Michigan – *Clinical Investigator*: Sharada A. Sarnaik, MD; *Study Coordinator*: Theresa Spranger, MS; *TCD Examiner*: Sally Thompson-Reid, RD, MS; *Study Nurse Coordinator*: Mary Murphy, RN, MSN, PNP

Anna and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois – *Clinical Investigator*: Alexis A. Thompson, MD, MPH; *Study Coordinator*: Katherine Bianchi, MBA, CCRP; *TCD Examiner*: Katherine Bianchi

Children's Hospital of The King's Daughters, Norfolk, Virginia - *Clinical Investigator*: William C. Owen, MD; *Study Coordinator*: Sabrina Wigginton, BSN, RN, CPON; *TCD Examiner*: Anissa Lambert, BSN, RN; *Additional*: Lorrie Coggsdale, RN, CCRP, Annette S. Slade, MSN, PPCNP-BC

Nemours Children's Clinic - *Clinical Investigator*: Cynthia Gauger, MD; *Study Coordinators*: Mary Lawlor-Barry, RN, Hollie Bonnema; *TCD Examiner:* Judy Craig

University of South Carolina/ Palmetto Health, Columbia, South Carolina – *Clinical Investigator*: Carla Roberts, MD; *Study Coordinator*: Selena Lollar, RN; *TCD Examiner*: Kristen Inman, BA, RDCS, RVT; *Additional*: Carson Gross, LPN

Duke University Medical Center, Durham, North Carolina – *Clinical Investigator*: Jennifer A. Rothman, MD; *Study Coordinator*: Shelly E. Burgett, FNP; *TCD Examiner*: Lance Boland, BS, RVT; *Additional*: Heather A. Nicoletto, BS, RVT

Brody School of Medicine at East Carolina University, Greenville, North Carolina – *Clinical Investigator*: Beng Fuh, MD; *Study Coordinator*: Cynthia Brown, CCRP, Ashley Allsbrook, MPH; *TCD Examiner*: James Walker, RDMS; *Additional*: Charles Daeschner, MD

Medical Coordinating Center

Cincinnati Children's Hospital, Cincinnati, Ohio - *Principal Investigator*: Russell E. Ware, MD, PhD; Clinical Coordinator and *Medical Monitor*: William H. Schultz, MHS, PA-C; *Project Manager:* Susan Stuber MA, CCRP, RAC; *Study Monitors*: Sophie Perier, Lisa Campbell, Jan Englehart, Teresa Latham

Data Coordinating Center

UT School of Public Health, Houston, Texas - *Principal Investigator*: Barry R. Davis, MD, PhD; *Co-Investigator*: Sara Pressel, MS, Peng Wei, PhD, Seoun Kim, PhD; Project

Manager: Cecilia Lara, BS; *Safety*: Linda Piller, MD, MPH, Lara Simpson, PhD, Aliza Matusevich

