Primary prevention of stroke in children with sickle cell disease in Sub-Saharan Africa: rationale and design of phase III randomized clinical trial

Shehu U. Abdullahi[a], Binta J. Wudil[b], Halima Bello-Manga[c], Aisha B. Musa[a], Safiya Gambo[e], Najibah A. Galadanci[f], Hauwa Aminu[a], Aliyu Tijjani Gaya[e], Surayya Sanusi[g], Musa A. Tabari[g], Aisha Galadanci[d], Awwal Borodo[h], Muhammed S. Abba[i], Abdu H. Dambatta[g], Lawal Haliru[j], Awwal Gambo[e], Holly Cassell[k], Mark Rodeghier[l], Djamila L. Ghafuri[m], Brittany V. Covert Greene[m], Kathleen Neville[n], Adetola A. Kassim[m,o], Fenella Kirkham[p], Edwin Trevathan[k], Lori C. Jordan[q], Muktar H. Aliyu[k], and Michael R. DeBaun[m]

aDepartment of Pediatrics, Bayero University, Aminu Kano Teaching Hospital, Kano, Nigeria; bDepartment of Pediatrics, Hasiya Bayero Specialist Hospital, Kano, Nigeria; cDepartment of Hematology and Blood Transfusion, Barau-Dikko Teaching Hospital, Kaduna, Nigeria; dDepartment of Hematology and Blood Transfusion, Bayero University, Aminu Kano Teaching Hospital, Kano, Nigeria; eDepartment of Pediatrics, urtala Mohammed Specialist Hospital, Kano, Nigeria; fDepartment of Epidemiology, School of Public health, University of Alabama at Birmingham, Birmingham, Alabama, USA; gDepartment of Community Medicine, Bayero University, Aminu Kano Teaching Hospital, Kano, Nigeria; hDepartment of Medicine, Murtala Mohammed Specialist Hospital, Kano, Nigeria; iDepartment of Pharmacy, Bayero University, Aminu Kano Teaching Hospital, Kano, Nigeria; jDepartment of Pediatrics, Kaduna State University, Barau Dikko Teaching Hospital, Kaduna, Nigeria; kVanderbilt Institute for Global Health, Vanderbilt University Medical Center, Nashville, Tennessee, USA; lRodeghier Consultants, Chicago, Illinois, USA; mDepartment of Pediatrics, Division of Hematology and Oncology, Vanderbilt-Meharry Center of Excellence in Sickle Cell Disease, Vanderbilt University Medical Center, Nashville, Tennessee, USA; nDepartment of Pediatrics, University of Kansas for Medical Sciences, Little Rock, Arkansas, USA; oDepartment of Hematology and Oncology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA; pDepartment of Pediatrics, UCL Great Ormond Street Institute of Child Health, London, UK; qDepartment of Pediatrics, Division of Pediatric Neurology, Vanderbilt University Medical Center, Nashville, Tennessee, USA

CONTACT Michael R. DeBaun m.debaun@vumc.org
Department of Pediatrics,
Division of Hematology/Oncology,
Vanderbilt-Meharry Center of Excellence in Sickle Cell Disease,
2525 West End Avenue,
Suite 750,
Nashville, TN 37203-1738, USA.

ABSTRACT

Strokes in children with sickle cell anemia (SCA) are associated with significant morbidity and premature death. Primary stroke prevention in children with SCA involves screening for abnormal transcranial Doppler (TCD) velocity coupled with regular blood transfusion therapy for children with abnormal velocities, for at least one year. However, in Africa, where the majority of children with SCA live, regular blood transfusions are not feasible due to inadequate supply of safe blood, cost, and the reluctance of caregivers to accept transfusion therapy for their children. We describe the Primary Prevention of Stroke in Children with Sickle Cell Disease in Nigeria Trial [Stroke Prevention in Nigeria (SPRING) trial, NCT02560935], a three-center double-blinded randomized controlled Phase III clinical trial to 1) determine the efficacy of moderate fixed-dose (20mg/kg/day) versus low fixed-dose (10mg/kg/day) hydroxyurea therapy for primary stroke prevention; 2) determine the efficacy of moderate fixed-dose hydroxyurea for decreasing the incidence of SCA-related hospitalization (pain, acute chest syndrome, infection, other) compared to low fixed-dose hydroxyurea. We will test the primary hypothesis that there will be a 66% relative risk reduction of strokes in children withSCA and abnormal TCD measurements, randomly allocated, for a minimum of three years to receive moderate fixed-dose versus low fixed-dose hydroxyurea (total n=220). The results of this trial will advance the care of children with SCA in Nigeria and sub-Saharan Africa, while improving research capacity for future studies to prevent strokes in children with SCA.

Introduction

Standard care for primary stroke prevention in children with sickle cell anemia (SCA) includes routine transcranial Doppler (TCD) measurement coupled with regular blood transfusion therapy for at least one year for children with abnormal timeaveraged maximum mean velocities (TAMMV) of TCD measurements (≥ 200 cm/ sec) in the terminal portion of the internal carotid artery (ICA) or the proximal middle cerebral artery (MCA).[1,2] However, blood transfusion therapy is not an acceptable or feasible strategy for primary stroke prevention in Nigeria and other parts of Africa. In Nigeria, regular transfusions require an indefinite family commitment to seek and sustain a pool of blood donors as the blood supply is limited. Given the challenges associated with regular blood transfusion in low-resource settings, the only current reasonable alternative for stroke prevention is hydroxyurea
therapy.

