Hydroxyurea for primary stroke prevention in children with sickle cell anaemia in Nigeria (SPRING): a double-blind, multicentre, randomised, phase 3 trial

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Summary

Background

In high-income countries, standard care for primary stroke prevention in children with sickle cell anaemia and abnormal transcranial Doppler velocities results in a 92% relative risk reduction of strokes but mandates initial monthly blood transfusion. In Africa, where regular blood transfusion is not feasible for most children, we tested the hypothesis that initial moderate-dose compared with low-dose hydroxyurea decreases the incidence of strokes for children with abnormal transcranial Doppler velocities.

Methods

SPRING is a double-blind, parallel-group, randomised, controlled, phase 3 trial of children aged 5–12 years with sickle cell anaemia with abnormal transcranial Doppler velocities conducted at three teaching hospitals in Nigeria. For randomisation, we used a permuted block allocation scheme with block sizes of four, stratified by sex and site. Allocation was concealed from all but the pharmacists and statisticians. Participants were assigned in a 1:1 ratio to low-dose (10 mg/kg per day) or moderate-dose (20 mg/kg per day) oral hydroxyurea taken once daily with monthly clinical evaluation and laboratory monitoring. The primary outcome was initial stroke or transient ischaemic attack, centrally adjudicated. The secondary outcome was all-cause hospitalisation. We used the intention-to-treat population for data analysis. The trial was stopped early for futility after a planned minimum follow-up of 3·0 years to follow-up for participants. This trial was registered with ClinicalTrials.gov, number NCT02560935.

Findings

Between Aug 2, 2016, and June 14, 2018, 220 participants (median age 7·2 years [IQR 5·5–8·9]; 114 [52%] female) were randomly allocated and followed for a median of 2·4 years (IQR 2·0–2·8). All participants were Nigerian and were from the following ethnic groups: 179 (82%) people were Hausa, 25 (11%) were Fulani, and 16 (7%) identified as another ethnicity. In the low-dose hydroxyurea group, three (3%) of 109 participants had

strokes, with an incidence rate of 1·19 per 100 person-years and in the moderate-dose hydroxyurea group five (5%) of 111 had strokes with an incidence rate of 1·92 per 100 person-years (incidence rate ratio 0·62 [95% CI 0·10–3·20], p=0·77). The incidence rate ratio of hospitalisation for any reason was 1·71 (95% CI 1·15–2·57, p=0·0071), with higher incidence rates per 100 person-years in the low-dose group versus the moderate-dose group (27·43 *vs* 16·08). No participant had hydroxyurea treatment stopped for myelosuppression.

Interpretation

Compared with low-dose hydroxyurea therapy, participants treated with moderate-dose hydroxyurea had no difference in the stroke incidence rate. However, secondary analyses suggest that the moderate-dose group could lower incidence rates for all-cause hospitalisations. These findings provide an evidence-based guideline for the use of low-dose hydroxyurea therapy for children with sickle cell anaemia at risk of stroke.

Funding

National Institute of Neurological Disorders and Stroke.

Introduction

The absence of stroke prevention programmes in children with sickle cell anaemia living in low-resource and high-resource countries results in high incidence rates of initial ischaemic strokes, recurrent strokes, and stroke-related mortality in this population.[1], [2], [3] The results of the Stroke Prevention Trial in Sickle Cell Anemia (STOP)[4] reported that standard care in high-income settings of annual transcranial Doppler ultrasonography screening to identify children with abnormal velocities (≥200 cm/s non-imaging) followed by initial monthly blood transfusion to lower maximum haemoglobin S (the abnormal haemoglobin variant occurring predominantly in haemoglobin sickle cell anaemia) concentrations from 90% to less than 30%, resulted in at least 92% relative risk reduction in the incidence of stroke.[4] However, in Nigeria and in other countries in Africa, initial monthly blood transfusion therapy for primary stroke prevention is not possible for most children.[5]

Research in context

Evidence before this study

Primary stroke prevention in children with sickle cell anaemia is effective when coupled with transcranial Doppler screening and initial blood transfusion therapy for children with abnormal velocities. In Africa, where regular blood transfusion is not feasible for most children, we tested the hypothesis that moderate-dose compared with low-dose hydroxyurea decreases the incidence of strokes for children with abnormal transcranial Doppler velocities. We searched PubMed for articles published before Jan 1, 2014, for the medical subject heading terms “sickle cell”, “stroke”, “brain infarction”, and “primary prevention”, and we searched titles and abstracts for the terms “stroke”, “prevention”, “sickle cell anemia”, “sickle cell anaemia”, “sickle cell disorders”, “sickle cell disease”, “sickle cell disorder”, and “sickle cell diseases”. We also searched ClinicalTrials.gov and reference lists in published articles. We refined references by quality and relevance (qualifying as a scoping rather than a systematic literature review). We did the same search after completion of the trial on April 1, 2021. We found no randomised controlled trial showing the benefit or harm of initial hydroxyurea treatment for abnormal transcranial Doppler measurements

in either a low-middle-income setting or high-income setting. Our Nigerian feasibility single-arm trial that began in 2013 (SPIN trial), coupled with pooled analysis before the start of the trial, showed that hydroxyurea lowered transcranial Doppler velocities and prevented strokes in a population that is at high risk.

Added value of this study

Our trial showed for the first time, to our knowledge, that in children with sickle cell anaemia with abnormal transcranial Doppler velocities, fixed-low dose (10 mg/kg per day) and moderate-dose (20 mg/kg per day) hydroxyurea have equivalent stroke incidence rates. Further, no laboratory monitoring is required for assessment of myelosuppression. The study also suggested that in Africa, an increase in hydroxyurea dosage from low to moderate dose, decreases the rate of in-hospital vaso-occlusive episodes and acute pain events at home.

Implications of all the available evidence

The results of this trial have changed clinical practice in three states in Nigeria already, with an estimated 40 000 children with sickle cell anaemia at risk for strokes. As standard care, these children now undergo transcranial Doppler assessments, and if abnormal, the state provides hydroxyurea free-of-charge at an initial fixed low dose of 10 mg/kg per day. If previous or future acute vaso-occlusive episodes (pain or acute chest syndrome) are frequent, shared decision making between the guardians and the health-care provider might lead to an increase in hydroxyurea dose to 20 mg/kg per day.

