CLINICAL PROTOCOL

PROTOCOL TITLE: Neuropsychological Dysfunction and Neuroimaging Abnormalities in Neurologically Intact Adult Patients With Sickle Cell Disease (SCD)

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1 SYNOPSIS

Title: Neuropsychological Dysfunction and Neuroimaging Abnormalities in Neurologically Intact Adult Patients With Sickle Cell Disease (SCD)

CSCC Protocol Chair: Elliot Vichinsky, M.D.

Intervention: In Phase I, approximately 156 subjects will participate in a cross-sectional study consisting of screening questionnaires, a neuropsychological testing battery, and MRI testing. In Phase II, a subset of approximately 30 participants will be asked to participate in a transfusion intervention. Half will be randomized to undergo a chronic transfusion regimen for six months, and the other half will be treated with standard care alone, guided by their disease symptoms.

Objectives:

Primary objective(s): To determine the extent of neurocognitive dysfunction in neurologically asymptomatic adult patients with sickle cell disease.

Secondary objective(s): To determine the association between neurocognitive dysfunction and imaging abnormalities.

Hypotheses/Estimates:

Primary hypothesis: Neurocognitive testing in neurologically asymptomatic adult sickle cell patients will be abnormal compared to community controls.

Secondary hypotheses:

1. Neurologically intact adults with SCD will have significantly lower scores on other neuropsychological (NP) tests of executive function than adult controls.
2. A larger percentage of adult patients with abnormal MRIs will have abnormal NP testing than those with normal MRIs.
3. Volumetric MRI will detect brain dysfunction in patients with abnormal neurocognitive tests but normal qualitative MRI.
4. Correction of anemia with transfusion therapy will improve the cognitive function in SCD patients with abnormal neurocognitive testing more than standard care.

Hypothesis testing will apply to the primary hypothesis only; all secondary hypotheses will address issues of estimation.

Criteria for Evaluation:
In the cross-sectional study, the primary endpoint is completion of baseline Weschler Adult Intelligence Scale-Third Edition (WAIS-III) PIQ studies in 120 subjects and 36 controls. The secondary endpoints include completion of the entire baseline NP battery and MRI studies in 120 subjects and 36 controls. In the pilot intervention trial, the primary efficacy endpoint is completion of the transfusion protocol and demonstration of 5-point improvement in WAIS-III PIQ from the standard core group in 30 patients.

Study Design: This study is a cross-sectional investigation designed to compare neuropsychological function of adult neurologically normal HbSS/SB^0 subjects with matched-peer controls. Additionally, a pilot intervention trial will randomize a subset of patients from the primary study to undergo either a chronic transfusion regimen or receive standard care alone, for six months**.**

Study Population: Neurologically intact adult subjects ages 21-55 years, with Hb SS/SB^0 will be enrolled in the cross-sectional study. A subset of these subjects with WAIS-III PIQ scores less than or equal to 90 will be eligible for randomization in the pilot transfusion trial.

Sample Size: 120 subjects and 36 controls are needed to assess the primary endpoint of completion of baseline WAIS III PIQ studies. The secondary endpoints would include completion of the entire baseline NP battery and MRI studies in 120 subjects and 36 controls. Assuming a 20% attrition rate, 150 subjects and 45 controls will be recruited. After consent and randomization, the final secondary endpoint is completion of the transfusion protocol with 30 patients and demonstration of 5-point improvement in WAIS-PIQ due to transfusion above any improvement observed in a standard care group. Again, assuming a 20% attrition rate, 36 patients will be recruited.

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Data Analyses: To test the primary hypothesis, we will use a GLM to model the data. At each site we will match the patients using a ratio of 10 patients to 3 community controls and control for site, age, sex, and education.
Phase I Completion: On February 22, 2008 enrollment into Phase I was completed with 120 cases and 32 controls. Enrollment and randomization into Phase II is dependent on the values obtained during Phase I, including the initial chemistry/hematology panel, MMSE, POMS, PIQ, and normal MRI. Subjects who enroll in Phase II more than 6 months (but less than 1 year) from completion of Phase I (as determined by date of WAIS-III PIQ) repeat selected assessments upon entry into Phase II. Subjects who were never enrolled in Phase I (or who completed Phase I more than 1 year ago) may also be enrolled in Phase II, per a revised set of inclusion/exclusion criteria that combine Phases I and II. The most non-invasive and least time-consuming screening assessments are completed first to ensure eligibility, before the subject moves forward with additional study procedures.

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2 STUDY VISIT SCHEDULE

2.1 Original Visit Schedule

PHASE I	Visit One	Visit Two/Visit Three	Visit Four
Consent Eligibility Screening History and Physical Exam MMSE POMS PSS	X X X X X X		
Neuropsychological Battery*		X	
MRI Testing*		X	
Exit Debriefing			X

- The sequencing of the Neuropsychological Battery and the MRI is flexible, and either component can occur at Visit 2. The component that is not completed at Visit 2 will occur at Visit 3.
- Enrollment into Phase I was completed on February 22, 2008.

PHASE II	Eligibility Visit[1]	Transfusion Visits [2] or Phone Calls/Visits [3]	NP Battery and SF-36 Visit	MRI Testing Visit	Exit Debriefing Visit
Consent Eligibility Screening SF-36 Randomization	X X X X				
Transfusions [2] Phone Calls/Clinic Visits [3]		X X			
SF-36 Neuropsychological Battery [4]			X X		
MRI Testing [4]			X		
Exit Debriefing					X

1 If a subject completed Phase I > 6 months, but < 1 year ago, the subject must repeat the WAIS-III PIQ portion of the Neuropsych Battery to confirm a score of ≤ 90. An additional MRI will also be done to confirm that the image does not have any new neurological events since the Phase I MRI.
2 Number of transfusions during the six-month period will vary between six and eight, and will occur at 3- to 4-week intervals.
3 Phone calls will occur during Months 1, 2, 4, and 5. In-person clinic visits occur during Month 3 and Month 6.
4 The sequencing of the Neuropsychological Battery and the MRI is flexible; the NP Battery can occur before or after the MRI, as long as the two-week visit windows are maintained.

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2.2 Revised Visit Schedule

Subjects who were never enrolled in Phase I (or who completed Phase I more than 1 year ago) may also be enrolled in Phase II per a revised set of inclusion/exclusion criteria that combine Phases I and II. The most non-invasive and least time-consuming screening assessments are completed first to ensure eligibility, before the subject moves forward with additional study procedures.

	Eligibility Visit	**Visit 2 MRI**	**Visit 3 NP Battery/ Randomiza -tion**	**Transfusion Visits [1] or Phone Calls/Visits [2]**	**Exit NP Battery Visit**	**Exit MRI Testing Visit**	**Exit Debriefing Visit**
Consent Eligibility Screening PIQ MMSE POMS	**X**						
MRI Testing		**X**				**X**	
SF-36 Neuropsycho-logical Battery (Randomization)			**X**		**X**		
Transfusions **or** Phone Calls/Clinic Visits				**X**			
Exit Debriefing							**X**

1 Number of transfusions during the six-month period will vary between six and eight, and will occur at 3- to 4-week intervals.
2 Phone calls will occur during Months 1, 2, 4, and 5. In-person clinic visits occur during Month 3 and Month 6.

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4 ABBREVIATIONS

ACS	Acute Chest Syndrome
AE	Adverse Event
BTPT	Benton Tactile Performance Test
CBCL	Child Behavior Checklist
CNS	Central Nervous System
CRF	Case Report Form
CSCC	Comprehensive Sickle Cell Centers
CSF	Cerebral Spinal Fluid
CSSCD	Cooperative Study of Sickle Cell Disease
CT	Computer Topography
CVA	Cerebrovascular Accident
CVLT-II	California Verbal Learning Test - Second Edition
D-KEFS	Delis-Kaplan Executive Function Test
DSE	Double Spin Echo
DSMB	Data Safety Monitoring Board
DSST	Digit-Symbol Substitution Test
EDAS	Encephaloduroarteriosynangiosis
EEG	Electroencephalogram
FDA	Food and Drug Administration
FET	Fischer's Exact Test
FLAIR	Fluid-Attenuated Inversion Recovery
FOV	Field of View
GCP	Good Clinical Practice
GLM	Generalized Linear Model
Hct	Hematocrit
ICH	International Conference On Harmonisation
ICV	Intracranial Volume
IQ	Intelligence Quotient

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IRB	Institutional Review Board
MMSE	Mini-Mental Status Examination
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
NHLBI	National Heart, Lung, and Blood Institute
NP	Neuropsychological
PET	Positron Emission Tomography
PI	Principal Investigator
POMS	Profile of Mood States
PRC	Protocol Review Committee
PSS	Perceived Stress Scale
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SB-Thalassemia	Sickle Beta-Thalassemia
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SD	Standard Deviation
SDMC	Statistics and Data Management Center, located at RhoFed, NC
TEA	Test of Everyday Attention
TIA	Transient Ischemic Attack
TMD	Total Mood Disturbance
TSE	Turbo Spin Echo
VMRI	Volumetric Magnetic Resonance Imaging
WAIS	Weschler Adult Intelligence Scale
WAIS-R	Weschler Adult Intelligence Scale - Revised
WAIS-III PIQ	Wechsler Adult Intelligence Scale - Third Edition Performance IQ
WISC-III	Wechsler Intelligence Scale for Children - Third Edition
WISC-R	Wechsler Intelligence Scale for Children Revised

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WCST	Wisconsin Card Sorting Test
WJ-R	Woodcock Johnson Revised
WML	White Matter Lesions
WMS-III	Wechsler Memory Scales - Third Edition
WMSH	White Matter Signal Hyperintensity
WRAT-3	Wide Range Achievement Test - Third Edition

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5 BACKGROUND AND RATIONALE

5.1 INTRODUCTION

The average life span for patients with sickle cell anemia (SCA) is now over 50 years, and 60 years for sickle variants [1-4]. Early intervention in life-threatening pediatric problems has successfully modified sickle cell disease (SCD). Once a fatal pediatric disease, it has become a chronic adult illness causing deterioration in quality of life due to progressive vital organ failure [3, 5-7]. Pediatric neurological studies and pilot data in adult sickle cell patients suggest brain dysfunction may be the most important and least-studied problem afflicting this aging population [3, 8-36].

During its first phase, the Cooperative Study of Sickle Cell Disease (CSSCD) included adult patients whose participation was eliminated before modern neurocognitive studies or imaging could be applied. In this early natural history study, over 24% of adults by the age of 45 years experienced a life-threatening central nervous system (CNS) event (hemiplegia, for example) [8, 10, 14, 16, 17, 23, 24, 28, 29, 32, 35, 37-49]. These observations led to prospective studies with neurocognitive and neuroradiographic components in children. The results demonstrated that children with SCD commonly have neurocognitive dysfunction and clinically silent CNS infarction on neuroimaging. Furthermore, the lesions are larger in older children [8, 19, 23, 25, 35, 50, 51]. It is now established that these children suffer from frontal lobe dysfunction syndrome, which can globally affect executive functioning in areas such as attention, concentration, information processing, and decision-making [33, 36, 38, 39, 45, 52-57]. Invasive treatment (including chronic transfusion and bone marrow transplant) has been initiated for some of these children [5, 58]. One would hypothesize that the problem increases with age and that adults are burdened with serious neurological injury. However, because neurocognitive impairment is not often associated with motor deficits, it is often not diagnosed or treated. In pilot neurocognitive and imaging studies of adult sickle cell patients, at least 50% had neurocognitive and neuroimaging damage. This is supported by small autopsy studies that found unsuspected widespread infarction [21]. In contrast, large neuroimaging/neurocognitive studies of normal age-matched individuals under 50 years of age found ischemic changes to be very rare [59-61].

Early explanations for developmental limitations identified in children with SCD such as social class, school absenteeism, psychosocial stress, and other environmental factors have recently been questioned. Several authors found that depression, self-perception, family adjustment, and maternal mental health were not significantly different than controls or predictive of neurocognitive dysfunction [36, 62, 63]. Other risk factors may be more predictive. In the CSSCD study of overt stroke (infarctive and hemorrhagic), multivariate analysis found predictive factors, which include prior Transient Ischemic Attack (TIA), recent acute chest syndrome, frequency of ACS, and systolic blood pressure [17, 19, 24, 64-73]. Other studies found acute anemic events or hypoxic events predictive of CNS events [74]. In the risk factor analysis of silent infarction in childhood

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SCD, low hemoglobin was among the predictive factors [19]. Anemia has also been shown to affect learning and memory tasks [75-79]. Steen, et al., compared children with SCD to controls utilizing quantitative MRI and psychometric testing and found that full-scale IQ of the sickle cell patients was a function of Hct as well as subtle T1 abnormalities on imaging [8, 70, 80].

Pilot data from our center on patients receiving serial neuropsychological (NP) testing from childhood through adulthood demonstrate significant neurocognitive injury in clinically asymptomatic patients (see Preliminary Results). Several clinically asymptomatic patients with normal conventional neuroimaging studies demonstrated neurocognitive impairment. We suspect that when utilizing advanced neuroimaging techniques such as volumetric MRI, areas of injury may be identified in patients in whom conventional MRI studies were negative. An objective of this study is to define the extent of neurocognitive and anatomical injury in the adult population, which can then serve as the foundation for future intervention with neuroprotective agents and other treatments.

5.2 BACKGROUND AND RATIONALE

5.2.1 PRIMARY HYPOTHESIS 1

Neurocognitive testing in neurologically asymptomatic adult sickle cell patients will be abnormal when compared to community controls.

In the last decade there have been major advances in documenting the serious problem of unrecognized brain damage in children with SCD. Newer and more sensitive imaging studies and improved methodology in neurocognitive testing have made detecting, treating, and preventing brain injury probably the most important clinical research goal of the 1990's in SCD [5, 8, 14, 19, 23, 25, 35, 47, 48, 56, 68, 80-83]. These studies have demonstrated a 32% prevalence of stroke in older adults [21, 24]. Neuroimaging in these patients and clinically neurologically asymptomatic patients have demonstrated a very high predilection for disturbances in the frontal lobes and other areas associated with executive functioning [8, 14, 21, 24, 32, 45, 59, 64, 84]. Steady-state metabolic flow studies have demonstrated a hyperemic state with increased perfusion and maximal adaptive vasodilation [85, 86]. A growing body of literature suggests this chronic dilated state lacks minimal if any reserve capacity for increased oxygen delivery or oxygen extraction, because all primary vessels and collaterals are already recruited [70, 75, 85-87]. As a result, a drop in perfusion pressure, metabolic changes, and other common events produce distal field metabolic dysfunction, ischemia, and eventual infarction of the cerebrovascular bed.

These physiological and anatomical observations support evidence of diminished selective and general cognitive functions in patients with SCD. Earlier studies with some methodological limitations found that deterioration in intellectual function was common [16, 34]. Recent data utilizing more sensitive neurocognitive tools have confirmed a high

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rate of executive function impairment, which appears to correlate largely with the propensity for frontal lobe injuries in these patients [8, 14, 40, 57, 64, 84, 88, 89]. Executive functioning refers to cognitive abilities in attention, volition, planning, purposeful action, and effective execution of plans. These are reflected in standardized tests that predict employability and higher-level skills of daily life such as financial management, cooking, safety, medication administration, utilization of community resources, and social function. Children and adolescents who have SCD without a history of Cerebrovascular Accident (CVA) or neurologic dysfunction demonstrate many of these cognitive impairments [14, 31, 33, 34, 36-39, 52-54, 90-92]. Specifically, children with SCD show problems with visual/motor speed and coordination, math, language, coding, block design, object assembly, design recall, and paired associative learning. Their scores deviate from their peers by age three and they perform more poorly in school readiness skills by 4 to 5 years of age [8, 37, 91]. Follow-up pediatric studies, while limited, suggest that as the children age, the neurocognitive dysfunction becomes more apparent [93, 94].