Based on the results of a feasibility trial, (Stroke Prevention in Nigeria (SPIN) trial,[3] NCT01801423), we designed the Stroke Prevention in Nigeria (SPRING) trial, NCT02560935] a three-center, double-blinded, randomized controlled Phase III clinical trial for primary stroke prevention in children with sickle cell anemia (SCA) in northern Nigeria. Funding for the trial started in 2015 for a planned 5 years. The primary hypothesis is that among children with SCA and abnormal TCD measurements, randomly allocated (n=220) to either moderate fixed-dose (20 mg/kg/day) or low fixed-dose (10 mg/kg/day) hydroxyurea for a minimum of three years, moderate fixed-dose hydroxyurea therapy is superior to low fixed-dose hydroxyurea therapy for primary stroke prevention. The rationale for low-dose hydroxyurea is that this dosing, if effective, would not require frequent laboratory monitoring for myelosuppression, which is recommended at higher doses.[4] Further, if fixed low-dose hydroxyurea is efficacious in preventing initial strokes, twice as many children could betreated with 10 mg/kg/day compared with 20 mg/kg/day dosing for the same cost of therapy and potentially without the requirement of laboratory monitoring. These two features, costs of hydroxyurea therapy in Nigeria (approximately $5.00 United States dollars per month for a child with SCA receiving 20 mg/kg/day) and complete blood count to assess myelosuppression of hydroxyurea (approximately $6.00 United States dollars) are critically important barriers for implementation of primary stroke prevention in a low-resource setting.

Methods
Trial objectives and organization
Main hypothesis

The overall goal of the SPRING trial (NCT02560935) is to determine the efficacy of moderate fixed-dose versus low fixed-dose hydroxyurea therapy for primary stroke prevention in children with SCA. We randomly allocated children with SCA and abnormal TAMMV for TCD measurements (≥200 cm/sec) to receive moderate-fixed dose versus low fixed-dose hydroxyurea. There will be a total of 110 participants in each treatment group followed for a minimum of three years. A comparison group of 220 children not treated with hydroxyurea that were screened but were deemed ineligible based on TCD velocity < 200 cm/sec will be followed for a minimum of three years to assess the potential for excessive mortality and morbidity (hospitalizations) associated with receiving hydroxyurea when compared to not receiving hydroxyurea.

Secondary hypothesis

Moderate fixed-dose hydroxyurea therapy is superior to low fixed-dose hydroxyurea therapy for decreasing the incidence of all cause-hospitalization (due to pain, acute chest syndrome, infection, or other). Using modified intention-to-treat analysis, we will compare the incidence rates of hospitalization for children receiving moderate fixed-dose and low fixed-dose hydroxyurea therapy.

Trial organization

Approval was obtained from the Ethics Committees of Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria Kano State Ministry of Health, Barau Dikko Teaching Hospital (BDTH), Kaduna, Nigeria; and the Vanderbilt University Institutional Review Board, Nashville, USA. The trial was also approved by the Nigeria National Agency for Food and Drug Administration and Control. All authors vouch for the fidelity of the trial protocol. A Data and Safety Monitoring Board (DSMB) appointed by the National Institute of Neurological Disorders and Stroke will review serious adverse events, trial progress, and safety. The trial is being conducted at three hospitals (two academic centers and one large community hospital) in northern Nigeria:

1. Aminu Kano Teaching Hospital (AKTH) is a 500-bed tertiary level facility affiliated with Bayero University and located in Kano, the second largest metropolis in Nigeria (2016 population: 3.9 million).5 AKTH the sickle clinic serves approximately 2,010 children with sickle cell disease, with an average of 70 chilidren evaluated once a week.

2. Murtala Muhammed Specialist Hospital (MMSH) is a community hospital affiliated with AKTH, also located in Kano. MMSH has the largest population of patients with sickle cell disease in Nigeria,6 with approximately 17,800 children with sickle cell disease registered at the pediatric unit and an average of 100 children with sickle cell disease seen four days a week in the clinic.

3. Barau Dikko Teaching Hospital (BDTH) is the teaching facility for Kaduna State University located 120 miles south in Kaduna, Nigeria (population: _1 million). The SCD Clinic serves approximately 1,600 registered patients with sickle cell disease, average of 40 children and 20 adults evaluated once a week.

Trial design and conduct of the trial
Overall trial design

SPRING is a multicenter, double-blind (pharmacist and statistician unmasked), but particpant, family members, and all health care providers were blinded, randomized controlled trial, in which we will assign children with SCA ages 5 to 12 years and abnormal TCD velocities to receive hydroxyurea into one of two treatment groups: fixed moderate-dose of 20 mg/kg/day or fixed low-dose of 10 mg/kg/day. Participants who are randomly assigned to receive either dose are evaluated monthly for hydroxyurea therapy adherence with monthly laboratory monitoring for SCA and hydroxyurea-associated adverse events. Assigned local trial monitors will review the laboratory values weekly.

Primary and secondary endpoints

The primary endpoint is the occurrence of an initial clinical stroke or transient ischemic attack (TIA), defined by the World Health Organization (WHO) as a clinical syndrome consisting of rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting >24 hours for stroke and or <24 hours for a TIA; or leading to death with no apparent cause other than that of vascular origin.7

Members of the neurology committee, who are unaware of the trial-group assignments, will adjudicate neurologic events based on a detailed history and neurological examination findings.