Neurology and TCD Core

MUSC Stroke Center, Charleston, South Carolina - *Principal Investigator*: Robert J. Adams, MD, PhD; *Others:* Judy Luden, Ellen Debenham, Corrine Hilbert
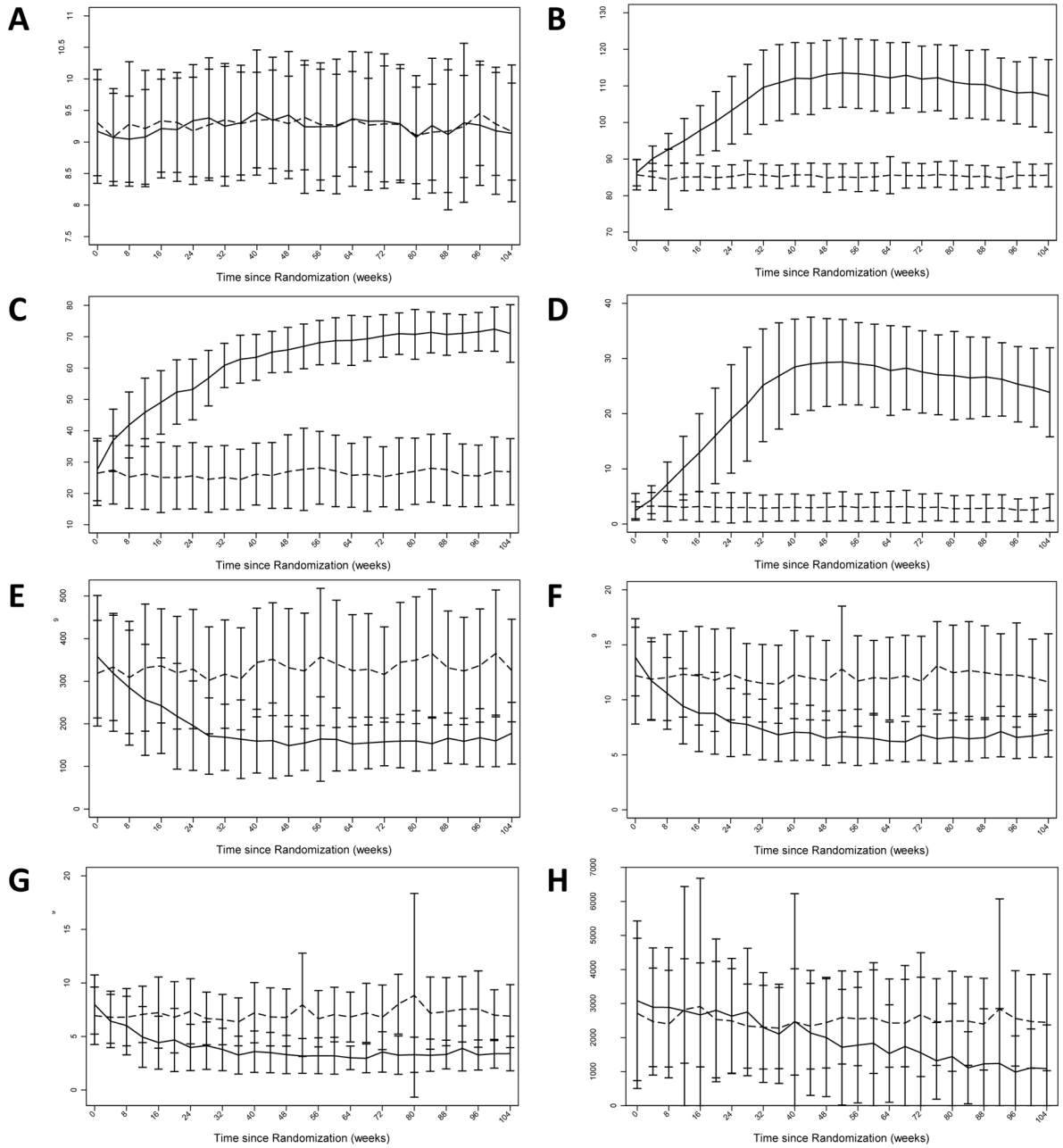
Central Laboratory