Approximately 50% of all infants with sickle cell anaemia in the world are born in Nigeria, 150 000 per year.[6] In Nigeria and USA, the incidence of strokes in children with sickle cell anaema is 0·88 and 0·79 strokes per 100 person-years, respectfully, before implementing primary stroke prevention programmes.[1], [3]

To address the gap for primary stroke prevention in a low-resource setting, we completed a single-arm feasibility trial, Stroke Prevention in Nigeria (SPIN), assessing the incidence of stroke in children with abnormal transcranial Doppler velocities treated with fixed moderate-dose hydroxyurea (20 mg/kg per day).[7] The SPIN trial showed the acceptability

and safety of hydroxyurea for children with abnormal transcranial Doppler measurements. In the SPIN trial, one child had a stroke (0·76 per 100 patient-years of observation)[7] compared with 14 expected strokes based on the incidence of strokes (10·7 per 100 patient-years) in the observation group of the STOP trial.[4] Previous studies in low-income settings have shown the benefit of low-dose hydroxyurea (10–15 mg/kg per day) in ameliorating complications related to sickle cell disease.[8], [9], [10] Given the strong preliminary data in the SPIN trial showing the benefit of moderate-dose hydroxyurea for preventing strokes in children with abnormal transcranial Doppler measurements, we tested the primary hypothesis that moderate-dose hydroxyurea (20 mg/kg per day) therapy for primary stroke prevention results in a 66% relative risk reduction (from nine to three events per 100 person-years) when compared with low-dose hydroxyurea (10 mg/kg per day) therapy.

Methods

Study design and participants

The SPRING trial was a multicentre, double-blind, randomised, controlled, phase 3 trial in children with sickle cell anaemia and abnormal transcranial Doppler velocities. Participants received either fixed low-dose or moderate-dose hydroxyurea.[11] The protocol is available in the appendix (pp 1–82). We conducted the clinical trial at three sites in northern Nigeria with a catchment area of at least 40 000 children with sickle cell anaemia (Aminu Kano Teaching Hospital, Kano; Murtala Muhammad Specialist Hospital, Kano; Barau Dikko Teaching Hospital/Kaduna State University, Kaduna, Nigeria; appendix p 88).[12] Ethical approval for the study was obtained from the Institutional Review Boards of each of the sites, and Vanderbilt University Medical Center (Nashville, TN, USA). The National Agency for Food and Drug Administration and Control in Nigeria approved the trial. A Data and Safety Monitoring Board (which included one member from Nigeria) appointed by the National Institute of Neurological Disorders and Stroke (NINDS; all members from USA and UK and one from Nigeria) reviewed serious adverse events and study progress.

The research team and the institutional Nigerian Ethics Committees did not believe an untreated (placebo) group of children with abnormal transcranial Doppler velocities was ethical because of this group's established high risk of strokes.

The Data Safety Monitoring Board recommended a comparison group of children with sickle cell anaemia that completed study screening procedures, had normal or conditional transcranial Doppler velocities as per STOP criteria,[4] were not treated with hydroxyurea, and agreed to be followed up prospectively for at least 36 months (n=220). The vital status of the participants in the comparison group was assessed monthly via phone calls and at least one study visit per year. Participants in the comparison group could crossover to the therapy group if, during routine care, annual transcranial Doppler measurements were 200 cm/s or more, and all other inclusion criteria were met.

The inclusion criteria were children aged 5–12 years, with diagnosed haemoglobin SS or haemoglobin Sβ⁰thalassaemia (referred to as sickle cell anaemia), and abnormal transcranial Doppler defined as time-averaged mean of the maximum velocity of 200 cm/s or more, measured by two separate transcranial Doppler certified ultra-sonographers or 220 cm/s or more measured once. We repeated the transcranial Doppler velocity typically on the day of the abnormal result because we wanted to shorten the time for treatment for children at risk for strokes, improve the convenience of enrolment for the family, and decrease the likelihood of a false-positive result due to the high coefficient of variation associated with transcranial Doppler velocities in children with sickle cell anemia.[13] All transcranial doppler measurements were completed in asymptomatic participants on the day of their baseline clinical visit.

Exclusion criteria were previous history of an overt stroke (a focal neurological deficit of acute onset), focal neurological deficit on standardised neurological examination, or concern for moderate or severe neurological deficit based on a positive ten-question screening,[14] and haemoglobin concentrations of less than 6 g/dL. No participants were prescribed hydroxyurea before the trial. The guardians of all participants provided written informed consent before screening and enrolment, and

children aged 7 years and older provided their assent. The clinical features of the population screened for eligibility are described elsewhere.[11, 15]

Randomisation and masking

Eligible children with sickle cell anaemia and abnormal transcranial Doppler velocities were randomly assigned to receive either low-dose or moderate-dose once daily oral hydroxyurea. Randomisation allocation tables were constructed by study statisticians using a permuted block allocation scheme, based on block sizes of four, stratified by sex, within site. The tables were loaded into Research Electronic Data Capture (REDCap) and used to randomly assign participants (1:1) by the unblinded study pharmacist after determining eligibility criteria. The participants went to the trial pharmacists who distributed the trial medication to the guardian. Allocation was concealed from all other study personnel except the statisticians because of the requirement to prepare interim reports for the Data Safety Monitoring Board (REDCap allows access to certain fields to be restricted).

Procedures

All participants with abnormal transcranial doppler velocities were offered regular blood transfusion therapy as standard care. For participants, research visits with a history, physical examination, and laboratory values were obtained monthly. Transcranial doppler ultrasound was completed at trial entry, 3 months, 12 months, and upon exit from the trial. Transcranial Doppler measurement in the trial served as a biomarker for hydroxyurea therapy response. The dosing scheme was dependent on the weight of the child using 100 mg, 250 mg, and 500 mg hydroxyurea capsules. Hydroxyurea was supplied by Bond Chemical (Ibadan, Nigeria).

The planned doses of treatment were approximately 10 mg/kg per day (for the low-dose group) or 20 mg/kg per day (for the moderate-dose group) of oral hydroxyurea daily for a minimum of 3 years. Participants were to be withdrawn if they were unable to tolerate hydroxyurea therapy or myelosuppression was suspected on the basis of the defined parameters and was unresponsive to manoeuvers described below.

The site investigators, the principal investigators, research staff at each site, and trial pharmacists met weekly to discuss any laboratory or clinical assessment of hydroxyurea-related toxicity or clinical trial defined adverse events and serious adverse events (scored using the Common Toxicity Criteria for Adverse Events). The three study monitors (AB, AG, AK) assessed the completed blood cell counts weekly and discussed the results on the weekly conference calls with the study site team investigators from all three sites and the leadership of the data coordinating centre. If myelosuppression secondary to hydroxyurea had occurred, the medical monitors would have been unmasked and a lower dose of hydroxyurea provided. An additional second weekly meeting occurred between the study coordinators and the leadership team of the data coordinating centre to ensure appropriate follow up for any laboratory concerns, serious adverse event monitoring that required hospitalisation, or serious adverse events including death, stroke, or transient ischaemic attacks. Haemoglobin F concentrations were measured at baseline, then annually, and upon exit from the trial. Follow-up visits were requested to repeat any laboratory measurements outside of the expected range, which was based on previous laboratory assessments of the cohort; the expected ranges were updated twice in the trial to include the upper and lower expected limits (appendix pp 83–87).