Secondary outcomes include, but were not limited to the incidence of hospitalizations for any cause and evaluation of long-term safety of moderate fixed-dose versus low fixed-dose hydroxyurea therapy. All clinical events were adjudicated without knowledge of the treatment assigment.

Mortality was a primary safety endpoint for the SPRING trial

The site investigator and panel of senior neurologists will review each stroke or acute neurological event, medical history, physical examination, and laboratory values. The neurology adjudication process optimizes the best opportunity to determine the presence of a stroke and whether a stroke was the proximal cause of death, in the absence of an autopsy. In this region of Nigeria, autopsies are not typically performed at the time of death.

Trial eligibility
Screening and randomization
Inclusion and exclusion criteria for screening and random allocation of participants are described in Tables 1 and 2, respectively. Children under 5 years of age were excluded due to the high mortality in this age group in Nigeria.8–11 Inclusion criteria for random allocation of eligible participants to moderate fixed-dose or low fixed-dose of hydroxyurea therapy include: TAMMV of TCD measurements _200 cm/second, measuredtwice by two different TCD certified ultrasonographers, or at least one TCD measurement greater than 220 cm/sec in the middle cerebral artery, internal carotid, or both vessels, with non-imaging TCD technique defined in Table 3;12 able to swallow an empty capsule and willing to be followed monthly for at least 36 months. Liquid formulation hydroxyurea is not feasible due to the absence of approval from the Nigerian National Agency for Food and Drug Administration and Control, the Nigerian equivalent of the United States Food and Drug Administration.

Comparison group
A cohort of children (n¼220) with SCA and normal TCD (<170 cm/sec) or conditional TCD (170-199 cm/sec) velocities1 that are not eligible to receive hydroxyurea therapy for primary stroke prevention based TCD criteria will be enrolled and followed with annual trial visits, including annual TCD assessments for at least 36 months.

Rationale for an untreated comparison group to the two hydroxyurea treatment groups
To assess the safety of hydroxyurea in Nigerian children with SCA, the trial is required to have a comparison group that was not initially treated with hydroxyurea. The randomized controlled trial design cannot determine if any increase in mortality rate is related to hydroxyurea because both treatment arms will receive hydroxyurea. The comparison group includes children with SCA and TCD values < 200 cm/sec not initially treated with hydroxyurea. These children have a lower risk of stroke due to non-elevated TCD values but should not be at higher risk for death or serious life-threatening events unless hydroxyurea treatment predisposes to these events. Treatment group participants will be seen monthly to assess for potential hydroxyurea associated myelosuppression with complete blood cell count (CBC) and to assess for stroke status with history and physical examination focused on the

neurological findings, interim hospitalizations, and interim medical treatment for acute illness, including acute vaso-occlusive pain that did not require hospitalization or a visit to a physician. Each of these events are considered secondary outcomes and will be adjudicated without knowledge of treatment assignment. Children in the comparison group will be contacted at every two weeks via phone to report hospitalizations and ascertain participant status.

Comparison group participants will be called every two weeks to assess for SCD related morbidity and vital status (alive or dead). Monthly visits for the comparison group are not necessary to collect the required safety information needed in the trial (vital status, interim hospitalizations, and interim treatment for acute illness, including vaso-occlusive pain that did not require hospitalization or a visit to a physician). During the trial, if participants in the comparison group develops abnormal TCD measurements, they are eligible to be enrolled in the trial and will be randomly assigned to receive either fixed moderate-dose or fixed low-dose hydroxyurea therapy.

Transcranial doppler assessment training
Two AKTH-based radiologists who participated in the feasibility trial3 recruited, trained, and certified research physicians to perform TCDs. For TCD certification, Spearman's rank correlation (r) will be performed on the two TCD velocity measurements made by the radiologist trainer on the right and left side. The minimum acceptable correlation for the trainer's two measurements on each side was set as 0.90, with an expected lower bound correlation between the trainer and the research physician trainee of 0.765 (85% of the 'trainer's correlation in the same individual performed only hours apart). Each trainee was expected to have a minimum of 40 paired TCD measurements with a trainer radiologist. Sample sizes of 42 and 31 paired measurements between the trainer and each trainee are associated with a power of 90% and 80%, respectively, based on the expected correlation between the trainer and trainee.Neurological examination training

All research physicians will be certified on the Pediatric National Institutes of Health Stroke Scale (PedNIHSS),13 a validated, standardized neurological examination. Two experienced board-certified pediatric neurologists (LJ and FK) will review a minimum of 10 paired neurological examinations with research physicians in children.

Participant assessment
TCD examination (non-imaging technique) performed for routine clinical care may be used as the first TCD required for the trial, if performed within three months before the informed consent, the TAMMV of TCD measurements meets the qualifying criteria with a measurement equal to or above 200 cm/sec, but less than 220 cm/sec TCD examiner.
However, the second TCD assessment after the first elevated TCD measurement must be completed by a certified TCD examiner. A single TCD measurement greater than 220 cm/sec was sufficient for eligibility.

Neurological evaluation
The PedNIHSS, is a validated, standardized neurological examination.13 The NIHSS is easily performed by non-neurologists, detects changes in neurological status well, and has excellent inter-rater reliability.14–17 The PedNIHSS will be completed on all

randomly allocated participants at baseline, annually, and as soon as possible following a suspected stroke event. The PedNIHSS evaluations after a possible stroke will be recorded, and videos, along with case report forms will be reviewed by the Neurology Committee.