In contrast to the ongoing investigation into neurocognitive injury in children with SCD, the adult sickle cell population has not been systematically studied. Limited information suggests that neurocognitive dysfunction may be an important factor in the relatively poor social, economic, and quality-of-life factors reported in adult patients when compared to matched controls [20, 21, 32, 39, 62, 80, 81, 84, 89, 95, 96]. Identification of injury will enable concrete steps to improve daily functioning and long-term preventive treatment [5, 68, 81, 97-101].

Important information on the probable incidence of MRI abnormalities in adult patients can be obtained from the CSSCD study of over 4,000 sickle cell patients with CVA [24]. This study documented clinical CVAs (defined as an acute neurologic event presumed to be secondary to occlusion of an artery or hemorrhage). While imaging studies were not required for all participants, this natural history study was of such size and duration that it defined the prevalence and incidence of hemiplegic strokes by age in SCD. The prevalence generally increased with age in HbSS patients (patients ages 2-5=2.29, 6-9=4.9, 10-19=5.5, 20-29=5.3, 30-39=7.22, 40-49=8.58). There appeared to be a high rate in children, the rate remaining stable until age 30. Similarly, the incidence of first CVA in participants in the study had two age peaks: one in young children and the other in older adults. While most people think of adult CNS disease as being only secondary to hemorrhage, infarction occurred in each age group, and the incidence in older adults was higher than in teenagers and young adults with SCD. Since there is a strong association between clinically silent MRI abnormalities and the development of future clinical symptoms, these data suggest smaller silent MRI lesions are ongoing and may occur with increased frequency in older patients [93]. The CSSCD study of a newborn cohort (n=312) followed children from ages 6 through 14 years. Seventeen percent of SCD patients had asymptomatic lesions, usually in the frontal lobe [19, 23, 93]. This imaging study only followed patients into early adolescence and therefore was not designed to detect the increased lesions expected in adults. However, even in this limited age range, it noted that older children had more lesions than younger patients [93].

There are several risk factors associated with ischemic brain injury in sickle cell that would increase the likelihood of new MRI lesions occurring with age. There is a greater chance of sudden events associated with MRI injury: number of episodes of acute chest syndrome (ACS), acute hypoxia, acute anemia, hypertension, and seizures [1, 19, 21, 24, 69, 102, 103]. For example, clinical neurologic events occurred in 20% of severe ACS cases, which were almost limited to older patients in a recent national trial of 600 cases [74]. It is likely that the number of patients with MRI lesions is much higher than reported for clinical neurologic events. Other risk factors such as hypertension and renal dysfunction increase as the patient ages [3, 69]. Low steady state hemoglobin is a strong risk factor for brain dysfunction in all studies [3, 17, 19, 24, 69, 85]. The resultant steady state of increased perfusion and maximal oxygen extraction appears to result in metabolic frontal lobe dysfunction in up to 50% of asymptomatic adults studied [86]. We hypothesize that these abnormalities lead to an increase in undetected border-zone/watershed lesions with age. The existing MRI data on asymptomatic adults with SCD are limited. Manfre, et al., recently studied 25 sickle/thalassemia disease patients with no history of neurologic symptoms or signs. They found that 52% of patients had abnormal MRIs consisting of atrophy and/or infarction; in 20%, the infarcts were multiple [21]. The incidence of brain injury increased with age and peaked after 35-40. These observations are supported by limited autopsy studies where clinically asymptomatic ischemic brain injury was present in the majority of cases [20].

Age has been shown to be a significant predictor of brain injury in non-SCD populations [1, 60, 104, 105]. Ischemic white matter changes in the aging adult brain of healthy volunteers have been studied. Generally, significant white matter hypointensities on T2 weighted scans in healthy volunteers younger than 50 are rarely reported [60]. The National Institutes of Health, in a report of incidental finding on brain MRI in 1,000 asymptomatic volunteers (median age 30, range 3-83), found 5 nonspecific T2 hyperintensity lesions overall and 12 subjects in the older age groups with age-related changes [60]. In a recent report, which estimated the incidence of silent stroke in the USA from several cardiovascular and stroke prevention studies, there are 6400 silent ischemic events (including high risk groups) per 100,000 people between the ages of 50 and 60 [59]. Other risk factors for non-sickle cell patients are: untreated hypertension, diabetes, hyperlipidemia, and coronary artery disease. While some of these risk factors are rare in SCD, the available data from the pediatric sickle cell studies and limited information in adult SCD patients suggest that the incidence of ischemic events in SCD may be similar to the highest-risk elderly population [21].

Neurocognitive data in adults with SCD are largely not available. In one report of 18 adults, neurocognitive dysfunction between MRI-positive and MRI-negative patients was compared [21]. They found neurocognitive injury in most of the patients regardless of MRI findings; some patients in both groups met cognitive criteria for dementia. This neurocognitive damage appears to reflect ischemia. Limited data from functional/flow studies have found that cerebral hypometabolism and disturbed blood flow are common in asymptomatic adult SCD patients [44]. Similar findings have been reported in non-SCD populations, but are rare in adults under age 50 [60, 81, 84, 86, 88, 89, 105].

Recognition and early identification of neurocognitive dysfunction in adult patients with SCD will allow helpful intervention, such as disability training, and may lead to research trials of the effects of transfusion, neuroprotective agents, anticoagulation, or even encephaloduroarteriosynangiosis (EDAS) [81, 100, 106, 107].

In preliminary data of 72 pediatric patients (see Preliminary Studies and Progress Report), all three groups of patients (those without evidence of stroke, those with silent infarct on MRI, and those with stroke) had mean scores on the WISC III Performance IQ scale at least one standard deviation below normative values. While the mean scores of adults with SCD on the WAIS-III Performance IQ will be compared to normative data, we feel that a control group of adults without SCD is essential for interpretation of the data collected in patients. The WAIS and other neurocognitive tests were developed and shown to be reliable and valid in a wide range of adult populations, but there are concerns about the interpretation of scores in a population of predominantly African-Americans. Additionally, socio-economic factors may affect performance on various tests. Therefore, we plan to study community controls matched for age and educational level.

5.2.2 SECONDARY HYPOTHESIS 1

Neurologically intact adults with SCD will have significantly lower scores on other neuropsychological tests of executive function when compared to adult controls.

There has been a paucity of CNS research with adult patients but abnormal studies in sickle cell children raise concern for widespread, presently undetected neurocognitive damage in adults. Several recent studies examined neurocognitive functioning and MRI in children with SCD and found these patients to be at marked increased risk for cognitive impairment [8, 14, 28, 30, 33, 45, 48, 52, 53, 108]. Specifically, investigators have identified a high frequency of frontal lobe cognitive deficits. There is strong evidence that silent infarction is associated with neurocognitive deficits, particularly frontal lobe in children. Recently Schatz found 80% of those with silent infarcts had significant cognitive deficits [52]. Since almost a third of HbSS children have MRI lesions, cognitive deficits are very common [8, 19, 94]. In these studies of children there is a suggestion that as the patients age they may be at more risk. For instance, in these pediatric studies the size of the lesions is larger and the degree of atrophy is greater in older children [8, 19, 50, 51]. Recently, the CSSCD study released a pilot report of neurocognitive functioning in 373 asymptomatic children that showed a progressive and significant decline in neurocognitive scores, excluding those with silent or overt stroke [8, 14, 19, 39, 51, 94]. One European study administered neurocognitive and imaging studies to older (mean 34 yrs) SB-thalassemia patients and found that at least 50% had neurocognitive and neuroimaging damage [21]. Our own pilot data in young adults (21 yrs) described below suggest that executive dysfunction becomes apparent on a variety of neurocognitive measures in asymptomatic, image-negative patients as they enter adulthood.

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5.2.3 SECONDARY HYPOTHESIS 2

A larger percentage of adult patients with abnormal MRI will have abnormal NP testing than those with normal MRIs.

A large body of evidence supports neurocognitive impairment in children with SCD [8, 16, 19, 31, 33, 34, 37-39, 50, 52-54, 57, 90-92, 94, 109]. Abnormal brain magnetic imaging is the most powerful predictor of impairment for these children when the data is analyzed for risk factors [8, 19, 23, 59, 90, 93]. The CSSCD multi-center study evaluated 373 sickle cell children from a natural history cohort with MRI and NP evaluation [8, 19, 59, 93, 94]. Utilizing a battery of standardized tests with normative data from children, they evaluated WISC-R, Woodcock-Johnson-R, Purdue, and Child Behavior Checklist (CBCL) among others. Data from subsets of WISC-R demonstrated poor results in patients with silent lesions when compared to those with normal MRI. Verbal, nonverbal, math and vocabulary scores, visual speed, and coordination were all affected. Similar results have been seen in several pediatric SCD studies including those utilizing normal siblings as controls. In addition, there is a relationship between the site of the lesion and the deficits found, with frontal lobe lesions predominating [8, 19, 47, 51, 94]. NP and MRI studies of patients with SCD with overt stroke compared to the general population found more severe dysfunction associated with increased lesion size [8, 47, 59, 81, 94, 110]. There appears to be strong evidence that adult sickle cell patients will show similar associations between neurocognitive dysfunction and the extent of brain injury found on MRI.

5.2.4 SECONDARY HYPOTHESIS 3

Volumetric MRI will permit detection of brain dysfunction in patients with abnormal neurocognitive tests but normal conventional MRI.

Early diagnosis of neuronal loss before the onset of disabling neurologic dysfunction is a major goal in SCD research. The detection of brain injury by neuroimaging techniques has improved significantly over the past 15 years. Computed topography (CT) is generally not helpful because acute and sub-acute ischemia due to progressive arterial occlusion are not visible on CT as hypodense lesions [23, 81, 111]. The development of MRI increases the resolution of ischemic injury of white matter lesions that appear hyperintense on T2 weighted views and hypointense lesions in T1 weighted studies [51, 112]. In one study of 22 HbSS neurologically “normal” children had infarcts detected by these studies [8, 19, 23]. The lesions were usually located high on the cerebral convexity and white matter borderzone area. Unfortunately, these lesions identified by MRI are already associated with irreversible and often progressive neurocognitive dysfunction [8, 93]. Eventually, 38% of these asymptomatic MRI-positive lesions in children become overtly symptomatic with focal exam findings [51, 93]. The combination of transcranial Doppler and MRI are synergistic and increase the sensitivity for early detection of injury in high-risk patients, but many of the patients identified have already suffered

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neurocognitive damage and neuronal tissue loss [48, 82]. In addition, transcranial Doppler screening in adults is not possible due to thickness of the skull.

New neuroimaging techniques provide a great source of quantitative information about the state of brain tissues. These more sensitive approaches may enable investigators to diagnose brain dysfunction before it becomes severe. This would enable early intervention and neuroprotective treatments to be evaluated. Volumetric MRI (VMRI) studies of the brain are an important tool for correlating ischemic injuries with neurocognitive deficits, and appear to have prognostic and diagnostic importance [47, 66, 113-116]. In general, studies of ventricular enlargement, white matter lesions, and lacunae numbers have been descriptive. Recently, new generation VMRI software has been used [114]. These findings have improved MRI resolution and increased the predictive nature of the studies. Hippocampal volume reduction, atrophy and ventricular dilation may be predictive of cognitive impairment independent of the lacunae number and size change [114]. While such detailed volumetric studies have not been done in adult SCD patients, their validity in vascular dementia has been accepted.

In 30 children with cognitive impairment, VMRI was abnormal despite normal conventional MRIs [47, 114, 117]. Another pediatric sickle cell study with 50 patients and age-matched controls used quantitative MRI T1. This study found asymptomatic sickle cell patients had abnormalities in the caudate, nucleus pulvinares, and cerebral cortex by age four. Conventional MRI was insensitive in detecting these abnormalities in the study population [46]. Mild mental deficiency was common in the pediatric population compared to a published prevalence of 1.5%. Supporting the possible effect of anemia on neurocognitive function were the observations of cognitive deficits and T1 abnormalities associated with low Hct. This finding supports our proposed pilot transfusion study on neurocognitive functioning.

5.2.5 SECONDARY HYPOTHESIS 4

Correction of anemia with transfusion therapy will improve the cognitive function in SCD patients with abnormal neurocognitive testing more than standard care.

We propose that a group of patients with NP abnormalities who undergo a transfusion regimen of six months duration will show more improvement in neurocognitive function than a group of patients with NP abnormalities who receive standard care. After baseline NP and MRI testing, we will randomize 36 patients to a six-month transfusion protocol or standard care alone, and then retest them on the NP battery. We expect that patients with baseline WAIS III PIQ scores ≤ 90, will show improvement when retested after six months of transfusion that is on average five points more than any average within subject improvement observed in a group of patients that receives standard care alone. We expect patients in the standard care group to have on average no improvement in WAIS-III PIQ scores after six months of standard care.

A growing body of literature suggests transfusions can improve cognitive function by increasing the delivery of oxygen to critical areas. Weiskopf evaluated the effects of transfusion on neurocognitive function in normal individuals. In his studies, adult volunteers were tested with verbal memory and standard, computerized neuropsychologic tests before and after reduction of their Hb from baseline of 14 to as low as 5 g/dl. These patients were retested after serial autologous transfusions. He found that reaction time for horizontal addition and digit-symbol substitution test (DSST) became progressively abnormal with worsening anemia, and returned to baseline the *morning* following transfusion. To understand whether the improvement in testing was caused by increased oxygenation of the brain, Weiskopf recently studied the relationship between arterial oxygen pressure, anemia, and neurocognitive functioning. His hypothesis was that increasing arterial oxygen pressure to 350 mmHg would supply the equivalent amount of oxygen as supplied via augmenting Hb concentration by 2-3 g/dl and thus reverse the effects of anemia. He tested 31 healthy volunteers with verbal memory and neuropsychological tests before and twice after reduction of their Hb to 5.7. Weiskopf found that inducing anemia resulted in abnormalities in the DSST and delayed memory degraded. Increasing the Pao2 to 406 mmHg reversed the DSST and delayed memory changes to values similar to baseline Hb concentration [118-120].

In renal patients, correction of the anemia by erythropoietin improves executive tasks. Small to moderate changes in Hb appear to improve neurocognitive functioning. Metry analyzed effects of normalization of Hct on cerebral blood flow, oxygen metabolism, and overall oxygen supply to brain tissue in healthy normals and renal patients. Results indicated a small change in Hct improved cerebral metabolism and oxygenation. By increasing Hb levels in renal failure patients from 31% to 41%, EEG frequency analysis shows significant decrease in EEG slowing [75, 121].

The auditory oddball and Continuous Performance Task and changes in P300 latency significantly correlated with increased Hct. Correction of anemia to normal Hct levels may result in improved ability to sustain attention in easier tasks and enhanced ability to recognize, discriminate, and hold stimuli in memory for more difficult tasks [121]. In controlled studies, IQ measured by the WAIS-R, improved by a mean of 7.2 points when compared to an untreated control group, showing a 0.3-point improvement [122]. Concentration and speed of information processing were assessed by the Paced Auditory Serial Addition Task and also improved in the treatment group ($P < 0.05$). Memory assessed by the Rey Auditory Verbal Learning Test tended to improve in the treatment group with amelioration of anemia.

The study of the effects of blood transfusion of brain dysfunction in non-sickle cell hemoglobinopathy patients has been limited. Overall, like SCD, there is concern for neurocognitive function in thalassemia. Manfre found 38% of thalassemia intermedia patients had asymptomatic brain injury that increased with age and correlated with the non-transfused Hb level [21]. Micheloyannis studied the effect of blood transfusion on alpha EEG activity in thalassemic patients. EEG spectral analysis was performed in 12

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young patients and 10 volunteer students with thalassemia before and after their regular blood transfusion. The aim was to test if the EEG analysis could detect signs of brain dysfunction due to the cerebral hypoxia as a result of anemia. EEG signals were computer analyzed and the results showed that after transfusion, the power spectral density of the alpha band showed a significant enhancement in most areas of the brain in thalassemia patient group as compared to the normals. The differences correlated with the hemoglobin levels of the patients and possibly reflected the degree of oxygenation to the brain [76].

