**Aim D: Characterization of risk of HIV and HIV outcomes in the Brazilian Sickle Cell Disease (SCD) population and comparison of SCD outcomes between HIV sero-positive and negative SCD patients**

## 1. Background

There is limited literature focused on HIV in sickle cell disease (SCD), although published reports show HIV is relatively rare in this population.  Most studies over the past few decades have consistently shown a lower prevalence of HIV in SCD compared to a non-SCD population[1-5], though this relationship was not seen in 1 study[6] (studies summarized in Table 1).  Other studies investigating HIV in SCD have reported a prevalence of 0-11%, but without a comparison population [3, 7-15].

Table 1: Studies Reporting HIV Positive Prevalence in SCD

| Publication Year/Author | HIV+ SCD Population | HIV+ non-SCD Population |
|---|---|---|
| 1989 Waweru [1] | 0 of 198 transfused SCD children | 3 of 54 (7.4%) non-SCD children transfused at same hospital/time period |
| 1990 Castro[4] | 0 of 88 SCD adults with at least 1 transfusion | None in paper Discussion cites 70-80% HIV+ of transfused hemophilia patients in same era as comparison |
| 1992 Waweru [2] | 1 of 44 (2.3%) transfused SCD children | 3 of 4 (75%) non-SCD children transfused at same hospital/time period |
| 2010 Batina Agasa [3] | 1 of 140 (0.7%) transfused SCD children/adults | 184 of 3390 (5.4%) blood donors in same community |
| 2012 Nouraie [5] | Discharge diagnosis of both HIV and SCD  51 of 3370 (1.5%) 1997 - 2003  43 of 3147 (1.4%) 2004 - 2009 | Discharge diagnosis HIV Without SCD  7726 of 222449 (3.3%)  p<0.0001 1997 - 2003  6030 of 194465 (3.1%)  p<0.0001 2004 - 2009 |
| 1988 Ouattara[6] | 15 of 67 (22.4%) transfused SCD children | 49 of 500 (9.8%) blood donors 2 of 320 (0.62%) transfused non-SCD children |

In addition to the reported lower prevalence of HIV in SCD, two studies have described HIV outcomes in this population.  Godeau et al identified 8 HIV+ of 283 (2.8%) screened adults with SCD and reported 0 of 8 had progressed to AIDS, even without antiretroviral therapy (ART), with a mean follow up of 4.6 years. [16].  Bagasra et al compared 18 HIV+ SCD patients (all HIV+ patients identified at 5 US SCD Centers) to 36 HIV+ non-SCD controls matched for age, race and gender.  They reported 8 of 18 (44%) SCD cases were long-term non-progressors (LTNP= asymptomatic with low viral load and CD4>500/mm without ART for at least 10 years) compared to 5 of 36 (13.9%) LTNP in controls with an average follow up of 10 years (p=0.0193).  Death due to AIDS occurred in 5 of 18 (23%) HIV+ SCD patients vs. 22 of 36 (61%) of HIV+ controls[17].  There is also a case report of spontaneous resolution of HIV associated nephropathy in a SCD patient not treated for HIV [18].  HIV associated nephropathy is typically a progressive disease with poor prognosis and spontaneous resolution in the

absence of ART has not been otherwise reported.

Speculated mechanisms for lower HIV prevalence and/or progression in SCD has included an inhibition of HIV replication due to the immunologic changes and pro-inflammatory component of SCD pathophysiology as well as changes in iron metabolism[5]. These hypothesized mechanisms have not been tested to our knowledge. Chies et al reported 5.1% of 79 SCD patients demonstrated the CCR5Δ32 allele that confers resistance against HIV1 compared to 1.3% of 112 race-matched healthy controls[19]. Other studies to confirm this or other genetic markers of HIV resistance in SCD have not been performed.

Limitations of studies demonstrating lower prevalence of HIV in SCD include small numbers of study subjects, insufficient matching of control population and no measurement of HIV risk factors in SCD populations. Outside the possibility of increased HIV risk due to transfusion, which is now exceedingly rare, risk of HIV in SCD may be modulated by delayed sexual maturation in these patients because of older age of sexual debut and perhaps lower risk behaviors. A 1984 survey administered to 52 females with SCD and 80 controls demonstrated 39% of SCD subjects were sexually active compared to 81% of controls, and the mean age at first sexual encounter was 17.7 years in the SCD group vs. 17.0 years in the control population[20]. Updated and more comprehensive assessments of HIV risk in this population have not been performed.