Definition of stroke and transient ischemic attack (TIA)
Definitions for a stroke or TIA are based on the WHO definitions:[7] a clinical syndrome consisting of rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting > 24 hours for stroke and or < 24 hours for TIA; or leading to death with no apparent cause other than that of vascular origin (Table 4).

Magnetic resonance imaging of the brain and computed tomography of the head are not readily available in Nigeria and other sub-Saharan African countries.

Hydroxyurea therapy (trial drug, from Bond Chemical, Awe, Oyo state, Nigeria).
The trial intervention is the random allocation to fixed low-dose hydroxyurea therapy at 10 mg/kg/day (range: 7–15 mg/kg/day) or fixed moderate-dose hydroxyurea therapy at 20 mg/kg/day (range 17.5–26 mg/kg/day). No dose escalation will occur, as 20 mg/kg/ day has efficacy in infants with SCA and is associated with rare myelosuppression.[18,19]

Further preliminary data from our feasibility trial,[3] indicates that hydroxyurea lowers abnormal TCD measurements (Figure 1) and prevents strokes. Hydroxyurea will be provided to each participant via the trial pharmacy at no cost for the duration of the trial.
Critical to sustainability was the availability of hydroxyurea produced within the country at a discounted cost of approximately $0.15 (United States dollar) per 500 mg capsule.

Double-blind randomized controlled clinical trial
The SPRING Trial is double-blinded because the paticipants, their family, and the health research team, were unaware of the random assignment. The participant's random allocation was known to the clinical trial pharmacists and statisticians. The trial monitors are masked but may be unmasked if the clinical situation warrants. During the monthly trial follow-up visits, the trial medication will be prescribed, distributed, and collected by the clinical trial pharmacist only. The clinical research team managing the participant's care will not have access to the assigned random allocation, nor will they distribute, collect, or handle the trial medication. This strategy prohibits the need for the trial physician to calculate the dose or determine the trial treatment assignment.

Randomization procedures
The project statistician will implement a permuted block allocation scheme, based on block sizes of 2 and 4, stratified by sex, within the clinical site. Randomization will be accessed at each site through REDCap,[20] a secure online data management tool that permits the trial pharmacist to click a "randomize" button. Eligibility will be confirmed, and a participant trial ID number will be assigned before random allocation. The ID number and the eligibility status will be recorded on a log sheet before the assignment.

This record will then be sent to the site pharmacist at each clinical site. The trial pharmacist will hold the sequence of assignment to fixed moderate-dose or fixed low-dose hydroxyurea at the trial site only. The medication will be provided by the pharmacist in 100, 250, or 500 mg capsules; the pharmacist will calculate the pills needed based on the weight of the trial participant at the time of the trial visit.

Adherence to hydroxyurea therapy

Adherence will be measured via mean cell volume (MCV). Based on our feasibility trial,3 we expect that the MCV will increase by at least 5 fL from baseline if participants are taking hydroxyurea regularly with a fixed moderate-dose. In the SPIN trial, participants had an increased 16 fL after 24 months of hydroxyurea therapy (85 fL to 101 fL).

We selected a minimum increase of 5 fL because, in a prospective cohort of adherence in adults with SCD, Queioz et al. demonstrated that any positive growth in the MCV was associated with a decrease in the incidence of vaso-occlusive pain episodes.21

Rather than stating any increase in MCV was associated with adherence to hydroxyurea, we selected a measure above estimated background laboratory assessment error. We arbitrarily decided an increase of 5 fL from baseline was a minimum measurement of adherence to hydroxyurea in both arms.

In addition to an increase in MCV of at least 5 fL, we also will measure hemoglobin F levels annually (too expensive to measure with each visit). Monthly hydroxyurea pill counts returned to the pharmacists are also included as an adherence measure. Together, all measures will be used to assess adherence to therapy. Participants will be educated on the importance of daily hydroxyurea. Adherence will be reviewed and discussed during each monthly trial visit. The trial pharmacist will also document the hydroxyurea dose and dispensing date at each reserach visit.

Laboratory and safety monitoring

Participants will undergo monthly laboratory monitoring to assess for potential SCA or hydroxyurea-associated adverse events. A local monitor in Nigeria will be paired with a second monitor from the coordinating center to review all laboratory values weekly. The trial monitors will meet weekly with the site investigators, principal investigators and research coordinators via webinar teleconference to discuss out-of-range laboratory values. Based on the laboratory values of participants in our initial feasibility trial3 and the laboratory values that will be collected in the SPRING trial (total of 6000 laboratory values for both studies), we selected the 10th and 90th percentile for each laboratory value to determine if a value is outside of the expected range and requires repeat laboratory evaluation, clinical evaluation, or both.

Steps to limit adverse events associated with participation and exit after completion of the trial

A CBC with differential will be obtained two weeks after hydroxyurea therapy is started and every 4 weeks thereafter, unless toxicity occurs. In the case of suspected hydroxyurea myelosuppression, therapy will be stopped, and a CBC with differential will be obtained weekly, until resolution of the myelosuppression. Hydroxyurea therapy will then be restarted at the same dose, unless hematologic toxicity has previously occurred at this dose; if so, the dose will be reduced. Myelosuppression possibly related to hydroxyurea is defined as absolute

neutrophil count <1000_10 9/L or platelet count <80_109/L. If either threshold is reached, the caretaker will be asked to return for repeat CBC within a week. Caretakers will be given a trial card to always carry with them, providing instructions for any health care provider who sees the participant for an unscheduled medical care visit.