Participants received monthly laboratory monitoring to assess for myelosuppression throughout the trial. Myelosuppression possibly related to hydroxyurea was defined as an absolute neutrophil count of less than 1000×10^9 cells per L or a platelet count of less than 80×10^9 cells per L. Reticulocyte assessment was not done because of inadequate quality control measures. Adherence was measured with mean cell volume, hydroxyurea pill counts returned to the pharmacists monthly, and haemoglobin F concentrations assessed annually.

Malaria was diagnosed as per WHO's guidelines[16] or local study site clinical practice. Malaria was defined as a fever (≥100·4°F or ≥38·0°C) and positive light microscopy, a rapid diagnostic test that can detect *Plasmodium falciparum* completed (depending on test availability). Participants could have malaria with any of the primary reasons for admission.

For the duration of the study, participant hospitalisations were documented. When available, a de-identified source document was uploaded for review. The adjudication of all parties was documented via REDCap. Pain requiring hospitalisation was defined as a sickle cell anaemia-associated pain episode requiring admission to the hospital and treatment with opioids. Emergency department visits were not included in this definition due to the variation in outpatient pain management practices and to capture only acute severe pain events. Headaches treated with opioids were also not included in this definition due to the difference in the proposed pathophysiology of headaches as opposed to acute vaso-occlusive pain. Acute chest syndrome was defined as abnormal findings on lung examination and the presence of at least two of the following criteria:[17] temperature greater than 38·0°C, increased respiratory rate greater than the 90th percentile for age,[18] positive chest pain or pulmonary auscultatory findings, increased oxygen requirement (saturation of peripheral oxygen drop by ≥3% from a documented steady-state value on room air),[19] and a new radiodensity on chest roentgenogram. A diagnosis of pneumonia was considered an acute chest syndrome episode.

Outcomes

The primary endpoint was the occurrence of an initial clinical stroke or transient ischaemic attack, based on WHO's definition.[20] Evaluations after a possible stroke were recorded with a video and assessed by the Pediatric National Institutes of Health Stroke Scale;[21] the Neurology Committee reviewed the neurological examination video and case report forms of all suspected strokes and were blinded to group assignment.

The prespecified secondary outcome measure was admission to hospital for any cause. Events were centrally adjudicated without knowledge of treatment assignment. Each hospitalisation and unscheduled outpatient visit were adjudicated for the primary reason for admission in the following hierarchal and mutually exclusive categories: stroke, acute chest syndrome, pain, or fever (temperature ≥38·0°C), with each event assigned only one primary reason. The acute vaso-occlusive event was either pain or acute chest syndrome.

The adjudication process for the primary cause of hospitalisation includes the following algorithm. If the patient presents with neurological deficits confirmed to be a stroke or transient ischaemic attack and acute chest syndrome, pain, fever, or other symptoms, then the primary cause of the hospitalisation is stroke or transient ischaemic attack. If the patient presents with acute chest syndrome, pain, fever, other symptoms, and no indication of neurological deficits for stroke or transient ischaemic attack, then the primary cause of the hospitalisation is acute chest syndrome. If the patient presents with pain, fever, other symptoms, and no acute chest syndrome or indication of neurological deficits for stroke or transient ischaemic attack, then the primary cause of the hospitalisation is pain. If the patient presents with fever and other symptoms, and no pain, acute chest syndrome, or indication of neurological deficits for stroke or transient ischaemic attack, then the primary cause of the hospitalisation is fever. If the patient presents with other symptoms and no fever, pain, acute chest syndrome, or indication of neurological deficits for stroke or transient ischaemic attack, then the primary cause of the hospitalisation is other. The primary reason was provided after adjudication, along with all applicable secondary reasons for hospitalisation.

The adjudication process to determine the primary reason for admission was: (1) local site investigator, paediatrician, haematologist, and study coordinator decided the primary and secondary reason; (2) study contact principal investigator (MRD) independently reviewed the reason for admission; (3) if the primary reason for admission was concordant between the study site team and contact principal investigator no further discussion occurred; (4) if discordant, the research teams from all three sites, plus the contact principal investigator determined the primary cause of hospitalisation on the basis of consensus (approximately ten team members reviewed every discordant primary reason for admission).

A post-hoc secondary outcome of acute pain managed only at home was assessed without knowledge of the treatment assignment or clinical outcome. The diagnoses of acute pain managed only at home was based on whether the guardians provided a history during the monthly research visit of administering a non-steroidal anti-inflammatory drug (eg, ibuprofen and

diclofenac) alone or with both paracetamol and codeine for sickle cell anaemia-related pain excluding headache. Neither the guardian providing the history, nor the research coordinators, were aware that the interim medical history of pain medication administered in the previous 30 days would be used to determine an acute pain event at home.

Statistical analysis

To test our primary hypothesis, our sample size was based partly on the results of our SPIN feasibility trial demonstrating a decrease to less than abnormal velocities (<200 cm/s) in 15 (65%) of 23 participants after 3 months on moderate-dose hydroxyurea therapy and we decided to use a relative risk of reduction of 66%.[7] In the STOP trial, the incidence rate of stroke in untreated children with abnormal transcranial Doppler measurements was 10·7 events per 100 person-years and for the monthly blood transfusion group was 0·97 events per 100 person-years.[4] Two additional conservative assumptions were made. First, low-dose hydroxyurea would have some benefit, reducing the stroke incidence to below 10·7 events per 100 person-years in the untreated group in the STOP trial.[4] Second, moderate-dose hydroxyurea would be beneficial, but would not be as effective as the regular blood transfusion therapy group in the STOP trial.

We anticipated a recruitment window of 2 years, a minimum follow-up time of 3 years, and a 9% loss to follow-up in each group per year. A sample size of 220 (110 patients per group) provided at least 90% power to detect a 66% relative risk reduction of the incidence rate of stroke for the moderate-dose group, assuming nine events per 100 person-years for the low-dose group versus three events per 100 person-years for the moderate-dose group, with two-sided type 1 error, and an alpha of 0·05.