Although the summarized literature indicates that SCD may ameliorate HIV infection and/or progression, a recent review suggested that HIV may worsen SCD[21]. Both HIV and SCD are independent risk factors for certain diseases such as stroke, avascular necrosis, pulmonary hypertension and infections. It is possible that HIV and SCD may interact to increase the odds of these complications in SCD patients. A review of the Nationwide Inpatient Databases of the Healthcare Cost and Utilization Project in the US demonstrated that hospitalized children with SCD and HIV had a higher odds of bacterial infection and sepsis than those with SCD alone[22]. Further research is needed to elucidate the interaction between the two disease states that might impact SCD clinical outcomes.

The primary objective of this study is to compare HIV risk behaviors between SCD patients (cases) and age matched non-SCD controls. The secondary objectives are to describe HIV outcomes in SCD and compare SCD outcomes between HIV positive and negative SCD patients.

## 2. Objectives

Primary objective: Compare HIV risk behaviors between SCD patients and non-SCD controls

Secondary objectives:
2a: Describe HIV outcomes in HIV seropositive SCD patients
2b: Compare SCD outcomes between HIV+ and HIV- SCD patients

## 3. General Approach

This project is part of the Recipient Epidemiology and Donor Evaluation Study (REDS)-III research program. REDS-III is a U.S. National Heart, Lung, and Blood Institute funded multicenter research program that is conducted in the United States, Brazil, China, and South Africa. The objectives of REDS-III are to ensure safe and effective blood banking and transfusion medicine practices through a comprehensive strategy involving basic, translational and clinical research. One ongoing research project within the REDS-III Brazil program is the development of a cohort study of SCD patients. The goals of the cohort study are to

characterize SCD outcomes, blood utilization, and genetic determinants of alloimmunization in the Brazilian SCD population and perform targeted studies focused on transfusion and HIV outcomes within the cohort.   All necessary Brazilian and United States approvals were obtained in 2013 and enrollment into the cohort began in November of that year. The REDS-III infrastructure will be utilized to identify eligible patient populations for the proposed research.

For the primary objective, which is to compare HIV risk behaviors in SCD and non-SCD subjects, a case control study of 150 SCD patients and 150 controls will be performed. Selection of cases and controls will be stratified on Hemocenter and age, with age divided into 5-year intervals.  The goal is to have the number of controls in a stratum match the number of cases.  Cases will be selected from the REDS-III Brazilian SCD Cohort. Approximately 1200 SCD patients ≥18 years will be enrolled in the cohort by March 2015. Cohort patients were randomly selected from the REDS-III Hemocenters' population; therefore, we expect the cohort population to be representative of the overall SCD population at each Hemocenter.  Controls will be recruited from the SCD patient advocacy associations at each Hemocenter.  These associations host friends and family of SCD patients during appointments and coordinate outreach efforts in SCD patients' communities.  Therefore, we anticipate that the controls recruited though the advocacy associations will be from a culturally and socioeconomically similar population as cases. A questionnaire will be administered to cases and controls to measure HIV risk behaviors using a self-administered audio computer-assisted self-interview.

