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

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REW, BRD, and RJA designed the study, supervised the trial, analysed the results, and wrote the first draft of the manuscript. WHS, LP, JL, SES, and SP helped coordinate many critical aspects of the trial to ensure its safe and successful operational execution. RCB, BA, SS, IO, BF, AG, WO, LLL, ZRR, LH, CG, CP, MTL, JLK, SJ, STM, CR, MMH, TK, SN, IH, KN, OA, MR, AAT, and JR enrolled patients, collected data, and helped interpret the results. KJH, DR, JC, MB, AK, NP, JW, NAM, NLCL, and ARC performed critical laboratory analyses and consultation for specific aspects of the trial related to study treatments and protocol endpoints. PW and BRD performed statistical analyses for the trial. All authors participated in the editing of the manuscript and approved the final version, and fulfill authorship requirements as outlined in the ICMJE recommendations.

DECLARATION OF INTERESTS
Hydroxyurea is not approved by the US FDA for use in children with sickle cell anemia, and the TWiTCH trial was performed under FDA IND #67289 with cross-reference to FDA IND #111926. Dr. Ware is a consultant for Bayer Pharmaceuticals and Global Blood Therapeutics; receives research support from Bristol Myers-Squibb, Addmedica, and Biomedonics Inc.; and serves on a Data and Safety Monitoring Board for Eli Lilly. Dr. Odame serves as a consultant to Novartis, and sits on an Advisory Board to ApoPharma and Global Blood Therapeutics. Dr. Owen serves on the Speaker’s Bureau of Novartis. Dr. Rogers is a consultant to ApoPharma and on the Speaker’s Bureau for Bio-Rad Labs. Dr. Kwiatkowski is a consultant for Shire and Siders, and receives research funding from ApoPharma. Dr. Heeney serves on the Scientific Advisory Board of Sancilio and Company. Dr. Imran is on the Speaker’s Bureau of NovoNordisk. Dr. Nottage is now employed by Janssen Pharmaceuticals, Inc. Dr. Wood is a consultant to ApoPharma, Biomed Informations, ISIS Pharmaceuticals, Celgene, AMAG, and Pfizer; receives research support from AMAG and Philips Healthcare; and serves as a Medical Advisor for ApoPharma. Dr. Cohen is a consultant to Novartis and serves on a Data and Safety Monitoring Board for an ApoPharma-sponsored clinical trial. None of these disclosures is relevant to the results and conclusions of the TWiTCH trial. Nothing to disclose: BRD, WHS, RCB, BA, SS, BF, AG, LLL, LH, CG, CP, MTL, SJ, STM, CR, TAK, SN, OA, MR, AAT, JAR, KJH, DR, JC, MB, AK, NP, LP, PW, JL, NAM, SES, NLCL, SP, RJA

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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 × 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.

Keywords
sickle cell anaemia; transcranial Doppler; stroke; hydroxyurea

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 ± 1.6 × 10⁹/L (median 3.4, range 1.5–10.1 × 10⁹/L) and final average neutrophil count of 3.6 × 10⁹/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 × 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 × 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.

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References

1. Ohene-Frempong K, Weiner SJ, Sleeper LA, et al. Cerebrovascular accidents in sickle cell disease: Rates and risk factors. Blood. 1998; 91:288–94. [PubMed: 9414296]
2. Hulbert ML, McKinstry RC, Lacey JL, et al. Silent cerebral infarcts occur despite regular blood transfusion therapy after first strokes in children with sickle cell disease. Blood. 2011; 117:772–9. [PubMed: 20940417]

3. Adams R, McKie V, Nichols F, et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease. N Engl J Med. 1992; 326:605–10. [PubMed: 1734251]

4. Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusion in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med. 1998; 339:5–11. [PubMed: 9647873]

5. Adams RJ. for the STOP 2 Trial Investigators. Discontinuing prophylactic transfusions used to prevent stroke in sickle cell disease. N Engl J Med. 2005; 353:2769–78. [PubMed: 16382063]

6. Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. Management of sickle cell disease. Summary of the 2014 evidence-based report by expert panel members. JAMA. 2014; 312:1033–48. [PubMed: 25203083]

7. Bernaudin F, Verlhac S, Arnaud C, et al. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy outcome in a newborn sickle cell anemia cohort. Blood. 2011; 117:1130–40. [PubMed: 21068435]

8. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Engl J Med. 1995; 332:1317–22. [PubMed: 7715639]

9. Wang WC, Ware RE, Miller ST, et al. for the BABY HUG Investigators. Hydroxycarbamide in very young children with sickle-cell anemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet. 2011; 377:1663–72. [PubMed: 21571150]

10. Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood. 2010; 115:5300–11. [PubMed: 20223921]

11. Gulbis B, Haberman D, Dufour D, et al. Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events: the Belgian experience. Blood. 2005; 105:2685–90. [PubMed: 15604217]

12. Zimmerman SA, Schultz WH, Burgett S, Mortier NA, Ware RE. Hydroxyurea therapy lowers transcranial Doppler flow velocities in children with sickle cell anemia. Blood. 2007; 110:1043–7. [PubMed: 17429008]

13. Helton KJ, Adams RJ, Kesler KL, et al. Magnetic resonance imaging/angiography and transcranial Doppler velocities in sickle cell anemia: Results from the SWITCH trial. Blood. 2014; 124:891–8. [PubMed: 24914136]

14. Heeney MM, Ware RE. Hydroxyurea for children with sickle cell disease. Pediatr Clin North Am. 2008; 55:483–501. [PubMed: 18381097]