Upon completion of the trial, participants will have a TCD measurement, CBC and hemoglobin F measurements. The participant's guardians will be given written final results of the trial, along with the verbal description of the final results.

Statistical considerations
Sample size and statistical analysis

To test our primary hypothesis, based on data from the feasibility trial3 where approximately 66% (15 of 23) of participants had their elevated TCD measurements drop to normal measurements (<200 cm/sec in both vessels) after three months on hydroxyurea therapy, we estimated a treatment effect of 66% relative risk reduction. Power was estimated with a two-sided test of the difference between hazard rates in the moderate fixed dose versus low fixed-dose hydroxyurea groups, assuming that the data were approximately exponentially distributed. We tested the hypothesis that the rate of stroke will be 3.0 events/100 person-years and 9.0 events/100 person-years in the moderate fixed dose and low fixed-dose hydroxyurea groups, respectively. With a recruitment period of 2 years, minimum follow-up time of 3 years, and 9% loss to follow-up in each group per year, an overall sample size of 220 participants (110 in the fixed moderate-dose group and 110 in the fixed low-dose group) achieves at least 90% power at a 0.05 significance level. A modified-intention-to-treat (mITT) principle will compare incidence rates of stroke or TIA between the moderate fixed versus low fixed-dose hydroxyurea groups. The primary analysis, based on mITT, will include randomly allocated participants who receive at least a month of hydroxyurea. A month is selected because this is the minimum period for which hydroxyurea is expected to have any clinically relevant adverse laboratory or clinical outcome. For the multivariable data analysis, a generalized linear model and a Cox proportional hazard model will be used to adjust for important covariates, including the actual dose of hydroxyurea received and known confounders to assess risk factors for stroke.

Data management

Data will be entered and managed using REDCapVR data system hosted at Vanderbilt, with each participant having a unique identifier.20

Interim data and stopping rules and data and safety monitoring

There will be one 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 will be used for the interim analysis to assess futility. The significance boundary for the final analysis will be P_0.047. One interim analysis for futility, based on the stochastic curtailment method, will be performed by calculation of conditional power, an estimate of the probability that the trial shows a statistically significant effect on the primary endpoint, i.e., stroke rate, given the results to date and assumptions regarding outcome through the end of the trial. The analysis will occur at the halfway point of the person-year accrual. A recommendation to stop the trial for futility will require a conditional power below 30%, under the observed efficacy

trend at the time of interim analysis, with two-sided type I error <5%. The superiority of stroke rate will be based on the upper limit of O'Brien-Fleming at the interim analysis. For the first look stopping rule will apply an alpha = 0.005. The proposed sample size of 110 per arm is adjusted for the interim analyses, i.e., types I and II errors.

We will also calculate the 95% confidence interval (CI) of the 1-year mortality rate ratio.

At the interim analysis, if the lower boundary of the 95% CI is greater than 1.17 (equivalent to the non-inferior limit of 0.85), then the trial will be paused, and a meeting will be held with the DSMB to address the relative merits of stopping the trial or moving forward.

Discussion

Approximately 150,000 children with SCA are born every year in Nigeria,[22] 50% of all children born annually with SCA in the world.[23] No strategy to decrease the global burden of strokes in children with SCA can be considered without including an approachfor primary stroke prevention in children living in Nigeria. The long-term goal of the SPRING Trial is to determine the optimal dose of hydroxyurea therapy for primary stroke prevention in Nigeria. Based on the results of the SPIN trial,[3] and in the absence of further data, moderate fixed-dose 20 mg/kg/day is considered the standard care arm of the SPRING randomized controlled trial, and 10 mg/kg/day is the experimental arm of the trial. In the SPIN trial all participants had TCD _ 200 cm/sec at trial entry and received a fixed moderate-dose of 20 mg/kg/day, three months after beginning hydroxyurea, 80% of the participants had TCD measurement below 200 cm/sec and maintained a normal TCD value for more than 12 months.[24] Most importantly, in the SPIN trial, the stroke incidence rate was far less than expected for children with abnormal TCD measurements if 20 mg/kg/day of hydroxyurea was not effective in preventing strokes.

In preparation for this Phase III trial, we have strong preliminary data from our feasibility (SPIN) trial in Kano, Nigeria, and others[24–27] demonstrating the efficacy of at least moderate-dose hydroxyurea therapy (20 mg/kg/day) for primary stroke prevention (Figure 2). SPIN trial participants with abnormal TCD measurements experienced a drop in their TCD velocity to < 200 cm/sec in three months. In comparison, in the STOP trial, 52% of the participants had a drop in TCD velocity to a normal level, after approximately 4 months.[1] These data indirectly demonstrate the efficacy of moderate dose hydroxyurea therapy for primary stroke prevention[33] and when coupled with our prior results from our feasibility trial, we have reached a threshold where a phase III RCT for primary stroke prevention is compelling in children with SCA in Nigeria.