For the primary hypothesis and all outcomes, the intention-to-treat principle was used to compare incidence rates between the two treatment groups. We summarised descriptive statistics using median and IQR for continuous data and percentages for categorical data. The comparison of baseline and exit characteristics between the low-dose and moderate-dose groups was done using the Mann-Whitney test for continuous variables and Pearson's χ^2-square test for categorical variables. Paired Wilcoxon rank-

sum tests were done to examine the change in laboratory values from baseline to 3 months and from baseline to 12 months and 24 months. All tests used a two-tailed probability, with p values less than 0·05 deemed statistically significant. Seven postulated stroke risk factors (age, sex, baseline concentrations of haemoglobin[3] and haemoglobin F, transcranial Doppler velocities ≥200 cm/s at baseline and 3 months,[22] and random assignment to fixed low-dose and moderate-dose hydroxyurea) were evaluated with Cox regression, with each model including group assignment and another risk factor. The small number of strokes precluded constructing a model with all risk factors. A recently introduced, but not validated, surrogate marker of hydroxyurea efficacy in Africa was described and assessed after the completion of the trial, defined as haemoglobin concentration of 9·0 g/dL or more or a haemoglobin F concentration of 20% or more.[23] Interim data, stopping rules, and data and safety monitoring was based on one planned interim analysis for efficacy conducted using the Lan-DeMets procedure with an O'Brien-Fleming stopping boundary to account for the interim analysis and the final analysis. An intention to-treat analysis was used for the interim analysis to assess futility. The significance boundary for the first look stopping rule was p=0·005, the final analysis was p=0·047. One interim analysis for futility, based on the stochastic curtailment method, was done by calculation of conditional power, an estimate of the probability that the trial shows a statistically significant effect on the primary endpoint of stroke rate given the results to date and assumptions regarding outcome through the end of the trial. The analysis was planned at the halfway point of the person-year accrual. A recommendation to stop the trial for futility would have required a conditional power below 30%, under the observed efficacy trend at the time of interim analysis, with two-sided type 1 error. All analyses were done in SPSS version 26. The SPRING trial is registered on ClinicalTrials.gov number, NCT02560935.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The first participants were assessed for eligibility on Aug 2, 2016, and the last participants on June 14, 2018. Among 960 participants who were screened for eligibility in the trial, a total of 220 children were randomly assigned to receive low-dose (n=109) or moderate-dose (n=111) hydroxyurea (median age 7·2 years, IQR 5·5–8·9, 114 [52%] females; table 1). Among the group of children with abnormal velocities, two ultrasonographers independently measured abnormal velocity measurements of 200 cm/s or more and less than 220 cm/s in 159 participants, and one ultrasonographer measured one abnormal velocity of 220 cm/s or more in 62 participants. One child died before being randomly assigned to a group. The mean monthly visits in the two therapy groups were 27·1 (SD 8·9) in the low-dose groups and 27·6 (9·1) in the moderate-dose groups. The median number of monthly visits was 29·0 in both groups (IQR 23·0–34·0). The median follow-up of the treatment group participants was 2·4 years (2·0–2·8) because the trial was stopped early by NINDS. During the trial, six (3%) of 220 participants withdrew, three participants from each group (figure 1).

Table 1. Baseline characteristics

	Low-dose group (n=109)	**Moderate-dose group (n=111)**
Age at start of hydroxyurea, years	7·4 (5·7–9·6)	7·0 (5·5–8·4)
Sex		
Female	56 (51%)	58 (52%)
Male	53 (49%)	53 (48%)
Ethnicity		
Hausa	89 (82%)	90 (81%)
Fulani	14 (13%)	11 (10%)
Other	6 (6%)	10 (9%)
Height, cm	115·3 (108·4–125·0)	114·0 (106·0–121·8)
Weight, kg	18·0 (15·5–20·7)	17·0 (15·0–20·0)
BMI, kg/m^2	13·5 (12·7–14·4)	13·3 (12·5–14·3)
Transcranial Doppler velocity at screening, cm/s	207·0 (203·0–222·5)	206·0 (203·0–221·0)
Total haemoglobin, g/dL	7·0 (6·4–7·6)	7·1 (6·6–7·7)
White blood cell count, $10^9/L$	14·2 (11·4–18·8)	14·3 (11·8–17·5)
Platelet count, $10^9/L$	447·0 (345·5–522·0)	414·0 (285·0–496·0)
Absolute neutrophil count, $10^9/L$ (n=219)	6969·2 (5195·2–9076·6)	6628·5 (5310·8–8382·0)
Systolic blood pressure, mm Hg	100·0 (90·0–100·0)	100·0 (90·0–100·0)
Mean cell volume, fL	86·0 (81·8–90·0)	86·2 (79·9–90·6)
Median percentage haemoglobin F, (n=219)	8·5 (5·2–12·1)	8·2 (6·1–12·3)
Median percentage haemoglobin S, (n=217)	81·3 (76·7–84·8)	82·6 (78·1–86·0)

Data are n (%) or median (IQR).