In SCD, it is known that neurocognitive testing and imaging abnormalities correlate with Hct. Steen prospectively compared 50 patients with 50 controls with the Wechsler and MRI. Psychometric testing showed that 33% of patients were functioning in the rate of mild mental deficiency (IQ 50-70), compared with a published prevalence of 1.45% in inner-city black children. Thus, sickle cell patients in this study were associated with a 23-fold increase in the risk of mild mental deficiency. In 2003, Steen studied 49 asymptomatic sickle cell patients with neurocognitive testing and found significant abnormalities in patients with normal imaging studies [46, 123]. Multivariate analysis showed Hct was a predictor of full-scale intelligence. The European multi-center studies on neuropsychometric studies on SCD have found similar results. MRI was not the only factor of cognitive deficit: Verbal IQ, Performance IQ, and Full Scale IQ were strongly impaired with patients with severe chronic anemia [64]. The benefit of transfusion in improving cognitive functioning in sickle cell is very limited. In 1999, Powers demonstrated improved brain metabolism and perfusion with transfusion therapy. Nahavandi recently studied near infrared cerebral oxygen saturation in 27 patients with sickle cell disease and in 14 controls. Near-infrared cerebral oxygen saturation in SCD significantly decreased and correlated with the patients' baseline Hb. Simple transfusion therapy in seven patients improved oxygenation [124].

Therefore, the hypothesis that correction of the anemia will improve neurocognitive function more than standard care in this population is likely if the neuroischemia is reversible. New functional brain studies indicate that the brains of sickle cell patients suffer from reversible ischemic dysfunction. These studies have measured cerebral blood volume, brain blood flow, tissue perfusion, tissue oxygenation, and metabolic and biochemical changes [79, 88, 101, 125-141]. Recent studies using Positron Emission Topography (PET), including some from our own program, show frontal lobe hypometabolism in normal MRI areas of sickle cell patients [80, 89, 110].

The use of diffusion/perfusion MRI adds to the understanding of brain function and ischemic injury [139]. These perfusion/diffusion techniques measure many functions very rapidly. They have been used to produce images that reflect physiologic effects of acute ischemic disease. Recently, the observation of hemodynamic effects in symptomatic and asymptomatic patients with chronic cerebrovascular disease has been accomplished [7, 44, 139]. A significant portion of dysfunctional brain regions in patients with ischemic injury can be corrected by improved oxygenation.

5.3 PRELIMINARY STUDIES

The neurocognitive assessment and evaluation of patients with SCD at the Northern California Comprehensive Sickle Cell Center has involved a strong collaboration with neuroimaging and neurocognitive scientists locally and in multi-center trials. Listed below are three neuroimaging and neurocognitive function pilot studies:

A. Neurocognitive Testing and MRI Results in 72 Pediatric Patients with SCD

B. Preliminary Neurocognitive Testing: Asymptomatic MRI Adults

C. Neuroimaging Results in Adults with SCD

5.3.1 NEUROCOGNITIVE TESTING AND MRI RESULTS IN 72 PEDIATRIC PATIENTS WITH SCD

Analysis of the data on 72 children and adolescents are summarized in the Tables 5.1 through 5.4 below. It includes three groups of asymptomatic children with normal MRI/MRA/Doppler, children with silent watershed lesions unassociated with clinical or neurologic findings, and those with neurologic events (stroke, TIA, rind) with an associated positive MRI. Information has been collected in a standard and systematic manner, with a well-integrated neurocognitive battery that included the Wechsler Intelligence Scale for Children Revised (WISC-R) or III (WISC-III), the Woodcock Johnson Revised (WJ-R), the Purdue, and the Benton. Our attention has focused on cognitive aptitude, achievement, fine motor skills, and tactile perception. Additionally, we have conducted MRI/MRA scans that will enable us to examine the associations between stroke status (no stroke, silent, CVA), location (frontal, temporal, parietal, occipital), and their neurocognitive functioning. Our pilot neurocognitive and imaging data is reported below. It is a cross-sectional examination of the 72 children.

TABLE 5.1 Location of Strokes and Silent Infarcts

	Stroke (N=23)		Silent (N=13)*	
Area of Damage	N	%	N	%
Frontal Lobe	12	52.2	3	23.0
Temporal Lobe	4	17.4	0	0
Parietal Lobe	13	56.5	3	23.0
Occipital Lobe	2	8.7	3	23.0
Caudate nucleus	2	8.7	0	0
Internal capsule	0	0	0	0
Centrum semivole	7	30.4	3	23.0
Basal ganglia	1	4.3	0	0
Parafalicine	1	4.3	0	0
Cortical	4	17.4	0	0

*There were 13 events in 12 patients.

TABLE 5.2 Patients Without Evidence of a Stroke (N=36)

Cognitive	Min.	Max.	Mean	Std. Deviation

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WISC-R Verbal IQ	67	123	90.28	12.16
WISC-R Performance IQ	71	118	93.64	13.29
WISC-III Verbal IQ	67	121	87.22	20.47
WISC-III Performance IQ	54	117	86.56	20.20
Woodcock Johnson —R Achievement				
Letter-Word	12	142	91.06	23.79
Reading Comprehension	65	134	92.68	17.26
Arithmetic Problems	72	127	95.56	12.74
Calculations	72	132	96.90	17.46
Fine Motor				
Purdue (R)	5	15	10.56	2.67
Purdue (L)	5	15	10.50	2.68
Tactile				
Benton (R)	2	10	7.19	2.82
Benton (L)	0	10	6.73	3.23

TABLE 5.3 Patients With Evidence of a Silent Infarct (N=13)

Cognitive	Min.	Max.	Mean	Std. Deviation
WISC-R Verbal IQ	79	113	96.00	17.00
WISC-R Performance IQ	79	113	96.00	17.00
WISC-III Verbal IQ	70	109	91.67	19.86
WISC-III Performance IQ	58	100	78.60	18.89
Woodcock Johnson —R Achievement				
Letter-Word	64	94	74.83	10.65
Reading Comprehension	60	115	85.13	19.25
Arithmetic Problems	54	123	85.14	23.30
Calculations	66	107	84.40	19.09
Fine Motor	49	119	82.29	26.85
Purdue (R)	7	14	10.60	2.88
Purdue (L)	7	14	10.60	2.88
Tactile	8	12	9.25	1.89
Benton (R)	3	10	6.71	2.36
Benton (L)	2	10	6.43	2.99

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TABLE 5.4 Patients With Evidence of a Stroke (N=23)

Cognitive	Min.	Max.	Mean	Std. Deviation
WISC-R Verbal IQ	54	98	70.38	14.77
WISC-R Performance IQ	48	98	68.50	16.22
WISC-III Verbal IQ	60	92	79.29	10.83
WISC-III Performance IQ	52	87	71.14	11.78
Woodcock Johnson —R Achievement				
Letter-Word	61	109	85.20	13.50
Reading Comprehension	67	117	86.92	14.72
Arithmetic Problems	29	104	82.50	19.14
Calculations	55	113	80.83	16.98
Fine Motor				
Purdue (R)	4	15	10.18	3.09
Purdue (L)	6	12	8.92	2.19
Tactile				
Benton (R)	3	10	7.42	2.47
Benton (L)	0	10	7.27	3.07

The data on WISC-III Performance IQ and fine motor and tactile perception indicate all patients were below the mean when compared to their gender- and age-matched control groups. Even children with normal MRI were well below the mean for gender- and age-matched controls. Patients with evidence of a stroke on verbal and performance scores were consistently two standard deviations below the mean. Patients with evidence of a silent infarct were functioning approximately one standard deviation below the mean. Patients with SCD and no evidence of stroke were functioning within the lower average range of cognitive abilities and abnormal WISC-III Performance IQ. In summary, patients with evidence of stroke or silent infarct have lower cognitive scores than the no stroke group, reflecting difficulties in verbal and non-verbal thinking and reasoning skills. However, the group with a silent infarct performs higher on the WISC-R versus its WISC-III performance. This discrepancy may be due to the more stringent nature of the WISC-III.

Achievement scores in patients with evidence of stroke and silent infarct were one full standard deviation below the mean, while patients without evidence of a stroke performed within the normal range but trended downward. Patients with evidence of a stroke or a silent infarct generally had lower scores of achievement than patients without a stroke - particularly in areas related to math conceptualization, written numerical operations, reading comprehension, and borderline functioning on word problems.

As expected, patients with evidence of a CVA are at high risk for developing cognitive impairments. However, it is important to note that patients with silent infarcts and no stroke status are functioning below the mean in some areas of achievement, motor speed, and manual dexterity. Investigators have begun to address this area of neurocognitive research noting that patients with SCD and no evidence of a stroke are vulnerable to

increased difficulties in several domains of neuropsychological functioning (e.g., attention and concentration, arithmetic, memory, and reading) which may increase with age.

5.3.2 PRELIMINARY NEUROCOGNITIVE TESTING OF ADULTS WITH NORMAL MRIS

Recently, we evaluated eight young-adult patients with SCD (SS=7, SC=1) with a mean age of 21.5 years who had negative MRI findings and no neurologic evidence of dysfunction on exam. They were administered the following neuropsychological battery; the WAIS-III, the WJ-R, the Purdue Pegboard Test, the Benton Tactile Performance Test (BTPT), the Wisconsin Card Sort Test (WCST) and the Test of Everyday Attention (TEA). Findings are limited given the small sample size. However, there are some findings of interest that support our current area of proposed investigation and may be better illustrated with a larger sample.

On measures of intellectual functioning the patients were in the low range. Patients demonstrated difficulty on tests of executive functioning and attentional processes (sustained, selective, and switching) as measured by the TEA. These tasks are important in the efficient encoding, retrieval, and learning of new information. Tasks measuring the ability to fluidly shift attention were one standard deviation below the mean for the group. Specifically, some patients found it difficult to track their performance and made more errors as the task became more complex. A greater percentage of the errors occur towards the end of the task, suggesting a vulnerability to cognitive fatigue. Overall, patients also took longer to complete this task. Similar findings are observed on the WCST. Some patients demonstrate early fatigue, difficulties with mental flexibility, and impairment in mental flexibility. It is noteworthy that subjects who applied one particular strategy were prone to reapply that strategy in situations where it was no longer effective. In this sample 4/8 (50%) exhibited problems with attention, mental flexibility, sustained attention, and decision-making.

5.3.3 NEUROIMAGING RESULTS IN ADULTS WITH SCD

Over the last 24 months we evaluated adult patients with SCD with new onset non-focal neurologic/psychological symptoms including memory loss, poor living skills, personality changes, and non-specific neurologic complaints of headaches, tingling, and dizziness. Out of 157 patients, 33 met the criteria. These included 5 patients with previous neurologic disease (recently asymptomatic) and 28 patients with no history of CNS dysfunction: 30 patients had hemoglobin SS, 2 HbS thalassemia and 1 hemoglobin sickle cell variant. There were 14 males and 19 females; the age range was 20 to 52. MRIs utilized T1, T2, and coronal FLAIR plane, studies occurred at one center, and 2 neuroradiologists reviewed films. Over 50% of neurologically asymptomatic patients had a new ischemic abnormality on MRI. This included 3 of the 5 patients with prior disease, and 14 of 28 patients previously normal. In the positive MRI group, the mean

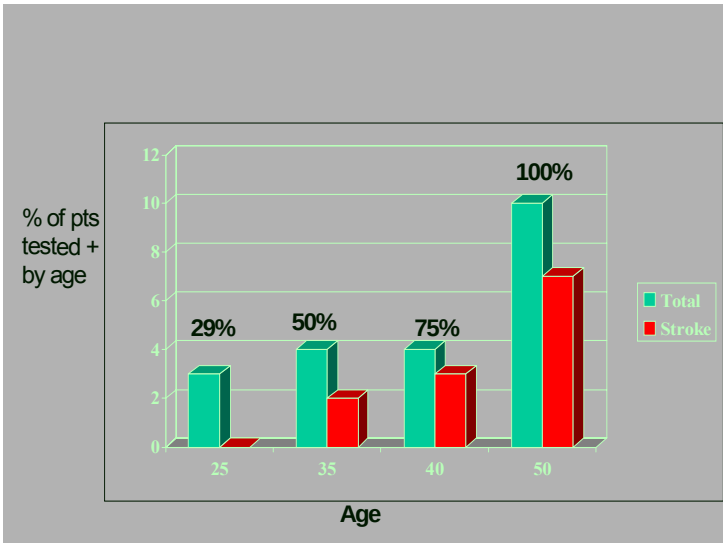
age was 37 in contrast to 29 years in the negative MRI group. These lesions were multifocal, bilateral, and involved watershed areas, with deep white matter in 12 of 17 patients. The frontal/parietal regions were most severely affected. Five patients demonstrated significant atrophy. In summary, adults with SCD commonly develop ischemic brain injury resulting in soft signs of neurologic dysfunction, which may lead to vascular dementia. Table 5.5 indicates the non-focal symptoms volunteered by patients upon interview. Memory loss such as forgetting appointments and prescriptions is common in adults, and usually attributed to psychosocial problems. For ten out of 32 patients, memory loss was the main non-focal symptom. Eighty percent of these patients had an abnormal MRI despite a normal neurological exam. In contrast, isolated psychiatric problems were not usually associated with MRI abnormalities. Figure 5.1 compares positive MRI with patient age. The results demonstrate an increasing rate of abnormalities with age.

TABLE 5.5 Predictor Values of Non-Focal Indications for MRI

Symptom	Total #	# Neg.	# Pos.	% Pos.
1) Memory loss	10	2	8	80%
2) Isolated psych*	8	7	1	13%
3) Non-specific neuro	10	4	6	60%
4) Headache	4	1	3	75%
total	32	14	18	56%

** Psych. symptoms affected most other groups.*

Figure 5.1 Asymptomatic Patients with Positive MRI



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6 STUDY OBJECTIVES AND PURPOSE

The purpose of this study is to determine the extent of neurocognitive dysfunction in neurologically asymptomatic adult patients with SCD and its association with neuroimaging abnormalities.

6.1 PRIMARY HYPOTHESIS

Given the evidence that NP testing is abnormal in pediatric patients with SCD and precipitating risk factors continue or may cumulatively increase with age, we propose that NP functioning in neurologically asymptomatic adults with SCD will also be abnormal. Specifically, we believe that the mean score of adult patients with SCD on the WAIS-III PIQ scale will be at least one standard deviation below the mean score of a group of community controls matched for age and educational level.

6.2 SECONDARY HYPOTHESES

Neurologically intact adults with SCD will have significantly lower scores on other neuropsychological tests of executive function when compared to adult controls.

A larger percentage of adult patients with abnormal MRIs will have abnormal NP testing than those with normal MRIs.

Volumetric MRI will detect brain dysfunction in patients with abnormal neurocognitive tests but normal qualitative MRI.

Correction of anemia with transfusion therapy will improve the cognitive function in SCD patients with abnormal neurocognitive testing more than standard care.

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7 STUDY DESIGN

7.1 DESCRIPTION OF PRIMARY AND SECONDARY ENDPOINTS

The primary endpoint is completion of baseline WAIS III-PIQ studies in 120 subjects and 36 controls. Assuming a 20% attrition rate, 150 subjects and 45 controls will be recruited.

Secondary endpoints include completion of the entire baseline NP battery and MRI studies in 120 subjects and 36 controls. After consent and randomization, the final secondary endpoint is completion of the transfusion protocol and demonstration of at least an average five-point within-subject improvement in WAIS-PIQ above any average within-subject improvement observed in the standard care group, total number of patients is 30 (15 in each group). Thirty-six patients meeting these criteria will be randomized, assuming 80% completion. Randomization in the transfusion phase of the study is appropriate in order to discriminate, as much as possible, intervention effects from re-test effects.

7.2 DESCRIPTION OF TYPE OF STUDY

Our primary hypothesis compares neuropsychological testing of neurologically asymptomatic adult SCD patients and controls. In addition, MRI is required on all of the neuropsychologically tested patients to determine the relationship between imaging and cognitive function. Our study design for the primary hypothesis will be a cross-sectional investigation of 150 adult neurologically normal Hb SS/SB^0 subjects. The 45 matched-peer controls will be recruited from the communities in which the subjects reside.