Abdullah Kutlar, MD and Niren Patel, MBBS

Consultants

Abdominal MRI: John C. Wood, MD PhD; *Neuroradiology*: Kathleen J. Helton, MD and Donna Roberts, MD; *Ultrasound*: Jamie Coleman, MD; *Neurocognitive*: Melanie J. Bonner, PhD; *Hematology*: Nicole Mortier, MHS, PA-C; *Transfusions*: Naomi Luban, MD; *Iron/ Chelation*: Alan R. Cohen, MD
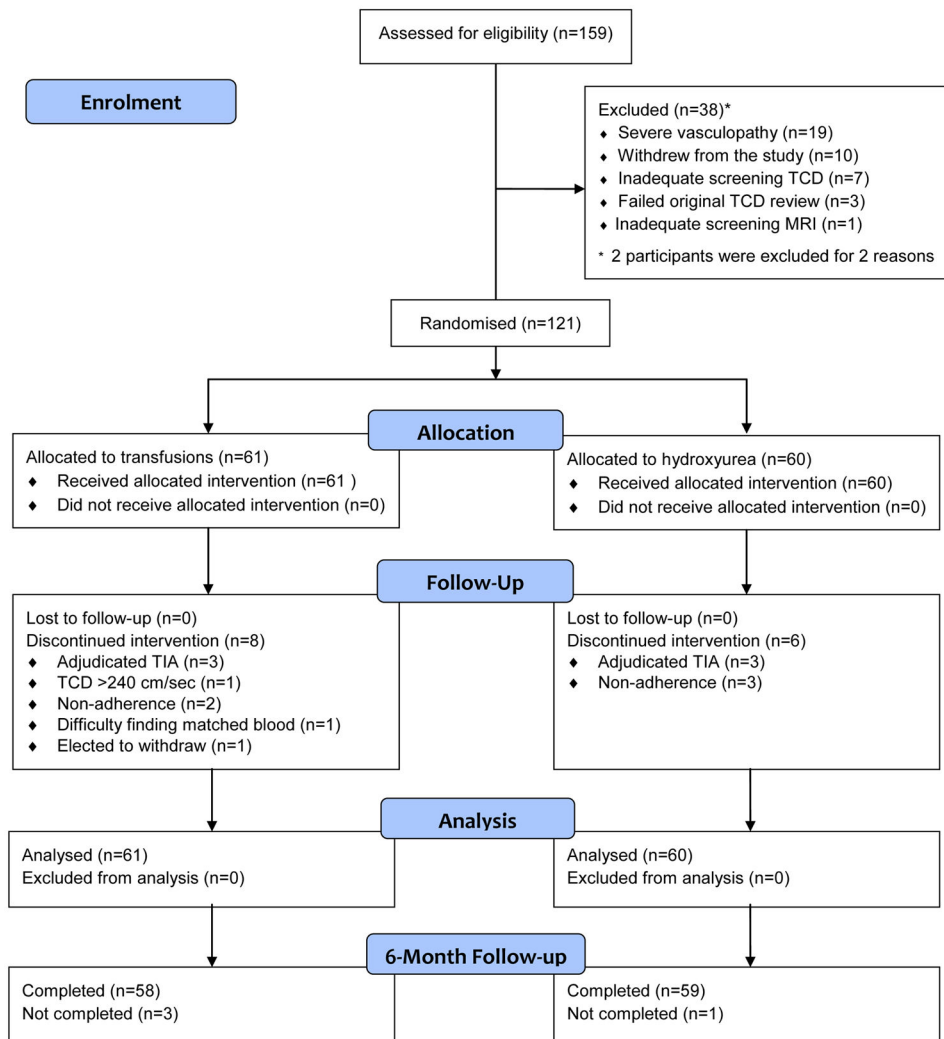
Figure 1.
TWiTCH CONSORT flow diagram showing the enrollment, randomisation, and follow-up of the TWiTCH study participants.

Figure 2.
Laboratory parameters based on intention-to-treat population: Panel A = hemoglobin concentration; Panel B = mean corpuscular volume; Panel C = %HbS; Panel D = %HbF; Panel E = white blood cell (WBC) count; Panel F = absolute neutrophil count (ANC); Panel G = absolute reticulocyte count (ARC); Panel H = serum ferritin. Complete blood counts and reticulocytes were measured locally, while hemoglobin electrophoresis and ferritin were analysed centrally. Data are illustrated as mean ± 1 standard deviation. The Standard Treatment Arm is portrayed in dashes while the Alternative Treatment Arm is shown by the