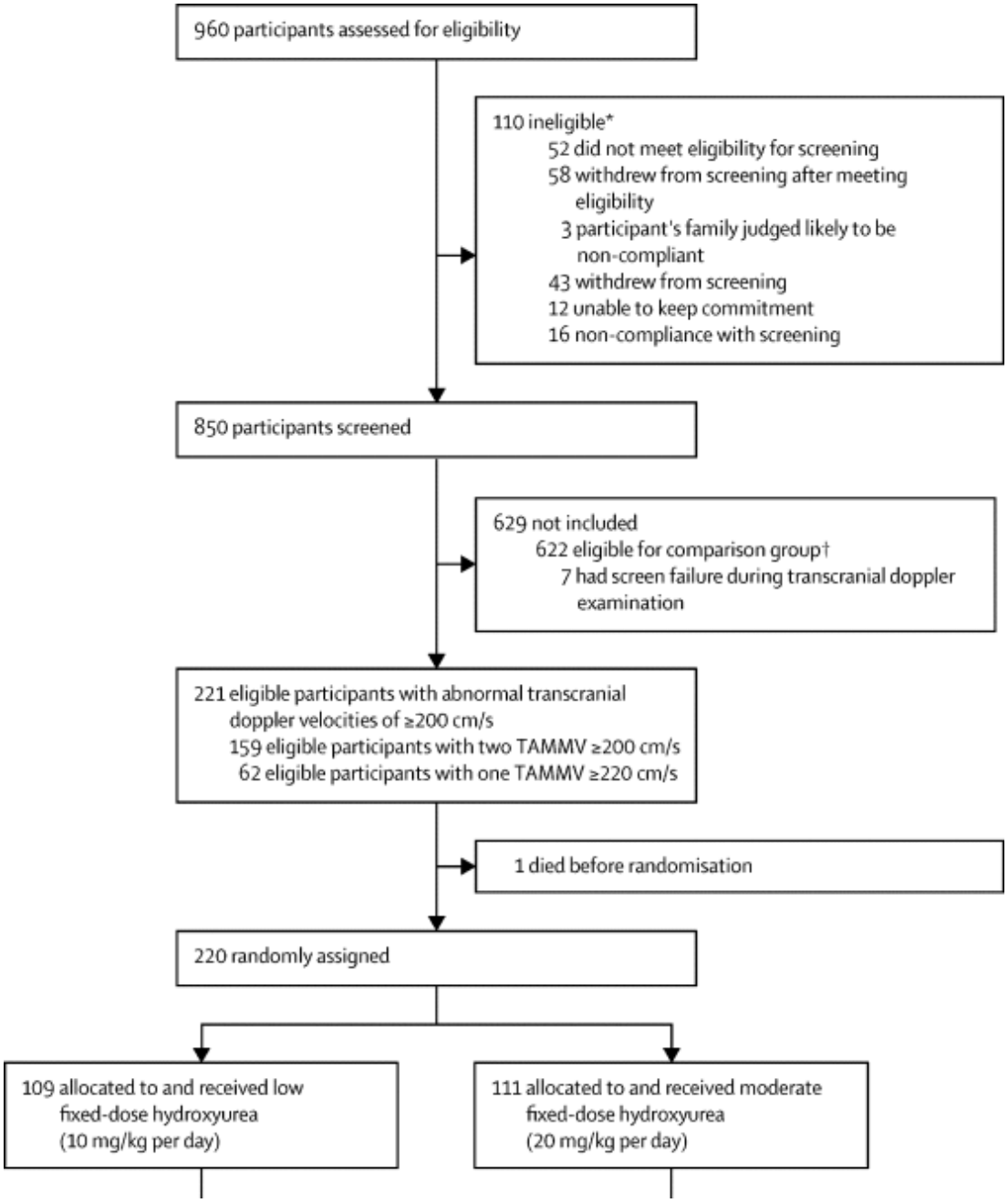

Figure 1. Trial profile

DSMB=Data Safety Monitoring Board. NINDS=National Institute of Neurological Disorders and Stroke. TAMMV=time-averaged mean of the maximum velocity. *Multiple reasons allowed. †For comparison profile, see appendix p 90. ‡One child who had a stroke also died shortly after and is therefore included in both categories.

The median prescribed dose for the low-dose group was 10·8 mg/kg per day (IQR 10·1–11·6) and for the moderate-dose group was 20·6 mg/kg (20·0–21·1) per day. In the low-dose group, the prescribed minimum dose was 9·5 mg/kg and the maximum dose was 15·4 mg/kg per day. In the moderate-dose hydroxyurea group, the prescribed minimum was 19·3 mg/kg per day and maximum dose was 23·9 mg/kg per day. A maximum of three capsules was provided for treatment.

A total of 220 children with sickle cell anaemia and transcranial Doppler measurements less than 200 cm/s were enrolled in the comparison group to determine whether hydroxyurea therapy was associated with serious adverse events. Nine crossed over from the comparison group to the therapy group during the course of the trial because of elevated transcranial Doppler measurement 200 cm/s or more and then were randomly assigned. The median follow-up of the participants in the comparison group was 2·8 years (IQR 1·5–3·1). In the comparison group, two participants developed strokes within 2 months after their last transcranial Doppler of 134 cm/s and 157 cm/s, respectively.

At a planned interim analysis in Aug 1, 2019, no difference was found in stroke incidence rates between the two treatment groups. Subsequently, a futility analysis was done using conditional power (the chance of finding a difference if the study was completed). Conditional power was 6·0%, under the prespecified decision boundary of 30%. Based on these results, the NINDS Clinical Trials leadership stopped the trial because of futility on Nov 6, 2019. The last contact for all participants was March 12, 2020. The lag time between stopping the trial and the last contact for the participants was due to two factors. First, the requirement for all communication to the participants to be approved by each of the Ethics Committee and second, scheduling of participants for exit visits that included transcranial Doppler evaluations.

Eight children had strokes, three in the low-dose group and five in the moderate-dose group (table 2). Strokes occurred at the following timepoints: one (13%) at 6 months, five (63%) at 12 months, and seven (88%) at 24 months after starting hydroxyurea therapy. The expected

stroke event rate for the low-dose group was nine per 100 person-years and for the moderate-dose group was three per 100 person-years; however, the actual stroke rate in the low-dose group was 1·19 per 100 person-years and in the moderate-dose group was 1·92 per 100 person-years, an incidence rate ratio of 0·62 (95% CI 0·10–3·20; p=0·77).

Table 2. Characteristics of therapy group participants who had strokes during the trial

	Time from baseline to endpoint (months)	Endpoint classification and description	Age (years)	Mean dose hydroxyurea (mg/kg per day)	Steady-state haemoglobin† (g/dL)	Haemoglobin at endpoint (g/dL)	Mean corpuscular volume* at baseline (fL)	Mean corpuscular volume at endpoint (fL)	Transcranial Doppler† at baseline (cm/sec)	Transcranial Doppler at 3 months (cm/s)
Low-dose group										
1	8·2	Stroke: slurred speech, right hemiparesis, face, and arm more than leg; PedNIHSS=5	9·1	11·2	6·7	6·7	76·7	90·1	309·0	291·0
2	12·2	Stroke: left hemiparesis, leg more than arm; PedNIHSS=3	10·0	9·8	7·1	7·0	91·7	92·8	293·0	232·0
3	10·0	Stroke: right face, arm, and leg weakness; PedNIHSS=4	7·4	9·8	8·3	7·5	90·0	96·0	231·0	218·0
Moderate-dose group										
4	0·3	Stroke: right hemiparesis, arm more than leg; PedNIHSS=3	6·2	22·6	7·1	NA	96·2	NA	204·0	NA
5	10·4	Stroke: left hemiparesis, face and arm more than leg, left visual field cut; PedNIHSS=5	8·3	20·4	6·7	8·6	85·1	108·6	243·0	247·0
6	8·0	Stroke: left hemiparesis, arm more than leg; PedNIHSS=2	12·4	19·9	6·0	7·8	83·4	118·4	202·0	143·0
7	14·8	Stroke: left-sided seizure, with persistent hemiparesis, face, and arm more than leg; PedNIHSS=7	5·1	23·5	8·1	8·4	88·8	119·7	232·0	254·0
8	30·7	Stroke: seizure, global aphasia, bilateral weakness, right more than left, bilateral strokes in setting of *Escherichia coli* sepsis; PedNIHSS=26	6·5	20·0	7·4	10·9	85·7	88·2	204·0	187·0

PedNIHSS=Pediatric National Institutes of Health Stroke Scale. NA=available. *All endpoint evaluations with the PedNIHSS occurred at 24 h or more after symptom onset.

Risk factors associated with a stroke included transcranial Doppler velocities at baseline (hazard ratio [HR] 1·04 [95% CI 1·02–1·06]; p=0·00013), and at 3 months (1·05 [1·03–1·08]; p<0·0001), controlling for the treatment group (appendix p 88). A higher proportion of strokes occurred when baseline transcranial velocities were 220 cm/s or more in five (8%) of 59 participants compared with when they were less than 220 cm/s in three (2%) of 161 (p=0·034; appendix p 89). After excluding the participant with a stroke before 3 months, similarly, a higher proportion of strokes occurred when the transcranial velocities were 220 cm/s or higher (four [50%] of eight) compared with less than 220 cm/s at 3 months (three [1%] of 205; p<0·0001; appendix p 89). The Pearson correlation between the baseline and 3 month velocities was 0·29 (p<0·0001).