The fourth secondary hypothesis will assess the benefit of transfusion therapy in improving neurocognitive function and be evaluated in a pilot intervention trial. A subset of 36 patients from the primary study phase with WAIS-III PIQ scores ≤ 90 will be randomized to undergo either a chronic transfusion regimen or receive standard care alone, for six months.

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8 SELECTION AND WITHDRAWAL OF SUBJECTS FOR THE CROSS-SECTIONAL STUDY

8.1 SUBJECT IDENTIFICATION AND RECRUITMENT

Subjects with HB SS/SB^0 will be recruited from the participating sickle cell centers from their clinic populations. Approximately 120 individuals and 36 controls are required to complete the cross-sectional study. The number of subjects required can easily be recruited by the centers. In order to obtain a broad population sample, subjects will be recruited from adult programs in approximately eight sites associated with the CSCC centers.

Assuming an 80% completion rate, 150 subjects and 45 controls will be enrolled in the cross-sectional study. For Phase II, the pilot transfusion study, a subset of 36 patients who complete the primary study will be randomized to either chronic transfusion or standard care, and 30 are expected to complete the protocol (allowing for 15 in each group). To eliminate selection bias, all eligible patients will be approached. A log of those enrolling and declining participation will be kept for both phases.

In Phase I, case group individuals will belong to one of four age-groups and one of four academic-group categories, resulting in 16 different possible categorizations for each subject. In order to maximize use of resources at the clinical site and ensure the usefulness of all data collected, sites will be requested to complete enrollment within a single category. Since the ratio of case to control group participants is 3:1, control subjects may not be enrolled until three cases within one category have been enrolled. For example, it is preferable to enroll three individuals who have completed high school in the 21-29 year old category, rather than to enroll one college-educated 21-29 year old, one 40-49 year old with high school only, and one 30-39 year old with only eight years of education, which will necessitate postponing recruitment of an appropriate control or possibly require an increased number of controls to maintain matching

In order to standardize procedures for identification, recruitment, and enrollment of case group members, eligible subjects presently at sickle cell clinics for previously scheduled, periodic and routine comprehensive visits will be the primary source for NP study participants. Targeting this group as the source population will increase the likelihood that study participation will be completed and full participation will occur since these individuals are motivated to follow through with scheduled, non-crisis related clinic visits. This approach will also eliminate the need to obtain informed consent at, or close to, the time of the occurrence of an acute event.

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8.2 COMMUNITY CONTROLS IDENTIFICATION AND RECRUITMENT

The study design requires that 36 control individuals complete the cross-sectional study. Control participants can be recruited from the subjects' church communities, their neighborhoods, or by word-of-mouth (i.e., acquaintances).

As an example of one recruitment strategy, subjects who have already completed study informed consent forms may be asked to list the church they attend and the name of their pastor. Community controls can then be recruited from the subject-reported church communities once the research assistants have contacted the pastors to determine their willingness to allow recruitment to occur at their church.

Previous outreach for church and community support for the sickle cell program has been documented. For example, an information table can be prepared with a research staff member available to provide additional information about SCD following services or other church-related events. Flyers describing the study, a sign-up sheet, contact names, telephone numbers, and hospital and email addresses can be provided for interested participants to contact the research team. The research team will be available to talk with interested individuals who have time to complete the screening materials, review and sign the informed consent forms, or who want to arrange a later meeting. The research team needs to be flexible and accommodating to the potential participants when answering their questions and addressing their concerns.

As an additional method, case subjects may assist in the selection of controls by choosing a neighbor or acquaintance for possible recruitment. It should be emphasized that this recruitment activity is completely voluntary; cases are under no obligation to approach anyone if they would prefer not to. If interested, case subjects can be provided with letters of introduction to the study at the time of enrollment that can be distributed to neighbors or close acquaintances who might be interested in volunteering to serve as a community control. The letters of introduction should briefly describe eligibility criteria, time commitment, and compensation. The letter of introduction could contain a tear-off portion on which the potential control can write their contact information (name, address, telephone number), age, gender, and years of education completed. The tear-off portion should be returned in a postage paid envelope to the study coordinator who will in turn, contact the potential control. If there is a need for a volunteer in the age/education level, they will be asked to schedule an appointment and invited to visit the center for additional information. If there is not a need for a control of the respondent's demographic, he or she can be asked to give permission to be contacted should there be a need at a later time.

Once an appointment has been scheduled the participant will meet with a research assistant at the hospital or clinic site for an evaluation. If appropriate, the researcher and the subject will review the informed consent form. Once community controls have demonstrated interest, they will complete the Visit 1 through Visit 4 events outlined in

sections 10.1.1 – 10.1.7. These events include screening measures, the NP test battery, an MRI, and a debriefing session.

Since the number of controls will be approximately one-third the number of subjects, an enrollment process has been designed that ensures comparable age by education joint distributions for subjects and controls for each gender. As the joint distribution of age and education develops during enrollment for the subjects, the following procedure will be used to enroll controls with the necessary age and education characteristics: First, controls will be enrolled so that the ratio of subjects to controls is roughly 3 subjects for every control. Second, age groups based on the study age range of 21-55 years (for example, 21-29 years, 30-39 years, 40-49 years, and 50-55 years) will be created. Third, education levels, such as 8 years of schooling or less, 9-11 years of schooling, completed high school, and > 12 years of schooling will be created. During enrollment, accumulating case counts by site, gender classification, and age group by education level combination will be tracked, with a set of tables similar to the following (e.g., for males at Site 1).

Males: Site 1	**≤ 8 yrs.**	**9 to 11 yrs.**	**Completed High School**	**> 12 yrs.**
21-29 yrs				
30-39 yrs				
40-49 yrs.				
50-55 yrs.				

Fourth, as individual subject cell counts accumulate in each table, one control will be enrolled once a cell count reaches a multiple of three. For instance, when any site's age by education combination for males reaches 3*x enrolled subjects, the site will have x-1 enrolled controls for that combination and will need to enroll the x^{th} control for that combination. To optimize matching of cases and controls in the broad (> 12 years) education category, a control that reflects the education level of three cases will be recruited. In addition, as addressed in greater detail in Section 13.2, we will also statistically control for site, age, sex and education using a GLM to model the data. Also, any subject cell count in the above table that is non-empty but contains less than 3 subjects at the end of enrollment period will also be matched with a control. The SDMC has the ability to automate this enrollment system, track the accumulated cases, and notify a site when it needs to enroll a control for a given gender and age group by education level combination.

8.3 INCLUSION CRITERIA FOR CROSS-SECTIONAL STUDY CASE GROUP

Individuals who meet all of the following criteria are eligible for enrollment as cases into the study:

1. Adult between the ages of 21 and 55
2. African descent
3. Proficient/fluent in English
4. Hemoglobin electrophoresis confirming hemoglobin SS or SB0 (%A ≤15)
5. Hemoglobin ≤ 10 g/dL
6. Capable of giving informed consent for the protocol

8.4 INCLUSION CRITERIA FOR CROSS-SECTIONAL STUDY CONTROL GROUP

Individuals who meet all of the following criteria are eligible for enrollment as community controls into the study:

1. Adult between the ages of 21 and 55
2. African descent
3. Proficient/fluent in English
4. Capable of giving informed consent for the protocol

8.5 EXCLUSION CRITERIA FOR THE CROSS-SECTIONAL STUDY CASE GROUP

Individuals who meet any of the following criteria are disqualified from enrollment in the case group of the study:

1. Overt stroke
2. Previous evidence of an abnormal MRI or CT other than small peri-ventricular or watershed lesions
3. History of head injury that resulted in neurological symptoms or medical visit
4. Abnormal neurologic exam with focal findings
5. Mini-Mental Status Examination (MMSE) score of < 20
6. Profile of Mood States (POMS) score on the Depression-Dejection Subscale suggestive of a clinical depression (score > 40)
7. Alcohol consumption exceeding 14 drinks/week if female, 21 drinks/week if male [142]
8. Drug abuse, defined as using non-prescribed medication
9. History of claustrophobia and/or presence of metallic implants such as pacemakers, surgical aneurysm clips, or known metal fragments embedded in the body

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10. Pregnancy
11. Baseline blood pressure > 140/90 on two repeated measurements. A second measurement is needed only if the first is > 140/90
12. History of uncontrolled hypertension
13. Any chronic disorder that may result in neurocognitive or brain dysfunction that is **not secondary to SCD** including:
    a) Inflammatory arterial disorders (lupus, polyarteritis)
    b) History of cancer requiring chemotherapy and/or radiation
    c) Untreated hyperlipidemia
    d) Diabetes
    e) Ongoing active infection such as HIV, tuberculosis, sarcoidosis
    f) History of chronic transfusion
    g) Chronic renal failure/Dialysis
    h) Chronic lung disease characterized by need for oxygen
    i) Morbid obesity (weight >115 kg)
    j) Heart disease: history of congestive heart failure, history of severe coronary artery disease characterized by angioplasty or surgery, or history of angina
    k) Active hepatitis or liver failure
    l) Acquired or congenital immune deficiency
    m) History of psychoses (delusions, hallucinations) and/or schizophrenia
    n) Neurodegenerative disorders
    o) Genetic disorder associated with neurocognitive dysfunction such as Down Syndrome
    p) Other chronic illness or disorder other than SCD that will adversely affect the subject's performance in the study
14. Currently on Procrit or related drug that stimulates red blood cell production

8.6 EXCLUSION CRITERIA FOR CROSS-SECTIONAL STUDY CONTROL GROUP

Individuals who meet any of the following criteria are disqualified from enrollment as community controls in to the study:

1. Hb electrophoresis other than AA
2. Abnormal Hb (females: < 12 g/dL; males: < 13.5 g/dL)
3. Overt stroke
4. Previous abnormal MRI or CT
5. History of head injury that resulted in neurological symptoms or medical visit
6. Abnormal neurologic exam with focal findings

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7. Mini-Mental Status Examination (MMSE) score of < 20
8. Profile of Mood States (POMS) score on the Depression-Dejection Subscale suggestive of a clinical depression (score > 40)
9. Alcohol consumption exceeding 14 drinks/week if female, 21 drinks/week if male [142]
10. Drug abuse, defined as using non-prescribed medication
11. History of claustrophobia and/or presence of metallic implants such as pacemakers, surgical aneurysm clips, or known metal fragments embedded in the body
12. Pregnancy
13. Baseline blood pressure > 140/90 on two repeated measurements. A second measurement is needed only if the first is > 140/90
14. History of uncontrolled hypertension
15. Any chronic disorder that may result in neurocognitive or brain dysfunction including:
    a. Inflammatory arterial disorders (lupus, polyarteritis)
    b. History of cancer requiring chemotherapy and/or radiation
    c. Untreated hyperlipidemia
    d. Diabetes
    e. Ongoing active infection such as HIV, tuberculosis, sarcoidosis
    f. History of chronic transfusion
    g. Chronic renal failure/Dialysis
    h. Chronic lung disease characterized by need for oxygen
    i. Morbid obesity (weight > 115 kg)
    j. Heart disease: history of congestive heart failure, history of severe coronary artery disease characterized by angioplasty or surgery, or history of angina
    k. Active hepatitis or liver failure
    l. Acquired or congenital immune deficiency
    m. History of psychoses (delusions, hallucinations) and/or schizophrenia
    n. Neurodegenerative disorders
    o. Genetic disorder associated with neurocognitive dysfunction such as Down Syndrome
    p. Other chronic illness or disorder that will adversely affect the subject's performance in the study
16. Currently on Procrit or related drug that stimulates red blood cell production

Questions or issues concerning eligibility for an individual case or control participant should be directed to the Protocol Chairman or the Medical Monitor.

8.7 SUBJECT DISCONTINUATION

Subjects may decide to discontinue participation at any time during the study. Investigators may discontinue any subject at their discretion if, in their professional opinion, the subject's health, safety, and/or well-being is threatened by continued participation in the study.

Adverse events caused by participation in the study may necessitate modifications to the subject's level of participation or discontinuation from the study.

Subjects who discontinue prematurely from the study for any reason will be encouraged to complete, at a minimum, all safety follow-ups and, if willing, all efficacy endpoint visits for safety and intent-to-treat analysis.

8.8 COMPLETION OF THE CROSS-SECTIONAL STUDY (PHASE I)

On February 22, 2008, enrollment into the cross-sectional study (Phase I) was completed. Patients who had signed consent and had completed Visit 1 were permitted to complete Visits 2-4. Enrollment and randomization into Phase II is dependent on the values obtained during Phase I, including the initial chemistry/hematology panel, MMSE, POMS, PIQ, and normal MRI.

Subjects who enroll in Phase II more than 6 months (but less than 1 year) from completion of Phase I (as determined by date of WAIS-III PIQ) repeat selected assessments upon entry into Phase II. Subjects who were never enrolled in Phase I (or who completed Phase I more than 1 year ago) may also be enrolled in Phase II, per a revised set of inclusion/exclusion criteria that combine Phases I and II. The most non-invasive and least time-consuming screening assessments are completed first to ensure eligibility, before the subject moves forward with additional study procedures.

9 TREATMENT OF PATIENTS ENROLLED IN THE PILOT TRANSFUSION TRIAL

9.1 PARTICIPATION IN THE PILOT TRANSFUSION TRIAL

Approximately 36 patients with WAIS III PIQ scores ≤ 90 will be asked to participate in an intervention study in which subjects will be randomized to either a transfusion arm or a standard care arm. Simple transfusion will be used, with a maximum of 3 units to be delivered. Exchange transfusion or undergoing pheresis will not be permitted.

Written informed consent will be obtained prior to randomization.