For the secondary objective, all SCD patients diagnosed with HIV at participating Hemocenters in the previous 10 years will be identified and recruited to perform a case series description of HIV outcomes in SCD patients. To compare SCD outcomes between HIV+ and HIV- SCD patients, we will randomly select age, gender, Hemocenter and SCD genotype matched HIV-SCD controls from the REDS-III cohort for each identified HIV+ SCD patient.  Approval will also be sought to perform a medical record review on all HIV+ SCD patients lost to follow up or deceased at time of study start to limit survival bias in comparison of SCD outcomes between HIV+ and HIV- SCD patients.  See figures 1-3 for overviews of study populations, procedures and outcomes for objectives 1 and 2.
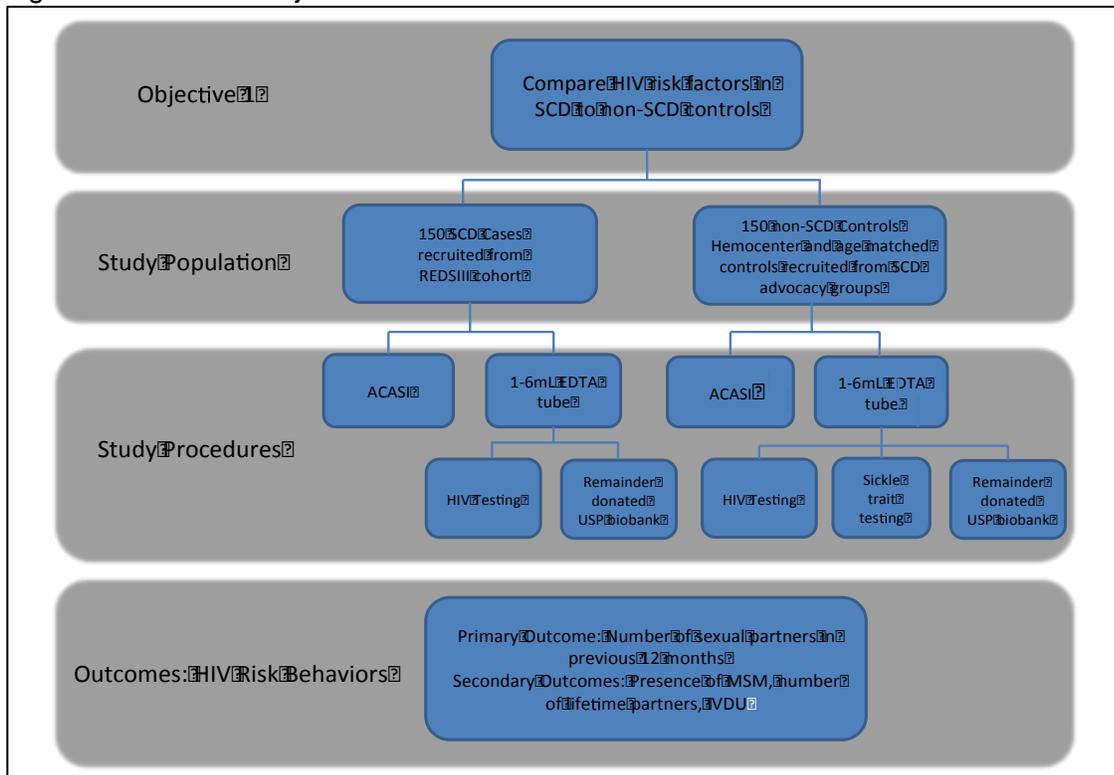
Figure 1: Overview Objective 1
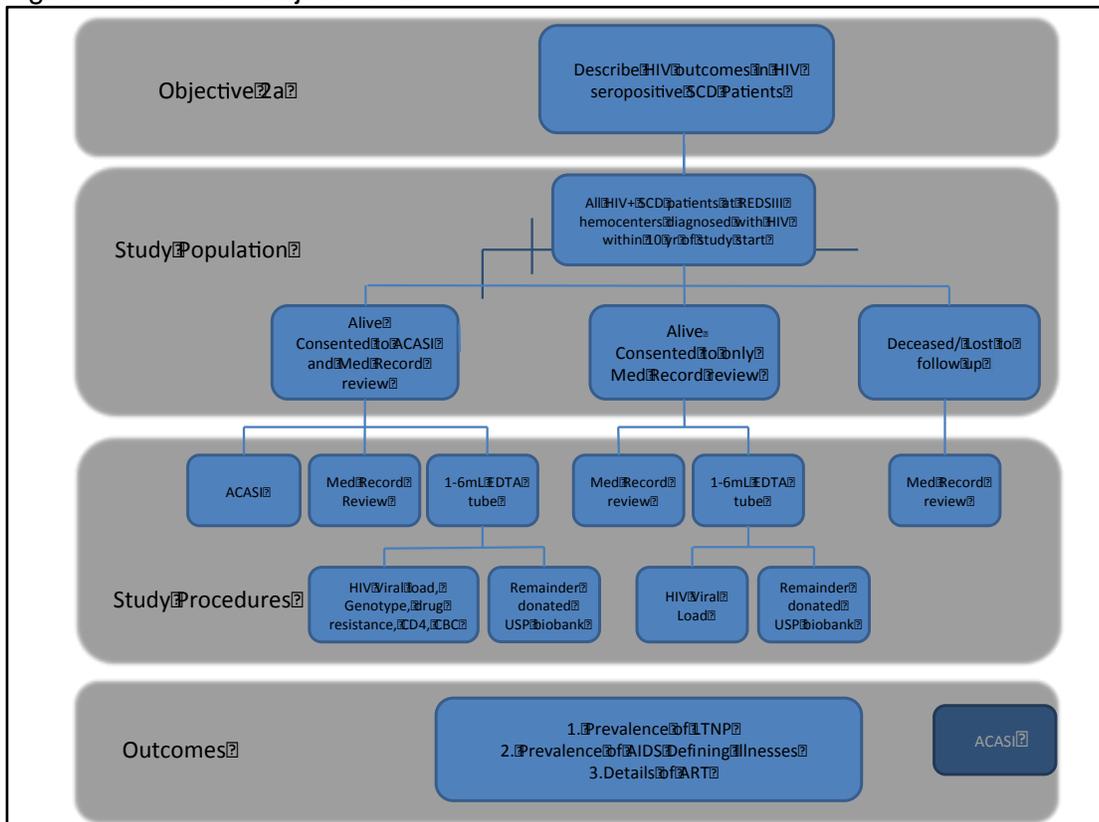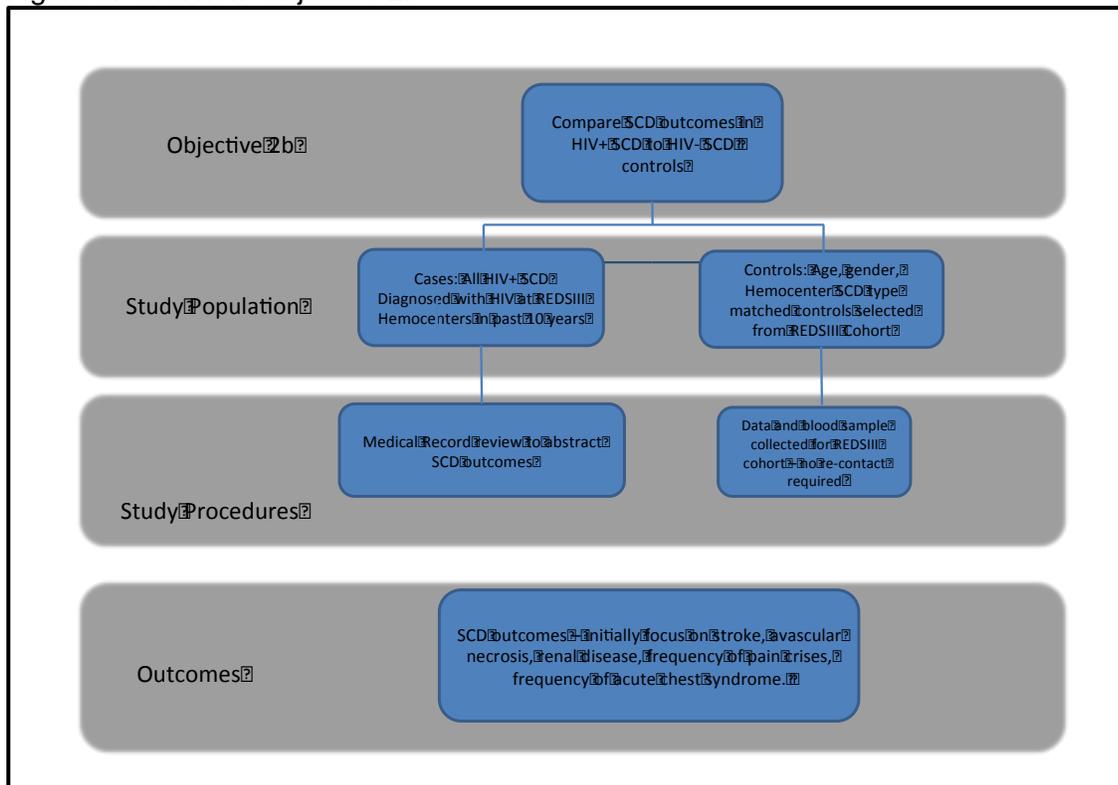