In participants who had Transcranial Doppler velocities at all three timepoints, velocities reduced to normal levels (<170 cm/s) at 3 months in 27 [29%] of 92 participants in the low-dose group and in 39 [40%] of 98 participants in the moderate-dose group (p=0·13) and upon trial exit in 45 [49%] of 92 in the low-dose group and in 70 [71%] of 98 in the moderate-dose group (p=0·0015; figure 2). For both low-dose and moderate-dose groups, change from study entry to 3 months and study exit was significant (p<0·0001). Upon trial exit, 32 (29%) of 109 participants in the low-dose group and 74 (67%) of 111 participants in the moderate-dose group had either a haemoglobin concentration of 9·0 g/dl or higher or a haemoglobin F concentration of 20% or more, which is a surrogate endpoint of hydroxyurea efficacy, with no difference in the incidence rate of strokes (those not meeting surrogate endpoint 2·40 [95% CI 0·88–5·23] per 100 person-years *vs* those meeting the surrogate endpoint 0·77 [95% CI 0·08–2·75] per 100 person-years; p=0·26). Additional laboratory values demonstrating the anticipated hydroxyurea response from baseline to exit are shown in the appendix (p 91). As a measure of adherence, returned pills were reported, with a median of 5·4% (2·9–7·4) in the low-dose group and 4·8% (2·2–6·7) in the moderate-dose group, p=0·14. Mean increase in mean cell volume from baseline to exit increased by 1·5 fL in the low-dose group and 7·2 fL in the moderate-dose group, p<0·0001. The median haemoglobin F concentration increased by 1·9% (IQR 0·0–5·2) in the low-

dose group and 10·0% (3·4–13·8) in the moderate-dose group from baseline to exit from the trial.

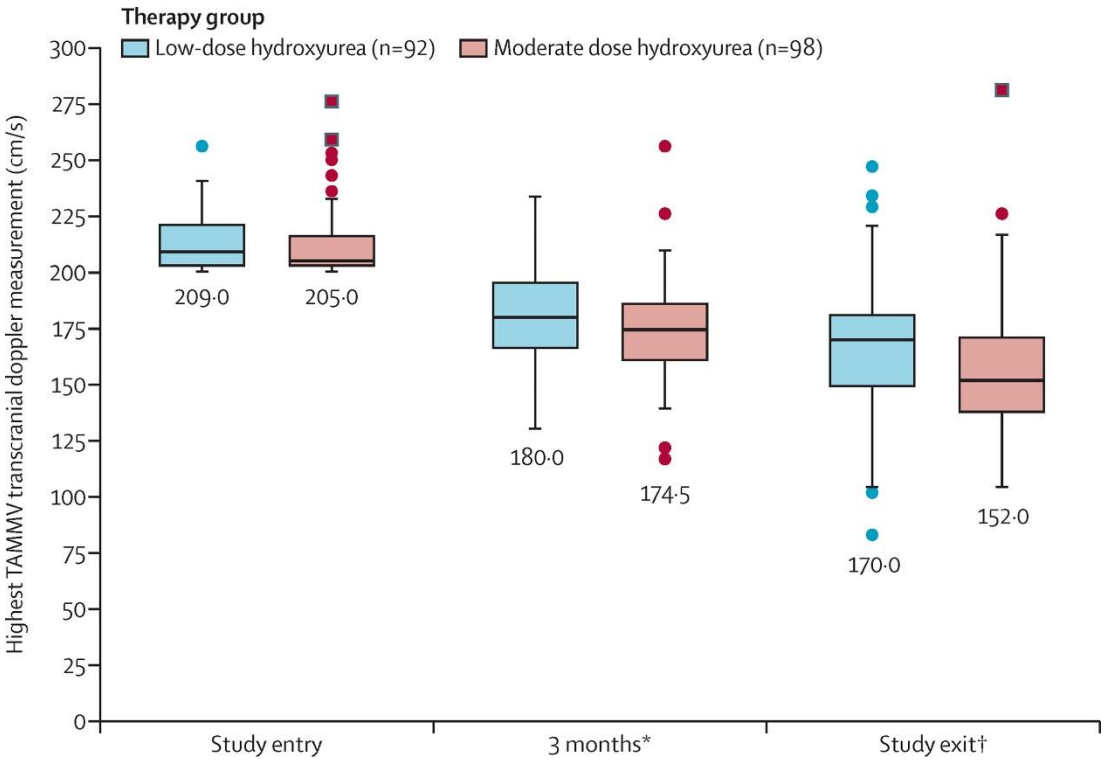

Figure 2. Boxplots of transcranial Doppler measurements at baseline, 3 months, and at exit, by therapy group

Includes only participants who had measurements at all three timepoints (n=190). The boxplot displays the 50th percentile (bar across the middle of the box), with the lower and upper ends of the box depicting the IQR, the 25th and 75th percentiles, respectively. The lower and upper whiskers denote the last data value which is within 1·5 times the IQR above the box borders. A circle represents an outlier that is between 1·5 and 3·0 times the IQR below or above the 25th or 75th percentile, respectively. A square represents an extreme outlier that is more than 3·0 times the IQR below or above the 25th or 75th percentile, respectively. For both low and moderate dose groups, change from baseline to 3 months and change from baseline to study exit were significant (p<0·0001). TAMMV=time-averaged mean of the maximum velocity. *Median time 3·4 months (IQR 3·1–3·9). †Median time 2·6 years (2·2–2·9).

Deaths occurred in both the low-dose and moderate-dose groups, but not with a greater incidence rate than in the comparison group. No deaths were treatment related. A total of ten deaths (3·98 per 100 person-years) were reported in the low-dose group, five deaths (1·92 per 100 person-years) in the moderate-dose group, and nine deaths (1·81 per 100 person-years) in the comparison group (table 3; appendix p 89). One child in the moderate-dose group died within a week of a stroke. The mortality incidence rate ratio when comparing the children treated with the transcranial Doppler group with velocities less than 200 cm/s (no hydroxyurea therapy, n=211) was 2·20 (95% CI 0·80–6·10; p=0·14) in the low-dose group and 1·06 (0·28–3·51; p=0·998) in the moderate-dose hydroxyurea group (appendix p 89). The incidence rate ratio of all-cause hospitalisation and hospitalisation for an acute vaso-occlusive event (acute pain or acute chest syndrome) was higher in the low-dose group than in the moderate-dose group (table 3).