9.2 INCLUSION CRITERIA FOR PATIENTS PARTICIPATING IN THE PILOT TRANSFUSION TRIAL

Individuals who completed Phase I and meet all of the following criteria are eligible for enrollment in the pilot transfusion study:

1. Completion of all components of the cross-sectional study
2. WAIS III-PIQ score ≤ 90
3. Hemoglobin ≤ 9.0 g/dL

Individuals who did not complete Phase I (or who completed Phase I more than 1 year prior to enrollment into Phase II) and meet all of the following criteria are eligible for enrollment in the pilot transfusion study:

1. Capable of giving informed consent for the protocol
2. Willing to undergo transfusion therapy for 6 months
3. Adult between the ages of 21-55
4. African descent
5. Proficient/Fluent in English
6. Hemoglobin electrophoresis confirming hemoglobin SS or SB^0 (%A ≤15)
7. WAIS III-PIQ score ≤ 90
8. Hemoglobin ≤ 9.0 g/dL
9. Mini-Mental Status Examination (MMSE) score of ≥ 20
10. Profile of Mood States (POMS) score on the Depression-Dejection Subscale ≤ 40

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9.3 EXCLUSION CRITERIA FOR PATIENTS PARTICIPATING IN THE PILOT TRANSFUSION TRIAL

Individuals who meet any of the following criteria are disqualified from enrollment in the transfusion study:

1. History of life threatening or serious transfusion complications
2. Lack of venous access
3. Current enrollment in the Arginine study
4. Pregnancy
5. Refusal of transfusion
6. History of unexplained severe hemolytic transfusion reaction
7. History of serious allergic, pulmonary transfusion reaction requiring hospitalization for the reaction
8. Positive auto-immune hemolytic anemia (direct coombs with IGG and complement)
9. Multiple (three or more) clinically significant allo-antibodies, due to common antigens (for example; EC, Kel)
10. Uncommon, clinically significant antibody that results in difficulty in finding matched units (for example; anti-JKB)
11. Currently taking Hydroxyurea and **not on a stable dose for ≥ 6 months**
12. Creatinine > 1.7 mg/dL
13. Ferritin > 1,500 ng/mL or quantitative liver iron > 7 mg iron/g > dry weight and **not** currently on iron chelation therapy. (This is a pilot transfusion in which only six months of transfusion will be utilized. The likelihood of iron overload induced toxicity from the transfusions over the six months is very small. Furthermore, ferritin is disproportionately elevated in SCD and overestimates the iron burden. Therefore, we have included a quantitative liver iron and/or ferritin as criteria for exclusion [143-145].)
14. Major infarct identified on Phase I MRI
15. Currently on Procrit or related drug that stimulates red blood cell production

In addition to the criteria listed above, individuals who did not complete Phase I (or who completed Phase I more than 1 year prior to enrollment into Phase II) are disqualified for enrollment in the pilot transfusion study if they meet any of the following criteria:

1. Overt stroke

2. Previous evidence of an abnormal MRI or CT other than small periventricular or watershed lesions
3. History of head injury that resulted in neurological symptoms or medical visit
4. Abnormal neurological exam with focal findings
5. Alcohol consumption exceeding 14 drinks/weeks if female, 21 drinks/week if male [142]
6. Drug abuse, as defined as using non-prescribed medication
7. History of claustrophobia and/or presence of metallic implants such as pacemakers, surgical aneurysm clips, or known metal fragments embedded in the body.
8. Baseline blood pressure > 140/90 on two repeated measurements. A second measurement is needed only if the first is > 140/90.
9. History of uncontrolled hypertension
10. Any chronic disorder that may result in neurocognitive or brain dysfunction that is **not secondary to SCD** including:
    a. Inflammatory arterial disorders (lupus, polyarteritis)
    b. History of cancer requiring chemotherapy and/or radiation
    c. Untreated hyperlipidemia
    d. Diabetes
    e. Ongoing active infection such as HIV, tuberculosis, sarcoidosis
    f. History of chronic transfusion
    g. Chronic renal failure/Dialysis
    h. Chronic lung disease characterized by need for oxygen
    i. Morbid obesity (weight >115 kg)
    j. Heart disease: history of congestive heart failure, history of severe coronary artery disease characterized by angioplasty or surgery, or history of angina
    k. Active hepatitis or liver failure
    l. Acquired or congenital immune deficiency
    m. History of psychoses (delusions, hallucinations) and/or schizophrenia
    n. Neurodegenerative disorders
    o. Genetic disorder associated with neurocognitive dysfunction such as Down Syndrome
    p. Other chronic illness or disorder other than SCD that will adversely affect the subject's performance in the study

9.4 TREATMENTS

Approximately 36 patients with WAIS-III PIQ scores ≤ 90 will be asked to participate in a transfusion intervention. Half of these patients will be randomized to undergo a chronic

transfusion regimen for six months, and the other half will be treated with standard care alone, guided by their disease symptoms. At the end of the six-month period, both groups of patients will be re-tested on the neurocognitive battery and the MRI will be re-administered. The mean change scores will be compared between the two groups.

The intervention will involve visits to the center for transfusions. Although it may be argued that benefits to patients receiving the intervention would include those due to the transfusion as well as those that might be due to increased interaction with center staff, it is not feasible to separate the effects of the two in the pilot study. To do so would require a more elaborate study design.

10 EVALUATIONS BY STUDY VISIT

10.1 VISIT 1 – SCREENING AND PSYCHOSOCIAL STRESS

After a potential study subject reads and signs the informed consent, the Study Coordinator or a trained research associate will conduct a brief screening to determine if the subject is eligible to participate. This involves the administration of the MMSE and the POMS. If the subject is not potentially eligible, he or she will be thanked for his or her time and dismissed. If the subject is eligible, he or she may begin to complete the psychosocial questionnaire (the PSS). The total time required to complete the basic demographic information and the three questionnaires is approximately 45-60 minutes. During this period the research assistant can conduct a medical chart review to assess whether the subject meets the inclusion and exclusion criteria. Following completion of the questionnaires, a history and physical exam utilizing the neurologic exam protocol will be obtained by one of the study staff. Two teaspoons of blood will be drawn to confirm a diagnosis of sickle cell disease (cases) or to confirm that the subject is not anemic (controls). The blood drawn will also be used for a hematology and chemistry panel. For females of childbearing potential, a urine sample will be collected to ensure that the subject is not pregnant. An appointment will then be scheduled with the neuropsychologist to begin neuropsychological testing.

10.1.1 SCREENING EVALUATIONS

For the purposes of screening, the research associate will assess each subject on the MMSE, the POMS, and a brief measure of alcohol and non-prescription drug use. The MMSE will be administered by the Study Coordinator, however, the POMS is a self-administered test. If the subject is unable to read, he/she may listen to the POMS on an audiotape using headphones. Instructions will be provided on the tape about how to record all answers.

The MMSE is a brief mental status examination designed to quantify cognitive status by assessing performance on the following cognitive domains: orientation, language, calculation, memory, and visuospatial reproduction [146]. The score on the MMSE is the total number of items answered correctly and can range from 0 to 30 points. A score of 20 points or less is considered indicative of possible cognitive impairment or dementia. The MMSE has high test-retest reliability and has moderate to high sensitivity. The MMSE is included as a brief screening tool for cognitive functioning.

The POMS is a 65 item five-point adjective rating scale that was developed for measuring six identifiable mood or affective states: Tension-Anxiety; Depression-Dejection; Anger-Hostility; Vigor-Activity; Fatigue-Inertia; and Confusion-Bewilderment, and a total mood disturbance score (TMD) [147]. The measure has been used with a wide range of clinical populations, and age and ethnically diverse samples. The measure demonstrates strong internal consistency with a Cronbach's alpha ranging

from 0.87 to 0.92. A Depression-Dejection subscale score of > 40 is suggestive of clinical depression and, as such, the POMS is included as a screening tool for depression.

10.1.2 EVALUATION OF PSYCHOSOCIAL STRESS

While it has been demonstrated that neurocognitive performance and functioning are impacted by psychological and social stress, the current protocol will employ a self-report measure to evaluate perceived stress. The measure will be administered following the consent procedure.

The PSS measures a global perception of stress during the previous month [148]. It has a short version comprised of 10 items (e.g., "In the last month how often have you felt difficulties were piling up so high that you could not overcome them?" Response options were assessed using a five-point Likert-type scale: "0"="Never" to "4"="Very Often"). The scores on four items that were worded in the opposite direction (e.g., "In the last month how often have you felt that things were going your way?") are reverse-scored. A total PSS score is computed by summing across all 10 items. The measure demonstrates strong internal consistency with a Cronbach's alpha of 0.88. It also has the virtues of being widely used, brief, and assessing stress response on a continuum from relatively mild to severe. It is at a sixth grade reading level.

10.2 VISIT 2 – EVALUATION OF NEUROPSYCHOLOGICAL FUNCTIONING OR MRI TESTING

Visit 2 must occur within four weeks of Visit 1. At Visit 2, either the Neuropsychological (NP) Battery or the MRI Testing will occur. The sequencing of these study components is flexible to allow for easier scheduling at participating sites.

10.2.1 NEUROPSYCHOLOGICAL BATTERY

A trained and supervised neuropsychologist will administer all neuropsychological battery tests as described below. This visit will take approximately 6 to 6 ½ hours to complete. At the conclusion of this meeting, an appointment for Visit 3 will be scheduled.

The neurological test battery in this study consists of a wide range of tests that investigate several aspects of neurocognitive functioning. All measures are individually administered and have been standardized on normative populations.

The Weschler Adult Intelligence Scale - Third Edition (WAIS-III) will be used to assess intellectual functioning [149]. Measurement of estimates of both nonverbal and verbal reasoning ability as well as general cognitive ability is provided. This version of the WAIS-III consists of 14 subtests that involve tasks such as having participants define

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words and using three-dimensional blocks to reproduce designs from models. The median reliability coefficient for the 14 subtests is 0.81, while the median reliability for the index scores is 0.87. Interscorer agreement is reported to be very high, averaging in the high 0.90s. Stability over time is reported to be adequate.

The Woodcock Johnson Revised: Test of Achievement (WJ-R) [150] will be used to measure academic achievement. On the WJ-R, participants will complete the core battery reading and mathematics subtest to measure reading decoding, reading comprehension, paper and pencil mathematics calculation, and applied mathematics problems. Internal reliability coefficients for the tests are reported to generally be in the high 0.80s and low 0.90s for the tests and in the mid 0.90s for the clusters.

The following neuropsychological measures will be administered to examine language, memory, executive functioning, and sensorimotor perceptual skills.

The Delis-Kaplan Executive Function System (D-KEFS) comprehensively assesses the key components of executive functions believed to be mediated primarily by the frontal lobe. The D–KEFS is the first nationally standardized set of tests to evaluate higher-level cognitive functions in both children and adults. The tests assess vital executive functions such as flexibility of thinking, inhibition, problem solving, planning, impulse control, concept formation, abstract thinking, and creativity in both verbal and spatial modalities. This instrument incorporates principles from cognitive science to evaluate the component processes of tasks thought to be especially sensitive to frontal-lobe dysfunction.

The Wisconsin Card Sorting Test (WCST) is a measure of nonverbal concept formation that is considered to measure executive functioning [153]. It requires strategy planning and the ability to use feedback to shift cognitive set. Intrarater and interrater reliability on the WCST have been reported to range from excellent to quite low.

The Test of Everyday Attention (TEA) examines attention and executive functioning [154]. These tasks are important in the efficient encoding, retrieval, and learning of new information.

The California Verbal Learning Test - Second Edition (CVLT-II) assesses memory for discrete auditory material by having the participant learn a list of 16 words over five trials [155]. The task also includes an interference list and delay and recognition components.

The Wechsler Memory Scales - Third Edition (WMS-III) [149] contains 11 subtests that assess aspects of memory and learning. Many of the subtests are separated into immediate and delayed conditions. Reliability of this measure is reported to be adequate.

10.2.2 MRI TESTING

Dr. Weiner at the University of California, San Francisco (UCSF) Neuroimaging Center, will supervise MRI testing. A standardized MRI protocol with matching software will be used. The MRI protocol is described in the following section.

MR scanners must meet the following minimal requirements to qualify for this study:

- 1.5T MR scanners of newer generation (not older than eight years)
- capable of volumetric T1-weighted MRI acquisition.

In addition, tissue segmentation requires that axially oblique, double-spin echo image acquisition can be performed with flow compensation.

10.2.3 MRI SCAN PROTOCOL

1. Scouts MRI

   3D planar scout: Quick acquisition in three orthogonal planes for anatomical orientation.

   Sagittal 2D Flash: Sagittal Flash to locate the left and right hippocampus; 15 contiguous slices with 3 mm thickness

2. Volumetric 3D T1-weighted (MP-RAGE, Spoil-Grass, etc.)

   Purpose: Volumes of brain structures at high resolution
   Orientation: Oblique Coronal (inplane)

   Angulation: Perpendicular (⊥) to the long axis of the hippocampus, as seen in the Sagittal Flash

   Resolution: 1 mm^2 inplane (Requires that the number of encodes (samples) = FOV in mm; i.e. 256 encodes for 256 mm). 1.5 mm through plane (which is achieved by number of encodes = 2/3 of slab thickness; i.e. 120 encodes for 180 mm slab).

   Timing: Acquisition Bandwidth = Maximum (195 kHz Siemens, 124 kHz GE)
   Full Echo acquisition
   Dephasing TE = between 8 and 4 ms
   Contrast TI/TR = 300/10 ms
   Flip angle = 15°

   Other recommendations:

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- MRI read encode along head to foot to avoid aliasing from shoulder regions
- Rectangular FOV (i.e. 6/8 with reduced FOV from left-right to shorten acquisition)
- Scan time: less than 8 minutes

3. Density/T2-weighted Spin echo (or Turbo)

Purpose:	Clinical reading and tissue segmentation
Orientation:	Oblique Transverse
Angulation:	Parallel to the AC-PC line seen in the Sagittal Flash
Resolution:	1 mm^2 inplane (requires number of encodes = FOV in mm) 3 mm slice thickness, interleaved excitation order Slice number: 47-51 or more for full brain coverage
Timing:	Echo timing: TE1 ≤ 30 ms TE2 = 80ms Repetition time: TR ≥ 2000 *(TR can be increased to approximately 5000 ms in order to include 48-50 slices within one acquisition)* Flow compensation gradients for spin echo Approximately 50 mm saturation Bands caudal to the MRI slices; positioned 15 mm below to the most inferior MRI slice to reduce inflow effects

Other recommendations:
- Standard double spin echo or turbo double spin echo
- Rectangular FOV (i.e. 6/8 to shorten acquisition time)
- Scan times: 15 minutes DSE, 5 minutes TSE

The MRI images will be electronically sent to Dr. Weiner at UCSF for volumetric analysis. The MRI reading panel will be comprised of one neuroradiologist and one additional qualified scientist masked to the participant's medical history or treatment. Reviews will be conducted independently with cases of disagreement reviewed by a third qualified researcher. The three will confer to produce a consensus. They will report the following results on MRI forms: side, size, location, and extent (periventricular and/or cortical) of all recorded infarcts. In addition, generalized atrophy, focal atrophy, and lesions will be noted.

Summary of Volumetric Outcome Measures

The following results (reported as volumes) will be delivered:

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- Total Intracranial Volume (ICV)
- Left and Right Hippocampus
- Cortical Gray Matter
- White Matter
- White Matter Lesions
- Cortical & Subcortical Lacunae
- Sulcal CSF
- Ventricular CSF

10.3 VISIT 3

Visit 3 must occur within four weeks of Visit 2. At Visit 3, the study component that was not conducted at Visit 2 will be completed. For example, if the Neuropsychological Battery was completed at Visit 2, then the MRI will be completed at Visit 3. If the MRI was completed at Visit 2, then the NP Battery will be completed at Visit 3.

10.4 VISIT 4 – EXIT DEBRIEFING

At Visit Four, the Exit Debriefing, both study controls and subjects will be given the opportunity to meet and discuss the results of their neuropsychological battery and their MRI. If the participants have a low average or below average score, the feedback they receive will include information about additional testing and intervention services that are available to them in their community but not as part of the study.

Once the debriefing has occurred, case group subjects who have completed the cross-sectional study may participate in the pilot transfusion study. If the subject is potentially eligible for Phase II of the study, he or she will be given the opportunity to complete the informed consent, eligibility screening, and SF-36 at this exit debriefing or he or she may choose to schedule an appointment to return at a later date for eligibility screening for Phase II.

10.5 VISIT SCHEDULE FOR THE PILOT TRANSFUSION TRIAL

Approximately 36 patients with WAIS III-PIQ scores ≤ 90 will be asked to participate in Phase II of the study. Half of these patients will be randomized to undergo a chronic transfusion regimen for six months, and the other half will be treated with standard care alone, guided by their disease symptoms.

During the first visit for Phase II, subjects who completed Phase I and went on to Phase II will sign informed consent, be screened for eligibility (including a blood draw for the hematology and chemistry panels), and will have their quality-of-life assessed by the self-administered SF-36. As described above, this can occur at the Visit 4 Debriefing or at a subsequent visit. The SF-36 assesses eight health concepts: 1) limitations in physical

activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitation in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions.

If > 6 months (but < 1 year) have passed since the subject's participation in Phase I of the study, the subject must FIRST repeat the WAIS-III PIQ portion of the Neuropsych Battery at the first visit of Phase II to confirm that the subject's PIQ score is ≤ 90. If the score is still consistent with eligibility requirements, the study coordinator will complete the screening blood draw on the subject, administer the SF-36, and will schedule a repeat MRI so that the image can be compared to the MRI conducted during Phase I. The site will locally confirm that the repeat MRI does not contain any new neurological findings when compared to the Phase I MRI.

If > 1 year has passed since the subject's participation in Phase I (or if the subject was not enrolled in Phase I), the subject must complete the MMSE and the POMS, as well as the WAIS-III PIQ portion of the Neuropsych Battery to confirm eligibility. If eligible, the study coordinator will complete the screening blood draw and schedule an MRI to be completed at Visit 2. (For subjects enrolled in Phase I, this is a repeat MRI that should be compared to the Phase I MRI to confirm there are no new neurological findings. If eligible to continue, the subject will return for Visit 3, during which the SF-36 and the remainder of the Neuropsych Battery is administered and the subject is randomized.