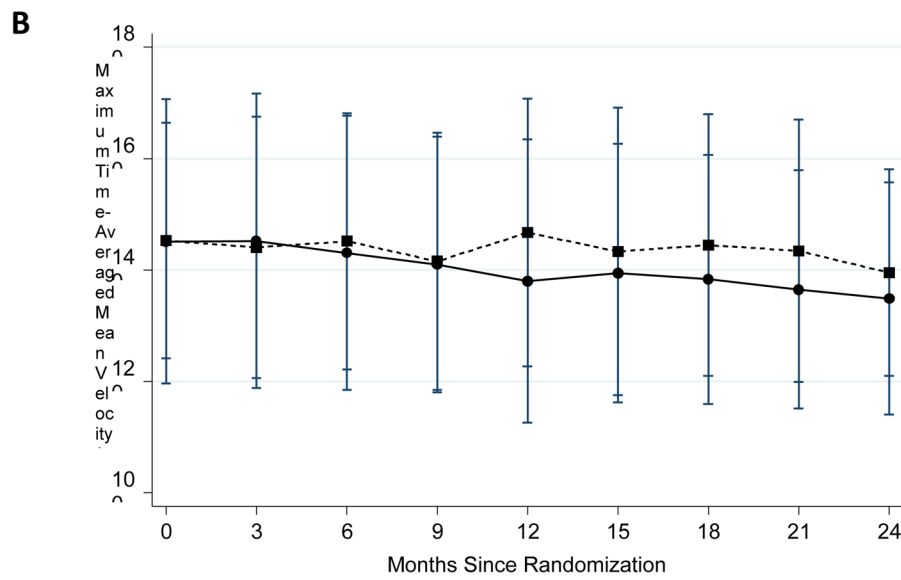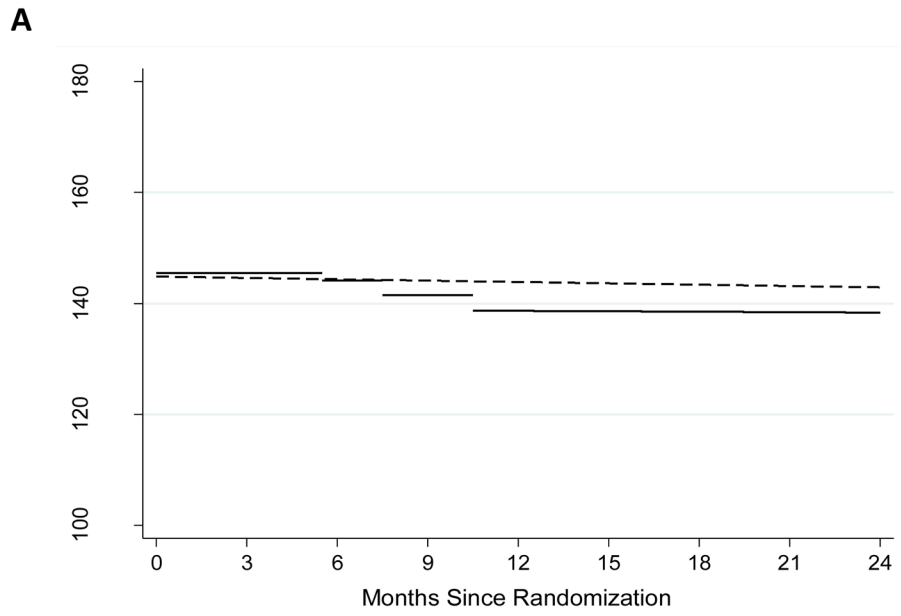solid line. All parameters are significantly different at exit ($p < 0.0001$) between treatment groups except Panel A, with $p = 0.800$.