Given the early success of the SPIN trial, which was initiated before TWITCH[19] and REACH[26] trials were started, we had no evidence that we should consider a maximum tolerated dose (MTD) of hydroxyurea for primary stroke prevention. Further, in young children with SCA, the REACH trial was conducted in multiple countries in Africa, titrated hydroxyurea to the MTD, with a median result of 22 mg/kg/day, which is not clinically different than our moderate fixed-dose of 20 mg/kg/day in the SPRING trial.

After the completion of the SPRING trial, we may able to determine whether there is a statistical difference in the incidence rate of strokes with fixed low-dose or fixed-moderate dose hydroxyurea. Based on the evidence that moderate fixed-dose hydroxyurea therapy lowers TCD values,[3,4, 19, 25, 34–39] and is safe in children

with SCA living in Africa,26 the remaining unanswered critical question is not whether hydroxyurea should be initially used for stroke prevention, but what dose maximizes benefits and minimizes cost, inconvenience, and toxicity.

Current recommendations for monitoring hydroxyurea associated myelosuppression are based on precedent, and not clinical trial data demonstrating an optimal CBC surveillance strategy. In the Baby HUG trial,4 fixed moderate-dose hydroxyurea therapy (20 mg/kg/day) was compared to placebo, hydroxyurea was not associated with an absolute neutrophil count less than <500/mm3 or platelet count <80_103/mm3. Given the evidence that moderate fixed-dose hydroxyurea therapy is not associated with myelosuppression in the vast majority of young children with SCA, we postulate that low fixed-dose hydroxyurea will require even less frequent laboratory surveillance. All 220 participants (110 participants randomly allocated to low and moderate-fixed dose hydroxyurea) will have planned monthly CBC assessments. The results of the SPRING trial may provide empirical evidence for determining the optimal CBC interval for hydroxyurea monitoring at low and moderate fixed dosing.

The SPRING trial will determine whether moderate fixed-dose versus low fixed-dose hydroxyurea therapy can potentially prevent thousands of strokes in children living in Africa, while simultaneously training the next cadre of physician-scientists and research staff in Nigeria. The results will provide evidence for hydroxyurea therapy as an initial alternative to blood transfusion therapy for children with abnormal TCD values living in low-resource settings, where blood transfusion therapy is not practical for the majority of children.

Authorship contributors
MD, LJ, MA, FK, NG, KN, AK, SA, MT designed the trial; and all coauthors wrote and reviewed the manuscript.

Disclosure statement
The authors report no conflict of interest.

References

1. Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med. 1998;339(1):5–11. doi:10.1056/NEJM199807023390102.

2. Adams RJ, Brambilla D. Optimizing Primary Stroke Prevention in Sickle Cell Anemia Trial I. Discontinuing prophylactic transfusions used to prevent stroke in sickle cell disease. The New England J. Med. 2005;353:2769–2778.

3. Galadanci NA, Umar Abdullahi S, Vance LD, et al. Feasibility trial for primary stroke prevention in children with sickle cell anemia in Nigeria (SPIN trial). Am J Hematol. 2017; 92(8):780–788. doi:10.1002/ajh.24770.

4. Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet (London, England). 2011;377(9778):1663–1672. doi:10.1016/S0140-6736(11)60355-3.

5. Prospects WP. Nigeria Population. 2019.

6. Galadanci N, Wudil BJ, Balogun TM, et al. Current sickle cell disease management practices in Nigeria. Int Health. 2014;6(1):23–28. doi:10.1093/inthealth/iht022.

7. Organization WH. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol. 1988;41:105–114. doi:10.1016/ 0895-4356(88)90084-4.

8. Ambe JP, Mava Y, Chama R, et al. Clinical features of sickle cell anaemia in northern nigerian children. West African J Med. 2012;31(2):81–85.

9. Grosse SD, Odame I, Atrash HK, et al. Sickle cell disease in Africa: a neglected cause of early childhood mortality. Am J Preventive Med. 2011;41(6 Suppl 4):S398–S405. doi:10.1016/j.amepre.2011.09.013.

10. Ikeh EI, Teclaire NN. Prevalence of malaria parasitaemia and associated factors in febrile under-5 children seen in Primary Health Care Centres in Jos, North Central Nigeria. The Nigerian Postgraduate Med J. 2008;15:65–69.

11. Ezeonwu B, Chima O, Oguonu T, et al. Morbidity and mortality pattern of childhood illnesses seen at the children emergency unit of federal medical center, asaba, Nigeria. Ann Med Health Sci Res. 2014;4(9):239–244. doi:10.4103/2141-9248.141966.

12. Neish AS, Blews DE, Simms CA, et al. Screening for stroke in sickle cell anemia: comparison of transcranial Doppler imaging and nonimaging US techniques. Radiology. 2002; 222(3):709–714. doi:10.1148/radiol.2223010524.

13. Ichord RN, Bastian R, Abraham L, et al. Interrater reliability of the Pediatric National Institutes of Health Stroke Scale (PedNIHSS) in a multicenter study. Stroke. 2011;42(3):613–617. doi:10.1161/STROKEAHA.110.607192.

14. Brott T, Adams HP, Jr., Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke. 1989;20(7):864–870. doi:10.1161/01.str.20.7.864.

15. Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. Stroke. 1994;25(11):2220–2226. doi:10.1161/01. STR.25.11.2220.

16. Muir KW, Weir CJ, Murray GD, et al. Comparison of neurological scales and scoring systems for acute stroke prognosis. Stroke. 1996;27(10):1817–1820. doi:10.1161/01.str.27.10.1817.