Patients randomized to the transfusion arm will receive six to eight transfusions over the course of six months. The first transfusion must occur within 2 weeks of randomization. The specific method of randomization is described in greater detail in the Statistical Analysis section of the protocol and the Study Information Manual for this study. The goal is to maintain a minimum of at least 2 g/dL rise of Hb over the baseline. Once transfusions are started, the pre-transfusion Hb obtained within three days of the transfusion should be 9.0 g/dL or greater, without exceeding a post-transfusion Hb concentration of 12.0 g/dL. All transfusions will be leukodepleted and Hb S negative. Phenotypically matched red blood cells for D, C/c, E/c, and Kell$_1$ antigens are required, along with any known antibody. Only simple transfusion will be allowed. Simple transfusion guidelines include the maximum delivery of 3.0 units of packed red blood cells (RBCs) per transfusion at 3-4 week intervals. Prior to each transfusion, an interval history form will be completed and women of childbearing potential will be asked whether they suspect they might be pregnant. If the answer is "Yes" or "Not Sure," a urine sample will be collected and a pregnancy test will be performed.

Patients randomized to the standard care arm will follow their normal care regimen, and will be asked to complete, on a monthly basis, an interval history form noting any new medications, neurological events, transfusions, hospitalizations, etc. the patient may have had since the last interval history. The interval history will be conducted via telephone during Months 1, 2, 4, and 5. During Months 3 and 6, the patient will make in-person

clinic visits to complete the interval history form, have blood drawn to measure Hb and ferritin levels, and for completion of a chemistry panel.

Within two weeks of the patient's final transfusion or clinic visit at month 6, the SF-36 will be re-administered in addition to the NP test battery. Within two weeks of this visit, another visit must occur and the patient will complete the MRI. The neuropsychological testing will again take about 6 to 6½ hours to complete, and the MRI will take approximately 30 minutes to complete.

The last visit of Phase II is for the Exit Debriefing. Once all of the tests have been completed, the patient will meet with a doctor or nurse to review a summary of the results and ask any questions he/she may have. It is possible that the tests used in this study (the NP questionnaires or the MRI) may show problems in the functioning of the patient's brain. If there are any problems, the patient will receive specific recommendations about what should be done. If requested by the patient, this information will be sent to his or her primary care doctor and will include recommendations for follow-up care.

10.6 LABORATORY ANALYSES

Each transfusion will be preceded by measurement of Hb S, A, F, A2, and other, Hct, red cell antibody screening, and Hb concentration.

10.7 TIMETABLE

The first three months will be used for standardization of NP and MRI studies at the institutions. During Months 6 to 18, all subjects will be enrolled into the cross-sectional study and will complete the MRI and NP battery. Concurrently, a subset of patients will be randomized to transfusion or standard care. By 24 months, all patients randomized to transfusion will have completed their six months regimen. When the last of these patients complete his/her second NP battery and MRI, the database will be finalized, and statistical analysis will follow.

Enrollment into Phase I was closed on February 22, 2008. The inclusion/exclusion criteria and the study procedures were modified so that patients willing to participate in 6 months of transfusion therapy can participate in Phase II, but still complete the baseline NP Battery and MRI previously obtained from Phase I.

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Phase I:

	Visit One	Visit Two/Visit Three	Visit Four
Consent	X		
Eligibility Screening	X		
History and Physical Exam	X		
MMSE	X		
POMS	X		
PSS	X		
Neuropsychological Battery[2]		X	
MRI Testing[2]		X	
Exit Debriefing			X

1. Enrollment into Phase I was completed on February 22, 2008.
2. The sequencing of the Neuropsychological Battery and the MRI is flexible, and either component can occur at Visit 2. The component that is not completed at Visit 2 will occur at 3.

Phase II – Original

	Eligibility Visit[1]	Transfusion Visits [2] or Phone Calls/Visits [3]	NP Battery and SF-36 Visit	MRI Testing Visit	Exit Debriefing Visit
Consent	X				
Eligibility Screening	X				
SF-36	X				
Randomization	X				
Transfusions [2]		X			
Phone Calls/Clinic Visits [3]		X			
SF-36			X		
Neuropsychological Battery [4]			X		
MRI Testing [4]			X		
Exit Debriefing					X

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Phase II timetable for subjects who were never enrolled in Phase I (or who completed Phase I more than 1 year ago):

	Eligibility Visit[1]	Visit 2 MRI	Visit 3 NP Battery/ Randomi-zation	Transfusion Visits [2] or Phone Calls/Visits [3]	NP Battery and SF-36 Visit	MRI Visit	Exit Debriefing Visit
Consent Eligibility Screening PIQ MMSE POMS	**X** **X** **X** **X** **X**						
MRI Testing		**X**				**X**	
SF-36 Neuropsychological Battery (Randomization)			**X**		**X**		
Transfusions [2] Phone Calls/Clinic Visits [3]				**X**			
Exit Debriefing							**X**

1 Patients must meet inclusion/exclusion criteria based on their Hemoglobin and Ferritin values, and their scores on the POMS and PIQ before an MRI and the NP Battery are administered.
2Number of transfusions during the six-month period will vary between six and eight, and will occur at 3- to 4-week intervals.
3 Phone calls will occur during Months 1, 2, 4, and 5. In-person clinic visits occur during Month 3 and Month 6.

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11 SAFETY MANAGEMENT

11.1 ADVERSE EVENTS (AEs) AND SERIOUS ADVERSE EVENTS (SAEs)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as provided in this protocol. Since the cross-sectional neurocognitive/MRI phase of the study is observational only, AEs and SAEs will not be collected. However, AEs and SAEs will be collected for all subjects participating in the transfusion intervention pilot study.

11.1.1 DEFINITION OF AN ADVERSE EVENT

An AE is defined for this study as any untoward medical occurrence in a subject who is administered clinical study material. The occurrence of this event does not necessarily have a causal relationship with study product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study product, whether or not related to the study product.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition, including either an increase in frequency or intensity of the condition
- Significant or unexpected worsening or exacerbation of the condition/indication under study
- A new condition detected or diagnosed after study product administration even though it may have been present prior to the start of the study
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (e.g., invasive protocol-defined procedures, modification of a subject's previous drug treatment regimen).

An AE does not include:

- Medical or surgical procedures (e.g., colonoscopy or biopsy). The medical condition that leads to the procedure is an AE
- Social or convenience hospital admissions where an untoward medical occurrence did not occur
- Day-to-day fluctuations of a pre-existing disease or conditions present or detected at the start of the study that do not worsen

11.1.2 DEFINITION OF AN SAE

An SAE is any adverse drug experience occurring at any dose that:

- Results in death
- Is life-threatening (at risk of death at the time of the event)
- Requires inpatient hospitalization or prolongation of existing hospitalization

  NOTE: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered to be an AE.

- Results in disability/incapacity

  NOTE: The term disability is defined as a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (i.e., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the above definition

11.2 CLINICALLY SIGNIFICANT LABORATORY ABNORMALITIES

All laboratory measurements will be evaluated for abnormalities. An abnormal laboratory finding is not by itself considered to be an AE or SAE unless the investigator considers the abnormal finding to be of clinical significance and should be reported in such a manner. The abnormal laboratory finding does not have to be associated with the use of the study product to be considered clinically significant.

For any significant changes noted by the investigator to be clinically significant or those that meet the criteria above, the clinical significance and relationship to the administration of study product will be established. This assessment will be recorded on the CRF. If the changes are clinically significant, the investigator will continue to

monitor the subject until the parameter returns to baseline or until the investigator determines that follow-up is no longer medically necessary.

11.3 Assessment of Adverse Event Severity

The investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the investigator's clinical judgment. The intensity of each AE and SAE should be assigned to one of the following categories:

Mild: Awareness of sign, symptom, or event, but easily tolerated; does not interfere with usual daily activities or tasks

Moderate: Discomfort enough to cause interference with usual daily activity; may warrant therapeutic intervention

Severe: Incapacitating; inability to perform usual activities and daily tasks; significantly affects clinical status; requires therapeutic intervention

Life-threatening: AE is life-threatening

Death: AE causes death

An AE that is assessed as severe should not be confused with an SAE. Severity is a category used for rating the intensity of an event; both AEs and SAEs can be assessed as severe. An event is described as 'serious' when it meets one of the pre-defined outcomes as described in "Definition of an SAE" section of this protocol.

11.4 Relationship of AEs and SAEs to Study Procedures

The investigator is obligated to assess the relationship between study mandated procedures and the occurrence of each AE/SAE. The investigator will use his/her clinical judgment to determine the degree of likelihood that the study design was responsible for the AE/SAE being reported. Alternative causes such as natural history of the underlying diseases (See Table 11.2 Sickle Cell Related Conditions), concomitant therapy, other risk factors, and the temporal relationship of the event to the study product will be considered and investigated. Table 11.2 serves as a guide to conditions typically associated with sickle cell disease, not an exclusionary list of conditions. If the investigator feels that one of the following conditions is related to the study procedures, he/she should report it as related.

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TABLE 11.2 Sickle Cell Related Conditions

Expected AE	Expected AE	Expected AE
Acute chest syndrome Anemia Aplastic crisis Aplastic crisis/anemia Arthralgia Avascular necrosis of hip/shoulder Avascular necrosis of the femoral head Bone infarction Cardiomegaly Cerebrovascular accident Cholecystitis, hepatic sequestration Cranial nerve palsy Decreased kidney function Decreased lung function Delayed growth/puberty Depressed ESR	Development of autoantibodies associated with hemolysis Development of clinically significant alloantibodies Elevated urinary urobilinogen Fever Hand-foot syndrome Hematuria Hemiplegia Hemolysis Hemolytic transfusion reaction Hepatosplenomegaly Hyperplastic bone marrow Hyposthenuria Hypoxemia (PO2 < 65mmHg) Infection, pneumococcal Jaundice Leukocytosis Meningitis	Pain, joint Pain, long bone Pain, severe abdominal Priapism Pulmonary embolism Pulmonary hypertension Pulmonary parenchymal infiltrates on chest x-ray Pyelonephritis Renal failure Renal insufficiency/albuminuria Renal papillary necrosis Reticulocytosis (∃10%–20%) Retinal disease Retinal hemorrhage Rhabdomyolysis Sepsis Skin ulcers Splenic sequestration

11.5 TIME PERIOD, FREQUENCY, AND METHOD OF DETECTING AES AND SAE

AEs and SAEs will be recorded for all subjects participating in the Phase II Pilot Transfusion study from randomization through the Exit Debriefing visit. At each visit, prior to transfusion or during the monthly call, an interval history recording hospitalizations, emergency room visits, and clinical events will be collected. Pre-transfusion measurements of hemoglobin (Hb) will be made, and ferritin will be measured at entry to the Pilot Transfusion Phase of the study if the ferritin lab data are more than three months old, at Month 3, as well as at the end of the study. During the course of the six-month transfusion period complications related to transfusions will be recorded, as will any observations of new endocrine or cardiac problems, specifically any new arrhythmia or cardiomyopathy.

Information to be collected includes the nature, date and time of onset, intensity, duration, causality, and outcome of the event. Even if the AE/SAE is assessed by the investigator as related to the subject's underlying sickle cell disease, its occurrence must be recorded in the source documents and on the appropriate page of the CRF.

11.6 RECORDING OF AES AND SAES

When an SAE occurs, the investigator is responsible for reviewing all documentation (e.g., hospital progress notes, laboratory test results, and diagnostic reports) relative to the event. The investigator or designee will then record all relevant information about an AE/SAE onto the appropriate page of the CRF and SAE Report Form, as applicable. It is

not acceptable for the investigator to send photocopies of the subject's medical records in lieu of completion of the appropriate CRF pages. Unless otherwise requested, transfer of subjects' medical records with the CRF should be restricted to hospital discharge summaries and autopsy reports, if available. When sending medical records, all subject identifiers (i.e., name, subject initials, and medical record number) must be obliterated prior to faxing the documents to the data coordinating center.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

11.7 REPORTING

11.7.1 PROMPT REPORTING OF SAES (SITES TO THE SDMC)

The investigator or designee must report all SAEs to the data coordinating center by the end of the next business day after becoming aware of the event. The SAE Report Form will always be completed as thoroughly as possible, with all available details of the event, signed by the investigator (or sub-investigator), and forwarded to the appropriate project contact within the designated time frames. If the investigator does not have all information about an SAE, he/she will not wait to receive additional information before notifying the data coordinating center of the event. The following information is a minimum set of information required for all initial SAE reports:

- Investigator name
- Subject identifiers
- Adverse event term(s)
- Reason why the event is serious

Facsimile transmission of the SAE Report Form is the preferred method to transmit this information to the data coordinating center. Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE CRF within the time frames outlined in the protocol.

11.7.2 FOLLOW-UP OF AES AND SAES

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information on the subject's condition.

All AEs and SAEs documented at a previous visit/contact that are designated as ongoing will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution (with or without sequelae), until the condition stabilizes, or until there is agreement between the investigator and the DSMB and/or NHLBI additional follow-up is not warranted.

New or updated information will be recorded on the originally completed SAE Report Form, with all changes initialed and dated. Once all updates have been made to the SAE report, the investigator must resign and date the report. The updated SAE CRF should be resent to the project contact for SAE receipt within the time frames outlined in the "Prompt Reporting of SAEs" section of the protocol.

11.7.3 REPORTING TO THE DATA AND SAFETY MONITORING BOARD (DSMB)

Safety issues that arise in this study will be brought to the attention of the CSCC Data and Safety Monitoring Board (DSMB), which will make recommendations to the National Heart, Lung, and Blood Institute (NHLBI) regarding possible suspension of the study. The NHLBI will consider the DSMB's recommendations, determine an appropriate action and notify the Principal Investigator and the CSCC Statistics and Data Management Center (SDMC). The Principal Investigator will notify all participating investigators who will implement the actions directed by NHLBI.

All data from this study will be critically and carefully monitored by an independent DSMB to ensure the safety of study subjects as follows:

- All SAEs reported with SAE Criterion of Death will be reported to the DSMB within 15 calendar days of the SDMC Safety Specialist becoming aware of the event.

- On a quarterly basis, Rho will analyze SAE data and if it is determined that the data triggers a suspension guidelines as outlined in Table 11.3 "*Summary of Procedures and Timing for Alterting the DSMB and NHLBI Project Officer of Possible Serious Safety Issues*", the DSMB will perform a review of the information.

- On a semi-annual basis, the DSMB will recieve statistical analyses and review of all safety reports to include AEs, SAEs, Tranfusion Reactions and Clinical Laboratory Results.

11.8 SUSPENSION GUIDELINES

11.8.1 OVERVIEW

SAEs, AEs, and abnormal laboratory measures will be monitored quarterly throughout the study and if a significant imbalance between the two treatment groups in any of these measures is observed, the DSMB and NHLBI will be notified immediately to consider a suspension of enrollment into the study. This section details the process for assessing imbalances in these measures.

11.8.2 SERIOUS ADVERSE EVENTS

Three months after the first patient is enrolled in the study, and at the end of each three months thereafter, a quarterly statistical analyses and review of all SAEs will occur pending there have been SAEs reported during these timeframes. The SDMC will:

- Use the current version of the MedDRA dictionary to code all SAEs that have been recorded on study AE case report forms.
- Make a "snapshot" copy of the SAE data, including MedDRA codes.
- Create frequency tables of treatment x occurrence (yes or no, since inception of the study) of all patients. One table will be created for each highest level MedDRA term for which SAEs have been reported. The counting units are patients, not events.
- Compute Fisher's Exact Test (FET) statistic to test the alternative hypothesis that occurrence of SAEs is not independent of treatment group. The FET p-value is not adjusted for multiplicity.
- If the FET p-value is less than the critical value shown in Table 11.3 and the active treatment group has a higher SAE rate, the SDMC will conduct further statistical analyses as indicated by the circumstances and report the results to the Chair of the DSMB subcommittee monitoring this study, the PI, and the NHLBI Project Officer.
- The SDMC will not file a report to the DSMB and the NHLBI Project Officer if the FET p-values is less than the critical value shown in Table 11.3.
- On a semi-annual basis the DSMB and the NHLBI Project Officer will receive a semi-annual report of all the SAEs reported regardless if a quarterly report was administered because a data trigger was found.