Figure 2: Overview Objective 2a

Figure 3: Overview Objective 2b



## 4. Study Populations

*4.1 Objective 1: SCD cases*
Inclusion Criteria
- SCD (any genotype) patients enrolled into the REDS-III Brazilian SCD Cohort
- Age ≥18 years

Exclusion Criteria
- None

*4.2 Objective 1: Non SCD-Controls*
Inclusion Criteria
- Age≥ 18 years with the number of controls matching the number of cases in each 5-year age stratum
- Matched by Hemocenter

Selection of cases and controls will not be stratified on gender. However, the gender distribution between the two groups will be monitored weekly and if significant imbalance is detected and persists when 50% enrollment has been achieved, recruitment will be adjusted to bring the numbers into approximate balance.

Exclusion Criteria
- Sickle cell disease by self report (controls will also have HPLC testing to confirm absence of sickle cell disease and presence/absence of sickle cell trait)

*4.3  Objective 2: HIV positive SCD patients*
Inclusion Criteria

- Sickle cell disease (any genotype)
- Treated by REDS-III Hemocenter for at least 1 year in the past 10 years
- Diagnosed with HIV within 10 years of study start.  Diagnosis of HIV must be confirmed by positive results on two separate HIV testing platforms including any two of the below:
    o HIV nucleic acid test (DNA or RNA)
    o HIV1 p24 antigen
    o HIV nucleotide sequence
    o HIV antibody testing
        ▪ Enzyme immunoassay
        ▪ Western Blot
    o Combination HIV antigen/antibody test
  Exclusion Criteria
    - None