Table 3. Primary and secondary outcomes for SPRING trial participants

	Number of events		Incidence rate per 100 person-years*		Incidence rate ratio† (95% CI)	p value‡
	Low-dose group (n=109)	Moderate-dose group (n=111)	Low-dose group (n=109)	Moderate-dose group (n=111)		
Primary outcome						
Ischaemic strokes	3	5	1·19	1·92	0·62 (0·10–3·20)	0·77
Death outcome						
Deaths during trial	10	5	3·98	1·92	2·08 (0·65–7·74)	0·19
Secondary outcome: hospitalisation						
Hospitalisation for any reason	69	42	27·43	16·08	1·71 (1·15–2·57)	0·0071
Vaso-occlusive event§	47	26	18·69	9·96	1·88 (1·14–3·16)	0·012
Acute chest syndrome	8	2	3·18	0·77	4·15 (0·83–40·14)	0·097
Acute vaso-occlusive pain	39	24	15·51	9·19	1·69 (0·99–2·93)	0·055
Malaria	42	27	19·89	16·08	1·62 (0·97–2·72)	0·065
Osteomyelitis	4	1	1·59	0·38	4·15 (0·41–204·50)	0·35
Secondary outcome: hospitalisation or outpatient visit						
Vaso-occlusive event	82	43	32·60	16·47	1·98 (1·35–2·93)	<0·001
Acute chest syndrome	10	2	3·98	0·77	5·19 (1·11–48·73)	0·033
Vaso-occlusive pain	72	41	28·63	15·70	1·82 (1·23–2·74)	0·0022
Malaria	49	41	19·48	15·70	1·24 (0·80–1·93)	0·36
Transfusion	19	12	7·55	4·98	1·52 (0·71–3·34)	0·32
Secondary outcome: home medication administered at least once in 30 days						
Vaso-occlusive pain, medication at home	289	226	114·90	86·54	1·33 (1·11–1·59)	0·0023
Malaria, medication at home	188	157	74·78	60·12	1·24 (1·00–1·55)	0·049

Data are n (%) unless otherwise stated. * Cumulative person-years in the low-dose group was 251·52 and in the moderate-dose group was 261·14. †Incidence rate ratio reference group is moderate hydroxyurea, the denominator of the incidence rate ratio. ‡ Fisher's exact test. §Vaso-occlusive events include both acute chest syndrome and severe acute pain events that required hospitalisation.

Most acute pain episodes occurred at home and not in a hospital or an outpatient visit with a health-care provider. An acute pain episode at home occurred in 73 (67%) of 109 children in the low-dose group and 81 (73%) of 111 children in the moderate-dose group. The incidence of acute vaso-occlusive pain at home was 114·90 person-years in the low-dose group and 86·54 person-years in the moderate-dose group, with an incidence rate ratio of 1·33 (95% CI 1·11–1·59; p=0·0023; table 3). Health-care provider treatment of acute vaso-occlusive pain (inpatient or outpatient setting) was higher in the low-dose group than in the moderate-dose group (incidence rate ratio of 1·82 [95% CI 1·23–2·74]; p=0·0022; table 3). No participant in either group had hydroxyurea stopped for myelosuppression or drug-related toxicity, and no participant required dose reductions. The baseline and exit haematology laboratory values per treatment group are in the appendix (p 89).

Discussion

To our knowledge, the SPRING trial is the first randomised controlled trial showing the benefits of initial hydroxyurea therapy for primary stroke prevention with no associated increase in hydroxyurea-related toxicities or death compared between dose groups and the reference group. Compared with low-dose hydroxyurea therapy, participants who received moderate-dose had no difference in the stroke incidence rate. In both hydroxyurea treatment groups, the stroke incidence rate was similar to the STOP trial's initial monthly transfusion group of approximately one stroke per 100 person-years.[4] However, moderate-dose hydroxyurea seems to be associated with lower incidence rates of all-cause hospitalisations, vaso-occlusive events requiring hospitalisations, and home management of acute vaso-occlusive pain events.

A significant strength of the SPRING trial was the initial focus on the sustainability of the stroke prevention teams after completion of the trial. The main obstacles to sustainability were the household poverty level of most children attending the clinic coupled with a predominantly self-pay health system. In Nigeria, approximately 53% of families (82·5 million people) live below the international poverty line of approximately US $1·90 per day.[24] Thus, many families cannot afford the cost of a complete blood

cell count for hydroxyurea myeloid suppression surveillance with a cost of about US$3·00 for a single measurement in Kano, Nigeria.

To address the sustainability of the trial results after completion, we identified a Nigerian pharmaceutical company that produces hydroxyurea at a subsidised cost (Bond Chemical, Ibadan, Nigeria) of $0·16 per 500 mg capsule. Additionally, we implemented task-shifting from physicians to nurses for continued free of charge transcranial Doppler screening; provided free of charge hydroxyurea for children with abnormal transcranial Doppler velocities or pre-existing strokes; and created state-supported stroke prevention teams at the teaching hospitals.[25] Together, these strategies ensured a seamless transition between completing the trial and implementing government-supported primary and secondary stroke prevention programmes in the three Nigerian states.[12]

The precise biological mechanism of hydroxyurea's beneficial effects for preventing ischaemic strokes in children with abnormal transcranial Doppler velocities is unclear. Plausible factors contributing to hydroxyurea's effect in primary stroke prevention are multifactorial. They include increasing concentrations of haemoglobin and haemoglobin F,[26] haemoglobin oxygen saturation percentage,[27] and nitric oxide via cyclic GMP and cyclic AMP concentations,[28], [29] while simultaneously decreasing white blood cell count, red blood cell count, and reticulocyte counts, and reducing red blood cell adhesion to the endothelium.[26]

In a systematic review of the literature before[30] and after cessation of the SPRING trial,[31] there has been no randomised controlled trial showing the benefit or harm of initial hydroxyurea treatment for abnormal transcranial Doppler measurements in either a low-middle-income or high-income setting. However, the team had substantial evidence that a moderate dose of hydroxyurea was effective in reducing transcranial Doppler measurements, based on the single-arm SPIN trial using moderate-dose hydroxyurea[13] and pooled analysis of seven studies before the trial indicating hydroxyurea therapy decreases transcranial Doppler velocities, with a mean decrease in velocities of 25 cm/s.[30] Together, these data were sufficient to initiate a randomised control trial for primary stroke prevention with initial low-dose and moderate-dose hydroxyurea.

Before this trial, Nigerian paediatricians participating in the trial were at equipoise as to whether low-dose or moderate-dose hydroxyurea should be used for primary stroke prevention. Collectively the paediatricians in Nigeria were unwilling to include an initial maximum tolerated dose of hydroxyurea group in the trial. The basis for their strong opinion was preliminary data from the SPIN trial showing the benefit of moderate-dose hydroxyurea for primary stroke prevention,[13] and their concern about the complex medical care and relatively expensive laboratory monitoring required to titrate children to the maximum tolerated dose in a setting where nurses were providing most of the clinical care without a paediatrician.