11.8.3 ADVERSE EVENTS

Six months after the first subject is enrolled in the study, and at the end of each 6 months thereafter, a semi-annual statistical analyses and review of all AEs will occur. The SDMC will:

- Use the current version of the MedDRA dictionary to code all AEs that have been recorded on study AE forms.
- Make a "snapshot" copy of the AE data, including MedDRA codes.
- Create frequency tables of treatment x occurrence (yes or no, since inception of the study) of all patients. One table will be created for each highest-level MedDRA term for which AEs have been reported. The counting units are patients, not events.
- Compute the FET statistic to test the alternative hypothesis that occurrence of AEs is not independent of treatment group. The FET p-value is not adjusted for multiplicity.
- If the FET p-value is less than the critical value shown in Table 11.3 and the active treatment group has a higher AE rate, the SDMC will conduct further statistical analyses as indicated by the circumstances and alert the DSMB to this finding in the semi-annual DSMB report.
- Collaborate with the Principal Investigator to incorporate the results into the study's semi-annual report to the DSMB and the NHLBI Project Officer.

11.8.4 TRANSFUSION REACTIONS

Six months after the first subject is enrolled in the study, and at the end of each 6 months thereafter, a semi-annual statistical analyses and review of all transfusion reactions will occur, regardless of the incidence rates. The SDMC will create a report that consists of:

- Development of clinically significant alloantibodies
- Development of autoantibodies associated with hemolysis that results in the inability to transfuse safely.
- Development of clinically significant hemolytic transfusion reactions.

11.8.5 CLINICAL LABORATORY RESULTS

Six months after the first subject is enrolled in the study, and at the end of each six months thereafter, a semi-annual statistical analyses and review of all clinical laboratory results will occur. The SDMC will:

- Make a "snapshot" copy of the study's clinical laboratory data.

- Perform an appropriate statistical analysis of clinical laboratory (hemoglobin and ferritin) change-from-baseline data for each clinical laboratory evaluation obtained in this study.

- Collaborate with the PI to incorporate the results into the study's semi-annual report to the DSMB and the NHLBI Project Officer.

11.8.6 PROCEDURES

This section specifies procedures for alerting the DSMB to serious safety issues that arise in this study. The incidences for alerting the DSMB are summarized in Table 11.3.

TABLE 11.3 Summary of Procedures and Timing for Alerting the DSMB and NHLBI Project Officer of Possible Serious Safety Issues

Situation or Event	Summary of Procedure (See text for details.)	Critical Value for DSMB "Alert"
SAEs reported with a SAE Criterion of Death	DSMB will be notified of all SAEs that are reported with a SAE criterion of Death within 15 days of the SDMC SAE Regulatory Specialist becoming aware of the event.	Death
SAEs (all)	1. SDMC performs quarterly analyses of MedDRA-coded SAEs, tabulates patients with SAEs classified by highest level MedDRA term. Report only when $p <$ critical value and active treatment group has higher AE rate. 2. SDMC performs semi-annual analyses of MedDRA-coded SAEs, tabulates patients with SAEs classified by highest level MedDRA term.	$p < 0.01$ p not adjusted for multiplicity
AEs (all)	SDMC performs semi-annual analyses of MedDRA-coded AEs, tabulates patients with AEs classified by highest level MedDRA term. Report every six months. Alert only when FET $p <$ critical value and active treatment group has higher AE rate.	$p < 0.01$ p not adjusted for multiplicity

11.9 REPORTS TO THE DSMB, STUDY INVESTIGATORS, IRBS, AND OTHER AUTHORITIES

11.9.1 SERIOUS ADVERSE EVENTS

The site investigator, the Principal Investigator, and the SDMC SAE Regulatory Specialist will collaborate to prepare a MedWatch 3500A report for any SAE that is fatal. The Principal Investigator will submit the report to (a) the Chair of the DSMB

Subcommittee appointed to monitor this study and to (b) the NHLBI Project Officer within 15 calendar days of initial notification to the SDMC SAE Regulatory Specialist.

If a safety signal is detected, a Dear Investigator Letter will be sent via the SDMC to all site study investigators. Each study investigator will submit the SAE report to the local IRB in accordance with the institution's regulations.

TABLE 11.3 Summary of Procedures and Timing for Alerting the DSMB and NHLBI Project Officer of Possible Serious Safety Issues

Situation or Event	Summary of Procedure (See text for details.)	Critical Value for DSMB "Alert"	Frequency
SAEs reported with a SAE Criterion of Death	DSMB will be notified of all SAEs that are reported with a SAE criterion of Death within 15 days of the SDMC SAE Regulatory Specialist becoming aware of the event.	Death	15 calendar days from SDMC notification
SAEs (all)	SDMC performs quarterly analyses of MedDRA-coded SAEs, tabulates patients with SAEs classified by highest level MedDRA term. Report only when p < critical value and active treatment group has higher AE rate.	$p < 0.01$ p not adjusted for multiplicity	Quarterly if meets critical alert value
SAEs (all)	SDMC performs semi-annual analyses of MedDRA-coded SAEs, tabulates patients with SAEs classified by highest level MedDRA term.	Report all cases	Semi-annual
AEs (all)	SDMC performs semi-annual analyses of MedDRA-coded AEs, tabulates patients with AEs classified by highest level MedDRA term. Report every six months. Alert only when FET p < critical value and active treatment group has higher AE rate.	$p < 0.01$ p not adjusted for multiplicity	Semi-annual if meets critical alert value
Transfusion Reactions	The SDMC will report the following events semi-annually to the DSMB, regardless of incidence rates: • Development of clinically significant alloantibodies • Development of autoantibodies associated with hemolysis that result in inability to transfuse safely. • Development of clinically significant hemolytic transfusion reactions.	Report all cases	Semi-annual
Clinical Laboratory Results	• SDMC performs semi-annual analyses of clinical laboratory (hemoglobin and ferritin) change-from-baseline data for each clinical laboratory evaluation obtained in this study.	Report all cases	Semi-annual

12 DATA COLLECTION AND DATA MONITORING

12.1 CRF AND SOURCE DOCUMENTATION

The site study coordinator will complete a case report form (CRF) for each subject. A CRF manual will be provided to each site to assist in correct CRF completion. A paper copy of this CRF will be provided as a worksheet. All data will be recorded into electronic CRFs. The study coordinator or data coordinator will use an internet browser to key data directly into Rho's internet-based remote data entry system. Univariate data validation tests are performed as the data are keyed and most implausible data values are resolved immediately. Data are not stored on the site's computer. At the end of each "page," data are submitted to Rho's secure web server and stored in the study's database. The database is backed up nightly; backup tapes are saved in a secure, off-site location. At any time site personnel can log into the system, review and correct previously entered data, key additional data, or lock records to prevent further inadvertent medications. The pages will be accessible via the CSCC website and require Center-specific user ID/password privileges.

CRF data must be currently maintained and up-to-date. Study participants must not be identified by name on any study documents. Patients will be assigned and identified by CSCC patient numbers assigned at the time of initial enrollment into a CSCC study.

12.2 DATA MANAGEMENT

The SDMC will provide statistical and data management support for the study. Validation rules will be applied at several points in the data management process. An error correction procedure will be applied to correct data values that fail validation rules.

12.3 TRAINING AND DATA MONITORING

Prior to the onset of screening, clinical staff (coordinators and psychologists) will be centrally trained to ensure adherence to the protocol and assure highest possible data quality. Training will be led by a combination of investigators and other staff from the clinical centers and the SDMC. Training presentations will address informed consent procedures, study operations and protocol requirements, data collection procedures, maintenance of source documentation, CRF completion and review, routine reporting requirements, electronic data capture, and CSCC and NHLBI policies and procedures. Re-training of existing staff or training of new project staff will occur at annual intervals in conjunction with annual meetings. When central training sessions cannot be arranged, study-specific training will occur over the telephone, either during teleconferences or during individual conversations. As needed and as time allows, face-to-face training will be provided by SDMC staff as part of periodic site visits.

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After central training, individual sites will monitor CRFs and source documents for accuracy, protocol compliance, subject safety, and adherence to guidelines outlined in the CSCC Manual of Procedures.

As referenced above, centers will be site-visited. Presently, plans for clinical data monitoring include, at a minimum, one site visit for each site during the cross-sectional and again for the pilot transfusion study phase once the study is underway. At each site visit, recruitment guidelines and study eligibility criteria will be reviewed. Additionally, completed data forms will be reviewed and compared to source documentation (medical or center records) to confirm accuracy. A detailed description of the activities and procedures for monitoring this study are described in the Neuropsychological Dysfunction and Neuroimaging Abnormalities in Neurologically Intact Adult Patients With Sickle Cell Disease (SCD) Clinical Monitoring Plan.

Apart from the training for clinical coordinators and psychologists, a procedure for site qualification for MRI completion will be implemented by scientists at the central reading center. The steps in qualification will consist of completion of a questionnaire about the local scanner, scanning a phantom and sending the reading to the central facility, and scanning and re-scanning a volunteer for test/retest reliability assessment.

The DSMB will receive reports concerning recruitment and other issues every six months. A month prior to the scheduled meeting time, the SDMC will synthesize all available data and generate a report of all relevant information. This report will be available to the DSMB at least two weeks prior to the scheduled meeting time. In addition to recruitment and participation data, this report will include all AEs (Section 12). Upon review of the study data, the DSMB will make recommendations to the PI. In addition to routine reports, the transfusion study will be stopped pending consultation with the DSMB in the event of the occurrences outlined in Section 12.

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13 STATISTICAL ANALYSIS

13.1 SAMPLE SIZE

The primary comparison is a difference in mean scores on the WAIS-III PIQ between adult SCD patients and community-based controls, after adjustment for age and years of education. Our preliminary data in children showed that even patients with normal MRI studies had a mean WAIS-III PIQ score of 87 (with a standard deviation of 20), close to one standard deviation below the normative mean of 100. The patients with silent infarctions on MRIs had a mean score of 75 (with a standard deviation of 11). We therefore would predict that our population of adults, some of whom will have abnormal MRI studies, will have a mean score of 85 or less and a standard deviation of 15. Although there are limited data, we would expect that the community control group of similar age and education level will have a mean of 100 with a standard deviation of 15. The discussion and tables below illustrate sample sizes (and associated power calculations) needed in order to detect varying magnitudes of differences in the mean score of our patients from the control group, with two different assumptions about the standard deviations. The calculations presented are based on the assumption that the ratio of patients to controls will be roughly 3:1.

For power calculations and statistical analysis, the primary hypothesis, adult SCD patients will have a mean WAIS III PIQ different from the mean of the community based control group adjusted for site, sex, age and years of education can be expressed:

Ho: $\mu_{control} - \mu_{SCD} = 0$

Ha: $\mu_{control} - \mu_{SCD} \neq 0$.

Although we will use a GLM to test the primary hypothesis, we used a 2-sample comparison t-test framework for power calculations. If the matching ensures homogeneity between the SCD patients and the control group except for SCD status, then for any choice of power the necessary sample size for the GLM will be smaller than what is predicted in this sample size analysis. To estimate the sample size needed per group to detect a 15 percentage point difference in two unequally sized groups using a 2-sided t-test (see Table 13.1), we used the following approximate sample size calculation algorithm:

1) Calculate m: $m = \dfrac{2\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2 \sigma^2}{\left|\mu_1 - \mu_2\right|^2}$.

2) Let n_1 = sample size for group 1, and n_2 = sample size for group 2.

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3) Calculate $n_1 = m(k+1)/2k$ and $n_2 = kn_1$.[1]

TABLE 13.1 Sample Size Calculations for a 2-Sided t-test with an SD of 15 and $n_2 = 0.3n_1$

Difference in mean WAIS-III Performance IQ between adults with SCD and the control group.	Sample sizes required in each group (2-sided) Cases, Controls*	Power
8	120, 36	0.80
8	137, 42	0.85
8	161, 49	0.90
8	198, 60	0.95
10	77, 24	0.80
10	88, 27	0.85
10	103, 31	0.90
10	127, 39	0.95
15	35, 11	0.80
15	39, 12	0.85
15	46, 14	0.90
15	57, 18	0.95

** Sample Size Estimates Assuming 3 Controls for Every 10 Cases (these do not take data loss into account).*

[1] Lloyd D. Fisher, Gerald van Belle, *Biostatistics A Methodology For The Health Sciences*, Wiley Science Paperback Series, (New York: John Wiley & Sons, Inc., 1993), 190-191.

TABLE 13.2 Sample Size Calculations for a 2-Sided t-test with an SD of 20 and $n_2 = 0.3n_1$

Difference in mean WAIS-III Performance IQ between adults with SCD and the control group.	Sample sizes required in each group (2-sided) Cases, Controls*	Power
8	213, 64	0.80
8	244, 74	0.85
8	285, 86	0.90
8	352, 106	0.95
10	137, 42	0.80
10	156, 47	0.85
10	183, 55	0.90
10	226, 68	0.95
15	61, 19	0.80
15	70, 21	0.85
15	81, 25	0.90
15	101, 31	0.95

** Sample Size Estimates Assuming 3 Controls for Every 10 Cases. (These do not take data loss into account).*

Observations:

1. If the standard deviation of the WAIS-III is no greater than 15 and the true difference in WAIS III group means is at least eight, then using 120 SCD patients and 36 controls results in at least 80% power for the primary hypothesis.

2. If the standard deviation of the WAIS-III is no greater than 20 and the true difference in WAIS III group means is at least 15, then using 101 SCD patients and 31 controls results in at least 95% power for the primary hypothesis (see Table 13.2).

In order to have sufficient power for the primary hypothesis and sufficient patients recruited for the 4th secondary hypothesis (transfusion study) we would need to enroll 150 patients and 45 controls. Assuming an 80% completion rate, 120 patients and 36 controls would complete the baseline evaluation. (Please refer to observations 1 and 2 above for power estimates.) Assuming a normal distribution, 60 of the 120 patients will have WAIS-III PIQ scores at or below the predicted mean of 85. These patients would be approached to participate in the transfusion study. Assuming 60% would agree to participate and again assuming a completion rate of 80%, 36 patients would be randomized to either the chronic transfusion arm or the standard care arm and 30 would complete the protocol (allowing for 15 in each group).

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For power calculations and statistical analysis the 4th secondary hypothesis, the transfusion arm will have a mean WAIS-III PIQ change score that is different than the mean change score in the control arm, can be expressed:

Ho: $\mu_{diff,trt} - \mu_{diff,control} = 0$
Ha: $\mu_{diff,trt} - \mu_{diff,control} \neq 0$.

We will use a 2-sample comparison t-test framework for power calculations. To estimate the power of the t-test given 15 subjects per arm, we used the following normal approximation:

1. Choose a mean change score difference under the alternative, $H_{a,diff}$.

2. Calculate the expected variance of the within subject change score:
$$V(d_i) = V(WAIS_{i,After}) + V(WAIS_{i,Before})$$
$$- 2 \cdot \rho_{WAISAfter,Before} \sqrt{V(WAIS_{i,After})} \cdot \sqrt{V(WAIS_{i,Before})}$$
where $V(WAIS_{i,x})$ is the WAIS variance for the i[th] subject at time x.

3. Calculate the expected variance of difference in mean change scores:
$$V(\bar{d}_{trt} - \bar{d}_{control}) = V(d_i)\left(\frac{1}{15} + \frac{1}{15}\right)$$

4. Using an alpha of 0.05 calculate the critical value needed to reject the hypothesis under the null: $0 + 1.96 \cdot \sqrt{V(\bar{d}_{trt} - \bar{d}_{control})} = a_{crit}$

5. Calculate power:
$$\Pr\left[\left|\bar{d}_{trt} - \bar{d}_{control}\right| > a_{crit} \mid E(\bar{d}_{trt} - \bar{d}_{control}) = H_{a,diff}\right] =$$
$$1 - \Phi\left[\frac{-a_{crit} - H_{a,diff}}{\sqrt{V(\bar{d}_{trt} - \bar{d}_{control})}} \leq Z \leq \frac{a_{crit} - H_{a,diff}}{\sqrt{V(\bar{d}_{trt} - \bar{d}_{control})}}\right]$$

where Φ is the cumulative distribution function for the normal distribution.