### 4.4 Objective 2b: HIV Seronegative SCD Controls
Inclusion Criteria
- Matched to an HIV+ case on the following criteria
    o Age $\pm$ 2 years
    o Hemocenter
    o Gender
    o SCD genotype

Exclusion Criteria
- Clinical history of HIV in REDS-III database
- HIV positive testing in REDS-III database

## 5. Study Enrollment and Specimen Procurement

### 5.1 Screening/Recruitment
5.1.1 Objective 1: SCD cases
A list of all subjects enrolled in the REDS-III Brazilian SCD cohort $\geq$ 18 years will be generated and placed in a random order.  Patients will be consecutively called according to the list.  Details of the study will be explained over the phone and interested subjects will be scheduled for a Hemocenter visit to complete informed consent and study procedures. Patients will be enrolled consecutively at all Hemocenters until 150 patients have completed enrollment.  No Hemocenter specific enrollment targets will be established.

5.1.2 Objective 1: Non-SCD Controls
The enrollment of a case into objective 1 will trigger enrollment of  a control from the same Hemocenter and in the same age stratum.  A list of required controls with eligible ages will be updated daily based on ages of enrolled cases at each Hemocenter. Flyers that describe the study will be distributed in the SCD patient advocacy association rooms and at advocacy events. REDS-III research staff will also attend functions sponsored by SCD patient advocacy associations to give presentations describing the study.  Any interested subjects will contact REDS-III staff to determine eligibility. Interested subjects who meet age eligibility will be scheduled for a research visit.  Research assistants will maintain a list of names and contact information of all interested potential controls not meeting eligibility criteria at this point of contact and call the subject in the future if s/he becomes eligible.

5.1.3 Objective 2a: HIV Seropositive SCD Patients

SCD patients followed at REDS-III Hemocenters are screened annually for HIV. Hemocenter records will be reviewed to identify all SCD patients with confirmed HIV in the 10 years prior to study start. As HIV is relatively rare in the SCD population, HIV seropositive cases will be recruited from the entire Hemocenter SCD population (not limited to patients enrolled in the REDS-III cohort) in order to capture the majority of HIV positive patients. All living HIV seropositive SCD patients receiving care at the Hemocenters will be recruited via phone or at routine Hemocenter visits. Details of study will be explained and interested subjects will be scheduled for visits to complete the informed consent and the HIV risk behavior questionnaire (only for HIV+SCD patients≥18 years). A review of patients' medical record will be performed to capture HIV outcomes. Eligible patients will be given the option to consent solely to the medical record review or to the medical record and the completion of the HIV risk behavior questionnaire if ≥ 18 year old. IRB/ethical committee approval will be requested to perform a medical record review of all HIV seropositive patients no longer living or otherwise lost to follow up at the Hemocenters. The two largest Hemocenters (Hemominas Belo Horizonte and Hemorio) have estimated that between 0.16-0.45% of their current SCD population are HIV seropositive (see Table 2). Therefore, we anticipate that 30-40 HIV positive SCD cases from the past 10 years will be eligible for objective 2.

Table 2: Current estimates of HIV at Two REDS-III Hemocenters

|  | Number HIV+ | Total SCD Population | Percent SCD Population HIV positive |
| --- | --- | --- | --- |
| Hemominas | 5 | 3211 | 0.16% |
| Hemorio | 17 | 3791 | 0.45% |

5.1.4 Objective 2b: HIV Seronegative SCD Controls

To compare SCD outcomes between HIV+ and HIV- SCD patients, controls matched for age (± 2 years), gender, hemocenter and SCD genotype will be randomly selected from the REDS-III cohort in a 2:1 ratio for each identified HIV+ SCD patient in objective 2a. These patients will already have comprehensive SCD data collected for the REDS-III cohort and will not require re-contact or additional medical record review.

*5.2 Sample Acquisition, Processing and Storage*

All cases and controls enrolled in Objective 1 and HIV+ cases enrolled in Objective 2a will have 12mL of whole blood collected in 2 EDTA tubes. No more than a maximum of 2.5% total blood volume of blood will be collected from any subject. Whole blood will be centrifuged at each Hemocenter within 6 hours of collection for separation into plasma and cellular components. Components will be divided into ~1mL aliquots. Aliquots required for testing as summarized in figures 1-3 will be separated and all other plasma and cellular aliquots will be stored at -80C at Dr. Sabino's lab at the University of Sao Paulo. Subjects will be given the option to donate blood leftover after testing required for this study is performed to the University of Sao Paulo's Biobank for future research. Consenting subjects will have samples transferred to the USP Biobank upon completion of this study. The repository created with the blood samples will allow future research focused on potential HIV resistance/control in patients with SCD.