15. Ware RE. Optimizing hydroxyurea therapy for sickle cell anemia. Am Soc Hematol Educ Program. in press.

16. Aygun B, Mortier NA, Kesler K, et al. Therapeutic phlebotomy is safe in children with sickle cell anemia and can be effective treatment for transfusional iron overload. Br J Haematol. 2015; 169:262–6. [PubMed: 25612463]

17. Reboussin DM, DeMets DL, Kyungmann K, Lan KK. Computations for group sequential boundaries using the Lan-DeMets spending function method. Control Clin Trials. 2000; 21:190–207. [PubMed: 10822118]

18. Brambilla DJ, Miller ST, Adams RJ. Intra-individual variation in blood flow velocities in cerebral arteries of children with sickle cell disease. Pediatr Blood Cancer. 2007; 49:318–22. [PubMed: 17243135]

19. Almeida CB, Souza LE, Leonardo FC, et al. Acute hemolytic vascular inflammatory processes are prevented by nitric oxide replacement or a single dose of hydroxyurea. Blood. 2015; 126:711–20. [PubMed: 26019278]

20. Raphael JL, Shetty PB, Lui H, Mahoney DH, Mueller BU. A critical assessment of transcranial Doppler screening rates in a large pediatric sickle cell center: opportunities to improve healthcare quality. Pediatr Blood Cancer. 2008; 51:647–51. [PubMed: 18623200]

21. Reeves SL, Fullerton HJ, Dombkowski KJ, Boulton ML, Braun TM, Lisabeth LD. Physician attitude, awareness, and knowledge regarding guidelines for transcranial Doppler screening in sickle cell disease. Clin Pediatr. 2015; 54:336–45.

*Lancet. Author manuscript; available in PMC 2017 December 11.*
22. Aygun B, Wruck M, Schultz WH, et al. Chronic transfusion practices for prevention of primary stroke in children with sickle cell anemia and abnormal TCD velocities. Am J Hematol. 2012; 87:428–30. [PubMed: 22231377]

23. DeBaun MR, Gordon M, McKinstry RC, et al. Controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. N Engl J Med. 2014; 371:699–710. [PubMed: 25140956]

24. Kwiatkowski JL, Yim E, Miller S, Adams RJ. for the STOP2 Study Investigators. Effect of transfusion therapy on transcranial Doppler ultrasonography velocities in children with sickle cell disease. Pediatr Blood Cancer. 2011; 56:777–82. [PubMed: 21370410]

25. Ware RE, Schultz WH, Yovetich N, et al. Stroke With Transfusions Changing to Hydroxyurea (SWiTCH): A Phase III randomized clinical trial for treatment of children with sickle cell anemia, stroke, and iron overload. Pediatr Blood Cancer. 2011; 57:1011–7. [PubMed: 21826782]

26. Ware RE, Zimmerman SA, Sylvestre PB, et al. Prevention of secondary stroke and resolution of transfusional iron overload in children with sickle cell anemia using hydroxyurea and phlebotomy. J Pediatr. 2004; 145:346–52. [PubMed: 15343189]

27. Greenway A, Ware RE, Thornburg CD. Long-term results using hydroxyurea/phaebotomy for reducing secondary stroke risk in children with sickle cell anemia and iron overload. Am J Hematol. 2011; 86:357–61. [PubMed: 21442640]

28. Flanagan JM, Frohlich DM, Howard TA, et al. Genetic predictors for stroke in children with sickle cell anemia. Blood. 2011; 117:6681–4. [PubMed: 21515823]

29. Flanagan JM, Sheehan VA, Linder H, et al. Genetic mapping and exome sequencing identify 2 mutations associated with stroke protection in pediatric patients with sickle cell anemia. Blood. 2013; 121:3237–45. [PubMed: 23422753]

30. Armstrong FD, Elkin TD, Brown RC, et al. for the BABY HUG Investigators. Developmental function in toddlers with sickle cell anemia. Pediatrics. 2013; 131:e406–414. [PubMed: 23296434]

APPENDIX

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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

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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

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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.
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**

| Characteristic                  | Standard (N=61) | Alternative (N=60) | p value |
|---------------------------------|-----------------|--------------------|---------|
| 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**

| Characteristic                  | Standard (N=61) | Alternative (N=60) | p value |
|---------------------------------|-----------------|--------------------|---------|
| Silent cerebral infarction (%)  | 25 (41.0)       | 22 (36.7)          | .764    |
| Mild-moderate vasculopathy (%)  | 6 (9.8)         | 4 (6.7)            | .762    |

**Transfusion History**

| Characteristic                  | Standard (N=61) | Alternative (N=60) | p value |
|---------------------------------|-----------------|--------------------|---------|
| 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**

| Characteristic                  | Standard (N=61) | Alternative (N=60) | p value |
|---------------------------------|-----------------|--------------------|---------|
| 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**

| Characteristic                  | Standard (N=61) | Alternative (N=60) | p value |
|---------------------------------|-----------------|--------------------|---------|
| 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 10⁹/L)                   | 319 ± 124       | 358 ± 144          | .113    |
| WBC (x 10⁹/L)                   | 12.2 ± 4.4      | 13.9 ± 3.5         | .024    |
| ANC (x 10⁹/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 \( \frac{\text{HbF}}{\text{HbF} + \text{HbS}} \) 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 | 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]      | 113 (10.1)       | 95.5 (8.7)           | <0.0001     | 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.