17. Goldstein LB, Samsa GP. Reliability of the National Institutes of Health Stroke Scale. Extension to non-neurologists in the context of a clinical trial. Stroke. 1997;28(2):307–310.doi:10.1161/01.str.28.2.307.

18. Wicherts JM, Dolan CV, Carlson JS, et al. Raven's test performance of sub-Saharan Africans: average performance, psychometric properties, and the Flynn effect. Learn Individual Differences. 2010;20(3):135–151. doi:10.1016/j.lindif.2009.12.001.

19. Ware RE, Davis BR, Schultz WH, et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWiTCH): a multicentre, open-label,phase 3, non-inferiority trial. Lancet (London, England). 2016;387(10019):661–670. doi:10.1016/S0140-6736(15)01041-7.

20. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)-a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377–381. doi:10.1016/j.jbi.2008.08.010.

21. Queiroz AMM, Lobo C, EMd N, et al. The mean corpuscular volume and hydroxyurea in Brazilian patients with sickle cell anemia: a surrogate marker of compliance. J Blood Disord Transfusion. 2013;2013:1–4.

22. World Health Assembly. Sickle-cell anaemia: report by the Secretariat. Geneva, Switzerland: World Health Organization; 2006. http://www.who.int/iris/handle/10665/20890. Accessed 1 October 2020.

23. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet (London, England). 2013;381(9861):142–151. doi:10.1016/S0140-6736(12)61229-X.

24. DeBaun MR, Kirkham FJ. Central nervous system complications and management in sickle cell disease. Blood. 2016;127(7):829–838. doi:10.1182/blood-2015-09-618579.

25. Park H, Bhatti S, Chakravorty S. Effectiveness of hydroxycarbamide in children with sickle cell disease - Analysis of dose-response metrics in a large birth cohort in a tertiary sickle cell centre. Pediatr Blood Cancer. 2019;66(7):e27615. doi:10.1002/pbc.27615.

26. Tshilolo L, Tomlinson G, Williams TN, REACH Investigators, et al. Hydroxyurea for children with sickle cell anemia in Sub-Saharan Africa. The New England J Med. 2019;380(2): 121–131. doi:10.1056/NEJMoa1813598.

27. Lagunju I, Brown BJ, Sodeinde O. Hydroxyurea lowers transcranial Doppler flow velocities in children with sickle cell anaemia in a Nigerian cohort. Pediatr Blood Cancer. 2015;62(9): 1587–1591. doi:10.1002/pbc.25529.

28. Galadanci NA, Umar Abdullahi S, Vance LD, et al. Feasibility trial for primary stroke prevention in children with sickle cell anemia in Nigeria (SPIN trial). Am J Hematol. 2018; 93(3):E83 doi:10.1002/ajh.25012.

29. Kratovil T, Bulas D, Driscoll MC, et al. Hydroxyurea therapy lowers TCD velocities in children with sickle cell disease. Pediatr Blood Cancer. 2006;47(7):894–900. doi:10.1002/pbc. 20819.

30. Zimmerman SA, Schultz WH, Burgett S, et al. Hydroxyurea therapy lowers transcranial Doppler flow velocities in children with sickle cell anemia. Blood. 2007;110(3):1043–1047. doi:10.1182/blood-2006-11-057893.

31. Thornburg CD, Dixon N, Burgett S, et al. A pilot study of hydroxyurea to prevent chronic organ damage in young children with sickle cell anemia. Pediatr Blood Cancer. 2009;52(5): 609–615. doi:10.1002/pbc.21738.

32. Lefevre N, Dufour D, Gulbis B, et al. Use of hydroxyurea in prevention of stroke in children with sickle cell disease. Blood. 2008;111(2):963–964. doi:10.1182/blood-2007-08-102244.

33. Kwiatkowski JL, Yim E, Miller S, STOP 2 Study Investigators, et al. Effect of transfusion therapy on transcranial Doppler ultrasonography velocities in children with sickle cell disease. Pediatr Blood Cancer. 2011;56(5):777–782. doi:10.1002/pbc.22951.

34. Hankins JS, McCarville MB, Rankine-Mullings A, et al. Prevention of conversion to abnormal transcranial doppler with hydroxyurea in sickle cell anemia: a phase III international randomized clinical trial. Am J Hematol. 2015;90(12):1099–1105. doi:10.1002/ajh.24198.

35. Bernaudin F, Verlhac S, Arnaud C, et al. Long-term treatment follow-up of children with sickle cell disease monitored with abnormal transcranial Doppler velocities. Blood. 2016; 127(14):1814–1822. doi:10.1182/blood-2015-10-675231.

36. Adegoke SA, Macedo-Campos RS, Braga JAP, et al. Changes in transcranial doppler flow velocities in children with sickle cell disease: the impact of hydroxyurea therapy. J Stroke Cerebrovasc Dis. 2018;27(2):425–431. doi:10.1016/j.jstrokecerebrovasdis.2017.09.020.

37. Lagunju I, Brown BJ, Oyinlade AO, et al. Annual stroke incidence in Nigerian children with sickle cell disease and elevated TCD velocities treated with hydroxyurea. Pediatr Blood Cancer. 2019;66(3):e27252. doi:10.1002/pbc.27252.

38. Ghafuri DL, Chaturvedi S, Rodeghier M, et al. Secondary benefit of maintaining normal transcranial Doppler velocities when using hydroxyurea for prevention of severe sickle cell anemia. Pediatr Blood Cancer. 2017;64(7):e26401. doi:10.1002/pbc.26401.