## 6. Measurements

*6.1 Objective 1: Confirmatory HIV Testing in Cases and Controls*

HIV testing will follow the Brazilian algorithm for HIV testing of blood donors. Samples will be screened with a 4th generation EIA (enzyme immunoassay). If repeat reactive, the sample will

be submitted to NAT (nucleic acid testing). If the NAT is negative the sample will be submitted to Western Blot. If Western blot is also negative the individual is considered non-infected by HIV. If the sample is NAT positive or Western blot positive, the individual will be considered HIV infected. Subjects considered HIV infected will be called back to the Hemocenter for the same counseling, repeat testing and referral that blood donors undergo upon confirmation of HIV positive blood donation.

### *6.2 Objective 1: Sickle Cell Disease Testing in Non-SCD Controls*
Each Hemocenter already has established protocols for sickle cell testing of family members of SCD patients. This infrastructure will be utilized to confirm there is no diagnosis of sickle cell disease in the non-SCD controls. One cellular aliquot will be transferred to the local Hemocenter lab for hemoglobin S testing by HPLC. Patients with sickle cell disease will not be eligible to serve as a control. Patients with sickle trait (hemoglobin AS pattern on HPLC) will be counseled about the presence and implications of sickle trait by hematologists at the Hemocenter who typically perform this counseling for family members of SCD patients. Patients with sickle trait will remain eligible.

### *6.3 Objective 1: Measurement of HIV Risk Factors in Cases and Controls*
A detailed HIV risk factor questionnaire will be administered to all subjects. This questionnaire will be based upon an instrument previously utilized and validated by the CDC in its HIV surveillance at U.S. blood banks. This tool has been modified for use in other HIV related REDS studies in Brazil, and has been further tailored to the SCD population with input from Brazilian SCD physicians. A self-administered audio computer-assisted self-interview (ACASI) on a laptop computer or netbook will be used in order to maximize reporting of stigmatized behaviors. A research assistant or nurse will provide the ACASI (including earphones to be able to listen to the questions confidentially) to each subject at the Hemocenter. The study subject will be shown how to use the computer to complete the interview by entering basic demographic data with the help of the nurse, but will be given privacy to complete the rest of the questionnaire. The research assistant or nurse will remain available to answer questions and provide help as necessary. We chose the ACASI to maximize reporting of stigmatized risk behaviors and to streamline the interview as the ACASI allows built in skip patterns depending on initial responses so that subjects are only prompted to answer questions about the details of a specific risk factor if they report having the risk. The ACASI format also utilizes electronic data capture which will reduce data entry errors. See appendix 1 for specific content of ACASI.

### *6.4 Objective 2a: Laboratory assessments in HIV+ SCD patients*
Laboratory assessments to be performed on HIV+ SCD patients to allow characterization of HIV outcomes in SCD will include measurement of viral load, HIV genotyping, drug resistance and complete blood count. A CD4 count will be requested if we are able to coordinate this testing with an appropriate local clinical laboratory.

### *6.5 Objective 2a: Measurement of HIV Outcomes in HIV Positive SCD Patients*
Medical records of HIV positive cases will be reviewed to record the route of acquisition (if known), CD4 count and viral load at specified points in time, presence of AIDS defining illnesses and details of antiviral therapy. See appendix 2 for specific content of HIV medical record case report form.

### *6.6 Objective 2b: Measurement of SCD Outcomes in HIV Positive and Negative SCD Patients*
The SCD clinical history case report form used to collect SCD outcomes for subjects enrolled in the REDS-III Brazilian SCD cohort (medical record questionnaire) will also be used to collect SCD outcomes for HIV+ patients enrolled in objective 2. The HIV- controls selected from the REDS-III Cohort will already have this form completed, therefore will not require re-contact or

repeat medical record review to collect outcomes. See specific content of medical record questionnaire in appendix 3. Outcomes to be compared between HIV+ and HIV- SCD patients will initially focus on stroke, avascular necrosis, pulmonary hypertension, acute renal failure, chronic renal failure and frequency of vaso-occlusive pain episodes and acute chest syndrome.