A

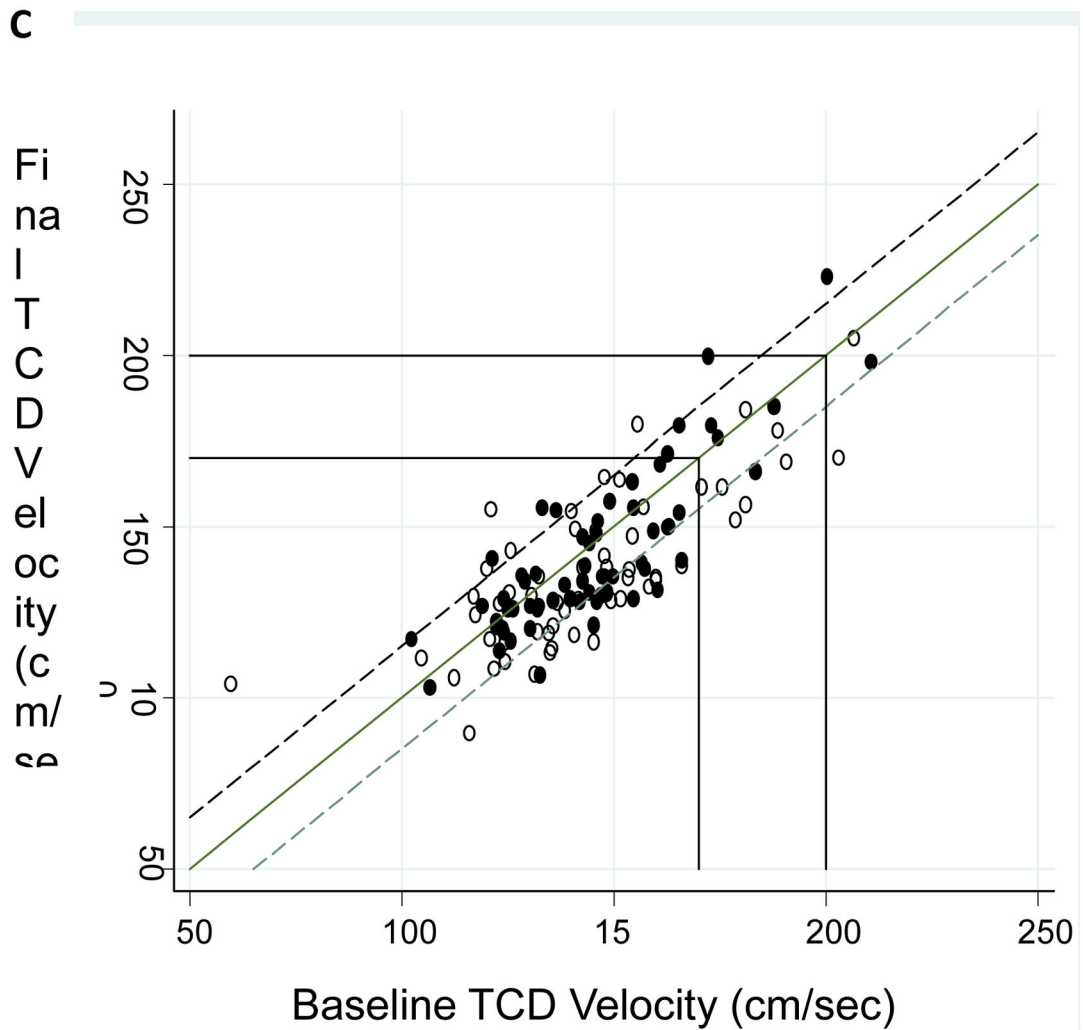Months Since Randomization

B

Months Since Randomization

Standard	61	59	59	59	58	44	56	49	43 31
Alternative	60	60	59	59	57	57	54	59	45

C

Figure 3.
Primary study endpoint analysis of TCD velocities. The Standard (Transfusion) Arm data are portrayed as dashes while the Alternative (Hydroxyurea) Arm data are shown as a solid line. Panel A illustrates the TCD data using the mixed model statistical analysis, with baseline equivalence but divergence over time with lower velocities in the Alternative Arm. The curves are significantly different using the per-protocol non-inferiority comparison (p=8.82 × 10^{-16}) and also by post-hoc analysis for superiority (p=0.023). Panel B illustrates the actual TCD velocity data in each arm as mean ± 1 standard deviation, including the number of participants evaluated at each 12-week time point. Panel C illustrates baseline (enrolment) and final (exit) maximum time-averaged mean TCD velocities for each participant. The lines at 170 and 200 cm/sec denote the normal and abnormal TCD boundaries, respectively. Open circles = Alternative Arm, Closed circles = Standard Arm.

Table 1

Enrolment Characteristics of the Intention-to-Treat Population

Selected demographic, clinical, and laboratory characteristics of the intention-to-treat population at study enrolment. Measures of central tendency are listed as mean ± SD; treatment group differences were assessed via t-test. Categorical values are summarised as Count (%); treatment group differences were assessed via Chi-squared tests, except Fisher's exact test used for sickle cell genotype.