After the SPRING trial was initiated, the results were published of the SCD hydroxyurea trial done in Angola, Uganda, and the Democratic Republic of Congo in children with sickle cell anaemia.[32] In that trial, the mean maximum tolerated hydroxyurea dose was 22·5 mg/kg per day, which was not clinically different from the mean moderate hydroxyurea dose in the SPRING trial, 20·6 mg/kg per day, and the fixed-dose of 20 mg/kg per day in the SPIN trial. In the previous completed hydroxyurea trial of 600 children with sickle anaemia, 5·1% of the participants had haematological dose-limiting toxic effects[32] compared with no participants in the SPRING trial or SPIN trial. Also, after the SPRING trial was completed, Lagunju and colleagues[33] described the results of the largest and most extensive experience with initial hydroxyurea therapy for primary stroke prevention in children with abnormal transcranial Doppler velocities. In this observational study, conducted in Ibadan, Nigeria, 396 children with sickle cell anaemia and abnormal transcranial velocities were treated with a maximum tolerated dose of hydroxyurea. Two strokes occurred in this cohort with a stroke incidence of 0·08 per 100 person-years. Equally important, in this observational study, the mean hydroxyurea dose was 23·7 mg/kg per day; however, no myelosuppression occurred resulting in the cessation of hydroxyurea therapy.

Three key trial outcomes will change regional clinical practice and might decrease the gap in primary stroke prevention in Nigeria and elsewhere in Africa. First, the absence of myelosuppression in either treatment group is important. At the SPRING trial sites, complete blood cell counts for

children on hydroxyurea have been reduced from monthly to biannually, per the new local standard. This change in monitoring dramatically reduces costs and burdens for families.

The second change in regional practice is the initial and 3 month transcranial Doppler velocities as biomarkers of hydroxyurea response. Higher Doppler velocities at baseline and 3 months were independently associated with future strokes. In the extended STOP trial, a threshold of 220 cm/s or more conferred high-risk for subsequent strokes.[22] All six strokes that occurred in an extended evaluation of STOP participants were in participants with initial velocities of 220 cm/s or more.[22] Thus, if the initial transcranial Doppler velocity is 220 cm/s or more, stroke risk is high, and at least moderate rather than low-dose hydroxyurea should be considered. At a minimum, the transcranial Doppler study should be repeated in 3 months. If the 3 month velocity remains 220 cm/s or more, an evaluation should be considered for adherence, increasing the hydroxyurea dose from low to moderate or moderate to the maximum tolerated dose, or both. Furthermore, the statistical relationship between strokes and the magnitude of the transcranial Doppler velocity supports the importance of the transcranial Doppler velocity as a risk factor for overt stroke in the Nigerian population, as in the STOP trial.
The third change in regional practice, which Nigerian paediatricians who participated in the trial now use, is an acute vaso-occlusive pain history specifically focused on acute pain at home and pain resulting in a subsequent health-care visit before and after starting hydroxyurea for primary stroke prevention. If there is a high incidence rate of vaso-occlusive events (acute pain or acute chest syndrome), shared decision making between the guardian and the health-care provider regarding the preferred hydroxyurea dose is initiated.

A perceived major weakness of the trial is the absence of brain MRI to diagnose a stroke. However, MRI of the brain confirmed strokes in the STOP trial, the presence of a stroke was based on a clinical diagnosis of all 11 participants after stroke adjudication.[4] Further, WHO's definition of a stroke does not include neuroimaging, and is an acceptable clinical endpoint in the American Society of Hematology Guidelines.[31] Further, a

clinical diagnosis of stroke as the primary outcome measure has been applied in a NINDS-sponsored sickle cell anaemia randomised controlled stroke prevention trial conducted in high-income countries.[34] Lastly, every month, participants were evaluated in-person with a standardised questionnaire to detect neurological impairment in children living in Africa,[14] coupled with a standardised neurological examination to detect strokes. A video of the neurological examination was centrally adjudicated for the presence of a stroke for any child with concern for stroke with a detailed recording of the neurological examination (table 2). Together, these strategies decreased the likelihood of an undetected and untreated stroke being missed in between the 30-day research visits. Another limitation was the absence of magnetic resonance angiography to detect cerebral vasculopathy, an exclusion criterion in one primary stroke prevention trial based in the USA[35] that was not applied in this trial. However, only approximately 20% of children with sickle cell anaemia and abnormal velocities have magnetic resonance angiography-defined severe vasculopathy.[36]

An inherent limitation in any clinical trial, including ours, is the inability to address the durability of the treatment effect. To address the long-term efficacy of low-dose hydroxyurea therapy for primary stroke prevention, we have initiated a stroke registry (NCT04800809) to follow up the neurological outcomes of all the participants now that the SPRING trial has been completed.

Primary stroke prevention with initial low-dose hydroxyurea therapy and biannual complete blood cell count is a new evidence-based strategy for children with sickle cell anaemia living in Africa. For children at risk for strokes with increased rates of all-cause hospitalisations, vaso-occlusive pain events at home, or requiring a physician visit, fixed moderate-dose hydroxyurea therapy and biannual complete blood counts could be considered after an informed discussion with the parents.

Contributors

MRD, MR, and JCS had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses. The trial was designed by MRD, SUA, MHA, LCJ, MR, NG, KN, FJK, and MAT. The transcranial Doppler Training was completed by AHD,

HBM, HI, AMT, AS, BWJ, SG, LH, SUA, MSH, AGT, and NI, LCJ, ET, and FJK verified the strokes with video of neurological examinations; BCG and DLG collected and audited data integrity; AK and AG and AB monitored the safety of the participants; JCS and MR performed the analyses. MRD, SUA, LCJ, and MHA interpreted the results; and SUA, LCJ, MA, FJK, MR, and MRD wrote the manuscript. Prior to submission, all authors reviewed the manuscript.

Declaration of interests

FJK received royalties from the MacKeith Press for her contributions to the *Cerebrovascular Disease and Stroke in Children* book. FJK received consulting fees from Global Blood Therapeutics for transcranial Doppler training for HOPE Kids and KOPE Kids 2. FJK received a payment from Johnson & Johnson for a presentation in October, 2019, and from Bluebird Bio for a presentation in December, 2019. FJK received payment for a speaker's bureau in November, 2019. FJK received payment for an institution discretionary for medicolegal. FJK received support for a European Paediatric Neurology meeting in Athens, in September, 2019, from BIAL. FJK was part of a group who received a DWL transcranial Doppler from Global Blood Therapeutics for training purposes in HOPE Kids 2. ET served as a member of the NINDS/NIH advisory council. MD and his institution are the sponsor of two externally funded research investigator-initiated projects. Global Blood Therapeutics will provide funding for the cost of these clinical studies, but will not a cosponsor of either study. MD is not receiving any compensation for the conduct of these two-investigator initiated observational studies. MD is a member of the Global Blood Therapeutics advisory board for a proposed randomised controlled trial for which he receives compensation. MD is the steering committee for a Novartis-sponsored phase 2 trial to prevent priapism in men. MD was a medical advisor for the development of the CTX001 Early Economic Model. MD provided medical input on the economic model as part of an expert reference group for Vertex/CRISPR CTX001 Early Economic Model in 2020. MD provided a one-time consultation to the Forma Pharmaceutical company about sickle cell disease in 2021.

Data sharing

Fully anonymised data can be shared on request from any qualified investigator with approved study and data transfer agreement between Vanderbilt University Medical Center and the Institution.

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Supplementary Material

Supplementary appendix.

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