## 7. Statistical Considerations

### 7.1 Planned Analyses

7.1.1 Objective 1: Measurement of HIV Risk Factors in Cases and Controls

For analysis of HIV risk behaviors in objective 1, the primary outcome to be compared between cases (SCD) and controls (non-SCD) will be number of sexual partners in the previous year. Other HIV risk factors ascertained by the ACASI will be compared between cases and controls including male to male sex, number of lifetime male and female sexual partners, use of condoms, age of sexual debut, intravenous drug use (IDU), sex with an IDU, and sex with an individual known to be HIV-positive. Most of the outcomes in the study will be binary or ordinal categorical variables. Logistic regression will be used to compare distributions between cases and controls, adjusted for Hemocenter and age stratum. Linear models will be employed for continuous outcomes with Hemocenter and age stratum treated as covariates.

7.1.2 Objective 2a: Case-series description of HIV outcomes in SCD patients

HIV outcomes will be reported with descriptive statistics (prevalence with 95% confidence limits) for HIV positive patients included in objective 2a. Specific outcomes to be summarized will include prevalence of long term non-progressors, prevalence of elite controllers (EC) if sufficient viral load and treatment data is available (EC=no evidence of viremia as measured by standard assays, <50 or <75 copies/mL with CD4 count >500 for at least a year), time to onset of AIDS, prevalence of AIDS defining illnesses and CDC classification of disease status at diagnosis and most recent point of contact (see table 3 for CDC classification and table 4 for AIDS defining illnesses).

Table 3: CDC Classification of Disease Stage

| Stage | Age at Time of CD4+ Lymphocyte Test* | | | | | |
|---|---|---|---|---|---|---|
| | < 1 year | | 1-5 years | | ≥ 6 years | |
| | Cells/microL | Percent | Cells/microL | Percent | Cells/microL | Percent |
| 1 | ≥1500 | ≥34 | ≥1000 | ≥30 | ≥500 | ≥26 |
| 2 | 750 – 1499 | 23-33 | 500-999 | 22-29 | 200-499 | 14-25 |
| 3 (AIDS)** | <750 | <26 | <500 | <22 | <200 | <14 |

*CD4 lymphocyte count takes precedence over the CD4 percentage. The percentage is considered only if the count is missing.

**If AIDS defining illness (table 4) is diagnosed, the stage is 3 regardless of CD4+ count.

Table 4: AIDS Defining Illnesses

| |
|---|
| Bacterial infections, multiple or recurrent* |
| Candidiasis of bronchi, trachea or lungs |
| Candidiasis of esophagus |
| Cervical cancer, invasive** |
| Coccidiomycosis, disseminated or extrapulmonary |
| Cryptosporidiosis, chronic intestinal (>1 month's duration) |
| Cytomegalovirus disease (other than liver, spleen or nodes), onset age>1 month |
| Cytomegalovirus retinitis (with loss of vision) |
| Encephalopathy, HIV related |

| |
|---|
| Herpes simplex: chronic ulcers (>1 month) or bronchitis, pneumonitis or esophagitis onset age>1 month |
| Histoplasmosis, disseminated or extrapulmonary |
| Isosporiasis, chronic intestinal (>1 month) |
| Kaposi sarcoma |
| Lymphoma, Burkitt |
| Lymphoma, immunoblastic |
| Lymphoma, primary brain |
| *Mycobacterium avium complex* or *mycobacterium kansaii*, disseminated or extrapulmonary |
| *Mycobacterium tuberculosis* any site |
| *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary |
| *Pneumocystis jirovecii* (previously Pneumocystis carinii) pneumonia |
| Pneumonia, recurrent** |
| Progressive multifocal leukoencephalopathy |
| Salmonella septicemia, recurrent |
| Toxoplasmosis of brain, onset age>1 month |
| Wasting syndrome attributed to HIV |

*Only among children aged < 6 years
** Only among children aged $\geq$ 6 years, adolescents and adults

7.1.3 Objective 2b Comparison of SCD Outcomes between HIV+ and HIV-

Many of the SCD events of interest in objective 2b will be modeled as binary outcomes, such as the presence/absence of a history of stroke, avascular necrosis, kidney failure, pulmonary hypertension, etc. For binary outcomes, conditional logistic regression will be employed for data analysis. With this approach, subjects are grouped into strata that are defined by the matching variables used to select controls for the study; for objective 2b, the strata are defined by age, hemocenter, sickle cell type and gender.

HIV acquisition and clinical progression status also require consideration. Among the HIV-positive subset, subjects with a history of a given SCD outcome may include a mix of those with the SCD event prior to HIV infection and those with the event after infection. Because the SCD population is annually tested for HIV as well as other transfusion-transmissible infections we will have date of HIV diagnosis to estimate age of HIV infection, and the age at which the SCD event first occurred. We will be able to include HIV status as a time-varying covariate in a survival analysis of time to the SCD event. Age will be treated as the measure of time in this analysis.