39. Lagunju IA, Brown BJ, Sodeinde OO. Stroke recurrence in Nigerian children with sickle cell disease treated with hydroxyurea. The Nigerian Postgraduate Med J. 2013;20(3):181–187.

Table 1. Inclusion and exclusion criteria screening phase of SPRING trial.

Inclusion Criteria for Screening:	Exclusion Criteria for Screening:
• Age 5 through 12 years	• Prior overt stroke (a focal neurological deficit of acute onset) based on obtained history, focal neurological deficit on standardized neurological examination, or concern for moderate or severe neurological deficit based on a positive "10 questions" screening (an established tool in low-resource settings)
• Diagnosis of hemoglobin SS or hemoglobin Sβ0 thalassemia confirmed by hemoglobin electrophoresis, high-performance liquid chromatography (HPLC), or isoelectric focusing (IEF)	• Hemoglobin < 6 g/dL
• S variant with baseline hemoglobin < 10 g/dL or other sickle cell syndromes apart from SC confirmed by hemoglobin electrophoresis, high-performance liquid chromatography (HPLC), or isoelectric focusing (IEF)	• Significant cytopenia (absolute neutrophil count < 1.5 × 109/L, platelets < 150 × 109/L, reticulocytes < 80,000/µl when hemoglobin level is > 9 g/dL)
• Written informed consent and assent	• Treatment with hydroxyurea therapy in the previous three months
	• A history of regular blood transfusion therapy
	• A history of a seizure disorder or diagnosis of epilepsy
	• Other chronic comorbid diseases other than asthma or any other condition illness, which in the opinion of the site' 's Principal Investigator (PI) makes participation ill-advised or unsafe
	• Participants of childbearing age who are pregnant or may become pregnant

Table 2. Inclusion and exclusion criteria for random allocation of participants to receive moderate or low-dose hydroxyurea therapy in SPRING trial.

Inclusion Criteria for Randomization	Exclusion Criteria for Randomization
• TCD velocity of ≥200 cm/second measured at least twice or least one measurement ≥ 220 cm/sec in the middle cerebral artery or	• Any other condition illness or reason, which in the opinion of the site investigastor makes participation ill-advised or unsafe
• Ability to swallow a capsule	• Participants of childbearing age who are pregnant or may become pregnant
• Acceptance of hydroxyurea and willingness to be followed monthly for at least 36 months	• Unable to commit to follow-up visits for the duration of the trial
• Written informed consent and assent	

Table 3. Qualifying Transcranial Doppler (TCD) assessment based on STOP criteria. [1]

Method	Threshold Range	Follow Up Assessment
Non-imaging TCD	Normal Range <170 cm/sec	Repeat TCD 12 months after initial screening
	High Conditional Range 170-199 cm/sec	Usual protocol of evaluation at study site, repeat TCD within three months of initial screening
	Treatment Range ≥ 200 cm/sec	Repeat TCD within 4 weeks of initial screening. However, we will typically, repeat the TCD on the same day with a second TCD certified examiner to increase efficiency of enrollment

Table 4. Definitions for clinical stroke or transient ischemic attack with clinical signs. [7]

Transient ischemic attack (TIA)	Clinical Stroke	Clinical Stroke	Neurological Deficit Due to Other Cause
< 24 hours Neurological Deficit*	> 24 hours Neurological Deficit*	> 24 hours Neurological Deficit*	> 24 hours Neurological Deficit*
Negative Head CT or MRI or no Head CT or MRI obtained	Positive Head CT or MRI if obtained for clinical indications (abnormality on CT which could explain deficit)	No Head CT or MRI or Negative Head CT or MRI or abnormality on CT or MRI does not explain a deficit	Based on investigations, physicians believe deficits are consistent with flaccid paralysis (polio), cerebral malaria, or other non-stroke etiology

* Neurological deficit in this setting means an abnormality consistent with a stroke. Usually, a stroke is associated with weakness of face, arm, or leg.

** The influence of blood transfusion on acute symptoms of stroke has not been well defined in SCD; some neurologic symptoms may last < 24 hours after the treatment with blood transfusion therapy obscuring the diagnosis of stroke. Transfusion therapy may decrease the persistence of neurological findings. Neurological deficit in this setting means an abnormality consistent with a stroke.

Figure 1. Preliminary data from the Primary Prevention of Stroke in Nigeria (SPIN) feasibility trial. The data indicates 23 participants with SCA treated with moderate fixed-dose hydroxyurea at a dose of 20 mg/kg/day and followed with serial TCDs, TCD velocity declined significantly after three months and was maintained at 12 months.

Figure 2. A pooled analysis of the impact of hydroxyurea and decreasing transcranial Doppler (published in Blood 2016, 127: 829-838, online): A pooled analysis of 8 studies documenting TCD measurement before and after hydroxyurea therapy. The pooled analysis is based on the random-effect model demonstrating the average drop in TCD measurement after starting hydroxyurea therapy of 25 cm per second.[27–32] The figure also includes the observation that the decrease in TCD measurements can be seen as early as three months after starting hydroxyurea therapy with a sustained impact of hydroxyurea therapy on decreasing TCD measurements for at least 36 months. The black diamond represents the results of random-effect models. The edges of the diamonds represent the 95% CI of the metaanalyses for the random-effect models.[24]