Observations:

1. If the standard deviation of baseline WAIS-III PIQ and six month WAIS-III PIQ is 15 in both arms and the correlation between repeated WAIS-III PIQ measurements is 0.70 in both arms, then the power to detect a five-point difference is 22%.

2. If the standard deviation of baseline WAIS-III PIQ and six-month WAIS-III PIQ is 15 in both arms but the correlation between repeated WAIS-III PIQ

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measurements is 0.80 in both arms, then the power to detect a 10-point difference is 82%.

13.2 STATISTICAL METHODS

The primary clinical hypothesis is that the mean score for adult patients with SCD on the WAIS-III PIQ scale will be at least one standard deviation below the mean score of a group of community controls matched for site, age, and education. We expect the adult patients with SCD will have a mean WAIS-III PIQ of 85 or less and the community control group will have a mean of 100. Although the primary hypothesis expresses the expected direction of effect, the statistical analysis will follow common practice and use a 2-sided hypothesis test with an alpha of 0.05.

To test the primary hypothesis, we will use a GLM to model the data. At each site participating in this study we will match the patients using a ratio of 3 patients to 1 community controls, but if necessary we will still statistically control for site, age, sex, and education. Model selection techniques will be used to determine the most parsimonious models. For statistical analysis we express the primary hypothesis, adult SCD patients will have an adjusted mean WAIS-III PIQ different from the community based control group adjusted mean WAIS-III PIQ (based on the final model):

Ho: $Adj\_\mu_{SCD} - Adj\_\mu_{control} = 0$
Ha: $Adj\_\mu_{SCD} - Adj\_\mu_{control} \neq 0$.

The first secondary hypothesis is the same as the primary hypothesis with other NP tests being used as the response; therefore, the analysis for these hypotheses will be the same as the primary hypothesis.

The 2nd secondary hypothesis is that patients with SCD and abnormal MRIs will have a higher proportion of abnormal NP test results than the patients with SCD and normal MRIs. The statistical analysis of this hypothesis will follow common practice and use a 2-sided hypothesis test with an alpha of 0.05. For statistical analysis we express this secondary hypothesis as:

> Cortical gray matter volume, hippocampal volume, white matter lesions (WML), cortical strokes, and lacunar infarcts will correlate with cognitive function (memory and executive function).

Ho: $\rho_{MRI\text{-}NPtest} = 0$
Ha: $\rho_{MRI\text{-}NPtest} \neq 0$.

For this analysis, we will use Spearman correlations, Pearson correlations, and/or multiple regression.
The 3rd secondary hypothesis involves comparing the ability of regular MRI and VMRI to predict neurocognitive injury. The 3rd secondary hypothesis is:

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> Cortical gray matter and hippocampal volume will demonstrate a more significant correlation with executive and memory cognitive functions than the correlation of WML or lacunar infarcts with these same cognitive functions.

The statistical analysis will not involve a formal hypothesis test, but in statistical terms the hypothesis can be written as:

Ho: $\rho_{MRI\text{-}Nptest\text{-}Vol} - \rho_{MRI\text{-}Nptest\text{-}Reg} = 0$
Ha: $\rho_{MRI\text{-}Nptest\text{-}Vol} - \rho_{MRI\text{-}Nptest\text{-}Reg} \neq 0$.

For this analysis, we will descriptively compare Spearman or Pearson correlations.

The last secondary hypothesis involves randomizing 36 people who had a baseline WAIS-III PIQ score ≤ 90 to two treatment groups. We will treat one group with transfusion for 6 months and the other group with standard care for six months. After the six months of treatment we will perform a before and after comparison of the WAIS-III PIQ results, using within subject WAIS-III PIQ change scores as the outcome. We expect that on average patients who receive transfusion will show at least a five-point improvement when retested after six months of transfusion. Although this secondary hypothesis expresses the expected direction of effect independently of the observed affect in the control group, the statistical analysis will follow common practice and first use a 2-sided hypothesis test with an alpha of 0.05 to compare the mean within subject WAIS-III PIQ change score in the two groups. Second, we will calculate a 95% CI for the true effect of transfusion on WAIS-III PIQ within subject change scores for the group who received transfusion. We will use either a t-test, a Wilcoxon Two-Sample test, or a regression model to test the hypothesis. For statistical analysis we can express this secondary hypothesis as the mean within subject change score in WAIS III PIQ for the transfusion group will be different than the mean within subject change score in the control group:

Ho: $\mu_{diff,trt} - \mu_{diff,control} = 0$.
Ha: $\mu_{diff,trt} - \mu_{diff,control} \neq 0$.

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14 HUMAN SUBJECTS PROTECTION

14.1 PURPOSE OF THE STUDY

The purpose of this research study is to determine the extent of memory and attention problems in adults with SCD. Adults who have not experienced a stroke or other brain damage will be asked to join the study. The study will also evaluate the link between these events or conditions and abnormal MRI**.** These findings will be compared to the results of healthy people with similar backgrounds.
For some participants, testing will show evidence of problems with memory and attention, potentially caused by the anemia that occurs among most patients with sickle cell disease. Correcting anemia has been linked in some non-sickle cell patients with better memory and attention.

A second part of the study will involve treating anemia for a six-month period in a subset of subjects who have completed the neuropsychological testing and MRI, in order to assess the effect of transfusion on memory and attention. This second study phase is a randomized clinical trial in which patients in the treatment arm of the study will receive transfusions over a period of six months as a potential treatment to improve memory. Patients in the non-intervention group will receive no transfusion therapy and will receive their standard treatment.

14.2 STUDY PROCEDURES

Hb SS patients and healthy adults are being asked to take part in this study. Patients will have NP testing, behavioral studies, and an MRI test. The schedule of events associated with the study is summarized below:

Visit 1:
Clinical site staff will review the informed consent process with the potential subject, will answer all questions, and will ensure that the consent is signed and dated by the subject. Following informed consent, clinical staff will conduct a brief screening (the PSS) and the potential study subject will be required to complete two short questionnaires (the POMS and MMSE). Medical records for potential participants with SCD will be reviewed. A history, physical exam, and neurological exam will be completed. For females of childbearing potential, a urine sample will be collected to ensure that the subject is not pregnant. Two teaspoons of blood will be drawn from all potential subjects; for adults with SCD, the blood will be used to confirm the diagnosis, and for the control group, the blood will be drawn to ensure the potential participant is not anemic. The blood drawn will also be used for a hematology and chemistry panel. If the blood draw confirms that the subject (case or control) is eligible for the study, then Visit 2 will be scheduled to conduct the NP testing.

Visit 2 – Neuropsychological Testing or MRI:

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The sequencing of the NP test battery and the MRI is flexible. Therefore, at Visit 2, either the NP test battery or the MRI can be performed. A trained and supervised professional will conduct the NP testing, which will require 6 to 6½ hours to complete. The individual administering the test will score and submit copies of the tests and the results to a study co-PI for validation. MRI testing will be done at the local hospital. The radiologist or imaging tech will document that completion of the MRI has occurred. Following testing, an appointment for Visit 3 will be scheduled.

Visit 3:

At Visit 3, the component of the study that was not completed at Visit 2 will be completed, i.e., either the NP Battery or the MRI.

Visit 4:

Upon completion of Phase I of the study, all subjects, including those with SCD and those recruited as controls, will be debriefed about the results of their evaluation. This debriefing should include a face-to-face discussion about the results of the evaluations.

Phase II visits:

A approximately 36 patients with sickle cell disease will be asked to participate in an intervention study (Phase II) in which subjects will be randomized to either a transfusion arm or a standard care arm. After informed consent and eligibility screening is complete, the SF-36 will be administered to all patients, followed by randomization to either the treatment or control groups. For females of childbearing potential, a urine sample will be collected to ensure that the patient is not pregnant.

If more than 6 months but less than 1 year has passed since the subject's participation in Phase I of the study, the subject must FIRST repeat the WAIS-III PIQ portion of the Neuropsych Battery at this visit to confirm that the subject still has a score of ≤ 90. If the score is still consistent with eligibility requirements, the study coordinator will complete the screening blood draw on the subject, administer the SF-36, and will schedule a repeat MRI so that the image can be compared to the MRI conducted during Phase I. The site will locally confirm that the repeat MRI does not contain any new neurological findings when compared to the Phase I MRI.

If > 1 year has passed since the subject's participation in Phase I (or if the subject was not enrolled in Phase I), the subject must complete the MMSE and the POMS, as well as the WAIS-III PIQ portion of the Neuropsych Battery to confirm eligibility. If eligible, the study coordinator will complete the screening blood draw and schedule an MRI to be completed at Visit 2. (For subjects enrolled in Phase I, this is a repeat MRI that should be compared to the Phase I MRI to confirm there are no new neurological findings. If eligible to continue, the subject will return for Visit 3, during which the SF-36 and the remainder of the Neuropsych Battery is administered and the subject is randomized.

At Visit 2, the subject will complete the MRI. The MRI will be read locally to confirm eligibility requirements. If the subject does not meet the criteria, he/she will be discontinued from the study without completing any additional assessments.

At Visit 3, the subject completes the remaining NP Battery tests (other than the PIQ), the SF-36, and is then randomized.

Individuals randomized to the treatment arm will receive simple transfusions and additional blood testing every 3 to 4 weeks. A blood sample will be sent to the study laboratory before each transfusion. Individuals randomized to the treatment arm will receive the same general care for SCD as those who are not randomized to treatment.

Individuals randomized to the standard care armwill have monthly contact with clinic staff to complete an interval history form, and to have blood drawn (at Months 3 and 6) for a Hematology and Chemistry panel and to measure ferritin levels. All patients will be taught the signs and symptoms of stroke and asked to report any such signs.

After 6 months of treatment, both groups will complete another NP Battery and SF-36. A new MRI must be completed within 2 weeks after the NP Battery.

14.3 EXIT DEBRIEFING

After the tests are completed, study participants will be encouraged to return to the clinic for an in-person debriefing. During the debriefing, study participants will receive a summary of their test results and will have a chance to review the results with a clinician and ask any questions they may have. It is possible that the tests used in this study (e.g., the screening questionnaires, the NP battery, or the MRI) could reflect changes in functioning. If any problems are present, participants will receive specific recommendations concerning follow-up care. In addition, this information and the recommendations will be sent to participants' primary care physicians upon request. Sample templates for uniform debriefings are available to site study staff through the SDMC.

If the study participant does not return to the clinic for an in-person debriefing, results of the testing should be mailed to him/her, along with a telephone number to contact study staff for additional follow-up.

14.4 RISKS OR DISCOMFORTS

Laboratory tests: Having blood drawn may cause brief pain from the needle stick and bruising.

Questionnaires and Neuropsychological Testing: There are no known risks for filling out the questionnaires or for participating in the neuropsychological testing. However, some

participants may find the process tiring or stressful. If a participant is concerned about a specific question, that item can be skipped. If a specific test cannot be completed, he or she will be allowed to complete the remainder of the NP battery. Failure to complete all components of the NP battery will not affect the medical care received.

MRI: There are no known risks associated with MRI studies. Radio frequency power levels and gradient switching times used in these studies are approved by the FDA. The levels of energy used to do the MRI are far less than those used in a single X-ray. Although MRI scanning is painless, there can be some discomfort. In particular, the participant may be bothered by the loud noise during the study. This noise is due to beeping and hammering sounds made when the scanner is taking measurements. Earplugs will be provided to reduce the noise. Also, some people become claustrophobic while inside the magnet. There may also be sensations on the skin, such as a gentle tap or a feeling of mild electric shock. If the participant does not feel well for any reason in the scanner, the test will be stopped. Because the MRI scanner attracts some metals, it could move metal objects within the MRI room during a test. This could possibly harm the participant, but safety measures have been taken to keep this from happening.

Transfusions: Most adult sickle cell patients have received transfusions in the past. The risks of this transfusion are the same as the risks of past ones. These risks are known to include allergies, fever, volume overload, and transfusion reaction. They may also include making antibodies that would make future transfusions difficult, infection, and iron overload. Several things will be done in order to make the red cells as safe as possible. All patients will get red cells that have had white cell contaminants taken out. Also, the red cells will be screened for several infections. They will undergo extensive cross-matching as well to decrease transfusion reactions. While the blood is safe, there is still a small risk of a serious complication.

14.5 BENEFITS

There may be no direct benefits from participation in this study. The study may improve what is known about attention and memory in adults with sickle cell disease. This could permit better understanding and treatment of sickle cell patients. It is possible that formerly unknown abnormalities may be found. This information may be useful to the participants with sickle cell for planning their medical care.

14.6 ALTERNATIVE TREATMENT

Alternatives include refusal to take part in this study. Whether or not the potentially eligible participant chooses to take part in this study, he or she will continue to receive the best medical care possible.

14.7 CONFIDENTIALITY

Medical and study records will be kept private to the extent allowed by law. Staff from government groups may need to look at the medical charts to make sure the study data are correct. Names will not appear in any research report or publication.

14.8 FEES FOR PARTICIPATION

There will be no charge for taking part in this study.

14.9 TREATMENT AND COMPENSATION FOR INJURY

If a participant is hurt from taking part in this study, he/she will be instructed to contact the Director of the Sickle Cell Center immediately. The Center Director will determine whether the injury is one caused by study activities. The sponsor of the study does not have to pay for the medical treatment of other injuries or illnesses. They also do not have to pay any other type of compensation. Medical care will be available at the hospital. However, the treatment will not be provided free of charge and the participant will not be compensated.

New information that might change a participant's mind about being in the study may become known during the study; or the participant's physician may feel it is best for him or her to be removed from the study. If this happens, the doctor in charge will explain this.

Each participant will be provided with a telephone number to contact the physician in charge of the study. Also, they will receive information about how to contact a physician who is not part of the study should they prefer not to discuss their participation with the doctor in charge of the study.

14.10 VOLUNTARY PARTICIPATION IN RESEARCH

It will be explained that participation in the study is voluntary and nothing will be lost if the decision is made not to take part in the study. They may also decide to quit the study at any time. The participant will be removed from the study for medical reasons or if he or she fails to follow instructions during the study. The sponsor (NIH/NHLBI) may stop the study before the participant has completed it.

If the subject leaves the study early for any reason, no benefits will be lost and his/her future care will not be affected.

The participant will be given a copy of the consent form.

14.11 DISCLOSURE OF DATA

The investigator, the investigator's staff and associates, and the appropriate regulatory agencies may use the information included in this protocol as necessary for the conduct of the trial and the safety of patients. Data from the trial are confidential and may not be disclosed without the written permission of NHLBI.

14.12 PUBLICATION OF RESEARCH FINDINGS

Manuscripts and abstracts prepared from the data collected during this trial will be prepared by the study investigators and the SDMC.

15 PARTICIPANT COMPENSATION

Participants will be reimbursed at the completion of all listed procedures. Upon the completion of tasks in Phase I, the subject will receive a total of $240 for the (approximately) eight hours of study participation. Subjects will be reimbursed for each of the following procedures; psychosocial questionnaire, $40.00; completion of the neuropsychological testing, $100.00; and the MRI, $100.00. If the subject discontinues participation at any time during the protocol, he/she will receive reimbursement for the part(s) he/she completed.

If the patient participates in the transfusion study, an additional $100.00 will be provided to cases and controls for completion of a second round of neuropsychological testing, and $100.00 for completion of a second MRI. Patients randomized to receive transfusions will receive $80.00 per visit for transfusion. The amount of $20 will be provided at each visit to cover the costs of transportation or parking, for both cases and controls. If the patient discontinues participation at any time during the protocol, he/she will receive reimbursement for the part(s) he/she completed

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