Characteristic	Standard (N=61)	Alternative (N=60)	p value
HbSS genotype (%)	59 (96.7)	60 (100.0)	.496
Male (%)	19 (31.1)	29 (48.3)	.053
Age at study enrolment (years)	9.5 ± 2.6	9.7 ± 3.2	.782
TCD History			
Age at index abnormal TCD (years)	5.7 ± 2.0	5.0 ± 1.8	.051
Average index TCD value (cm/sec)	226 ± 25	220 ± 17	.156
Average entry TCD value (cm/sec)	145 ± 21	145 ± 26	1.000
Brain MRI/MRA			
Silent cerebral infarction (%)	25 (41.0)	22 (36.7)	.764
Mild-moderate vasculopathy (%)	6 (9.8)	4 (6.7)	.762
Transfusion History			
Duration (years)	3.8 ± 1.8	4.5 ± 2.8	.072
Simple transfusions (%)	36 (59.0)	39 (65.0)	.806
RBC alloantibodies (%)	9 (14.8)	11 (18.3)	.632
RBC autoantibodies (%)	12 (19.7)	10 (16.7)	.814
Iron Overload Status			
Liver Iron (mg Fe/gm dry wt liver)	8.4 ± 7.6	11.3 ± 9.5	.072
Serum ferritin (ng/mL)	2713 ± 2206	3080 ± 2347	.254
Current chelation usage (%)	51 (83.6)	48 (80.0)	.607
Laboratory parameters			
Haemoglobin (gm/dL)	9.3 ± 0.8	9.2 ± 0.8	.363
MCV (fL)	86 ± 4	86 ± 4	.397
HbA (%)	67.8 ± 11.4	67.4 ± 10.4	.837
HbS (%)	26.5 ± 10.3	27.6 ± 9.9	.528
HbF (%) [*]	10.3 ± 6.5	8.8 ± 5.5	.763
ARC (x 10^9/L)	319 ± 124	358 ± 144	.113
WBC (x 10^9/L)	12.2 ± 4.4	13.9 ± 3.5	.024
ANC (x 10^9/L)	6.9 ± 2.7	8.0 ± 2.8	.035
ALT (U/L)	49 ± 53	47 ± 27	.412
Creatinine (mg/dL)	0.4 ± 0.2	0.4 ± 0.2	.562
Total bilirubin (mg/dL)	2.4 ± 1.1	2.9 ± 1.8	.039

Hb = hemoglobin, ARC = absolute reticulocyte count, WBC=white blood cells, ANC = absolute neutrophil count, ALT = alanine transferase. Mild-moderate vasculopathy was Grade 1–3 as described. [13]

*Due to the large amount of HbA present, the HbF was calculated as (HbF)/(HbF + HbS) as described. [14]

Table 2

Laboratory Evaluations of the Intention-To-Treat Population at Final Assessment

Laboratory evaluations of the Intention-To-Treat population upon final assessment, after completing study treatment (N=83), after truncated treatment (N=24), or upon early termination for other reasons (N=14). Mean (SD) results are provided. Treatment group differences for laboratory parameters were assessed via t-test.

Parameter	Final Assessment			Change from Baseline		
	Standard (N=61)	Alternative (N=60)	P-value [1]	Standard (N=61)	Alternative (N=60)	P-value [2]
Haemoglobin (g/dL)	9.2 (0.7)	9.1 (1.1)	0.800	−0.1 (1.0)	−0.2 (1.2)	0.828
MCV (fL)	86 (3)	107 (10)	<0.0001	0 (4)	21 (10)	<0.0001
HbF (%)	10.3 (7.3)	24.3 (7.9)	<0.0001	−0.1 (5.0)	15.5 (7.1)	<0.0001
HbS (%)	27.6 (10.1)	70.7 (9.6)	<0.0001	1.2 (10.7)	43.0 (15.5)	<0.0001
ARC (x 10^9/L)	329 (112)	181 (86)	<0.0001	9 (158)	−169 (157)	<0.0001
WBC (x 10^9/L)	11.8 (4.0)	7.2 (2.2)	<0.0001	−0.4 (2.9)	−6.6 (3.9)	<0.0001
ANC (x 10^9/L)	6.9 (2.6)	3.6 (1.6)	<0.0001	0.0 (2.6)	−4.4 (3.2)	<0.0001
Platelets (x 10^9/L)	378 (112)	333 (133)	0.050	−3 (86)	−73 (135)	0.001
Total bilirubin (mg/dL)	2.7 (1.4)	1.6 (1.3)	<0.0001	0.3 (0.9)	−1.3 (1.3)	<0.0001
LIC (mg Fe/gm dw) [3]	11.3 (10.1)	9.5 (8.7)	.0323	2.4 (8.7)	−1.9 (4.2)	0.0011
Ferritin (ng/mL)	2674 (1717)	1276 (1228)	<0.0001	−38 (2095)	−1805 (1651)	<0.0001
LDH (U/L)	547 (191)	475 (196)	0.044	−6 (215)	−140 (231)	0.001

[1] p-value based on final assessment;

[2] p-value based on change from baseline to final assessment;

[3] Values for LIC represent 52 subjects in the Standard Treatment Arm and 58 on the Alternative Treatment Arm.