Because event times may not be accurately captured in medical records, we recognize that there may be events for which a meaningful survival analysis is not possible. For example, pulmonary hypertension is diagnosed through examinations that may be performed sporadically on an irregular schedule in many patients, which may mean that we cannot perform survival analysis for this outcome. Before beginning any time to event analysis, we will evaluate the completeness of data for each endpoint of interest.

Some SCD events, such as VOC and acute chest syndrome, can occur repeatedly in the same subjects. Poisson regression will be employed to evaluate risk factors for both of these outcomes. The outcome variable will be the count of events in a defined period.

Regression analysis will be conducted using SAS. Conditional logistic regression will be employed for binary outcomes and generalized linear modeling will be used for Poisson regression.

*7.2 Sample size justification*
Number of sexual partners in previous year was chosen as primary outcome on which to power study.  A simulation study was conducted to explore statistical power for a comparison of rates of sexual activity among patients with sickle cell disease (cases) and age matched persons without sickle cell disease (controls).  It was assumed that subjects would be classified into three levels of sexual activity: 0, 1-2 or 3+ partners in the preceding year to produce a 3-level multinomial distribution. The distribution in the controls was based on the distribution observed in an HIV negative control population in a previous REDS-II study in Brazil:  6.3% in the zero activity group, 86.6% in the low activity group and 7.1% in the high activity group.  The simulations proceeded as follows:
1) Specify a distribution of activity among sickle cell patients.
2) Take random samples of 500 simulated subjects from the multinomial distributions for the cases and controls.
3) Perform a chi square test to compare the two distributions and note whether the resulting p value was <0.05.  Repeat using the first 250 subjects per group, the first 200 subjects per group, the first 150 and the first 100 subjects per group.
4) Repeat this 2,000 time; the proportion of the replicates with p<0.05 is an estimate of statistical power for the comparison in question at samples sizes of 500, 250, 200, 150 and 100 per group.

Results are summarized in the table below.  The estimates of statistical power are grouped according to the assumed proportion with zero activity in the cases.  Within each assumed proportion with zero activity, estimates of power are listed in order of descending proportions in the low activity group.  Estimates of power that are $\geq$0.80 are shown in bold.  Power calculations were restricted to situations in which the cases (SCD patients) have lower sexual activity than the controls meaning that (1) the sum of the percentages in the low and high activity groups was lower in the cases than the controls <u>and</u> (2) the percentage in the high activity group was lower in the cases than in the controls.

Table 5 Power Simulations

| Distribution in the cases | Power | | | |
|---|---|---|---|---|
| | 100 per group | 150 per group | 200 per group | 250 per group |
| 15/85/0 | **0.92** | **0.99** | **0.995** | |
| 15/84/1 | 0.76 | **0.92** | **0.98** | |
| 15/83/2 | 0.63 | **0.825** | **0.92** | |
| 15/82/3 | 0.53 | 0.72 | **0.86** | |
| 15/81/4 | 0.475 | | **0.80** | **0.885** |
| 15/80/5 | 0.415 | | 0.75 | **0.835** |
| 15/79/6 | 0.42 | | 0.715 | **0.818** |
| 15/78/7 | 0.41 | | 0.725 | **0.83** |
| | | | | |
| 14/86/0 | **0.90** | **0.99** | 0.995 | |
| 14/85/1 | 0.705 | **0.89** | **0.965** | |

| | | | | |
|---|---|---|---|---|
| 14/84/2 | 0.58 | 0.775 | **0.89** | **0.95** |
| 14/83/3 | 0.47 | | 0.79 | **0.885** |
| 14/82/4 | 0.405 | | 0.71 | **0.81** |
| 14/81/5 | 0.37 | | 0.66 | 0.765 |
| 14/80/6 | 0.355 | | 0.65 | 0.75 |
| 14/79/7 | 0.355 | | 0.645 | 0.74 |
| 13/87/0 | **0.89** | **0.99** | **>0.995** | |
| 13/86/1 | 0.665 | **0.87** | **0.95** | |
| 13/85/2 | 0.53 | 0.725 | **0.85** | **0.925** |
| 13/84/3 | 0.395 | | 0.73 | **0.830** |
| 13/83/4 | | | | 0.745 |
| 13/82/5 | | | | 0.66 |
| 13/81/6 | | | | 0.610 |
| 13/80/7 | | | | 0.625 |
| | | | | |
| 12/88/0 | **0.86** | **0.985** | **>0.995** | |
| 12/87/1 | 0.635 | **0.83** | **0.93** | |
| 12/86/2 | 0.465 | 0.655 | **0.80** | **0.89** |
| 12/85/3 | 0.35 | | 0.65 | 0.755 |
| 12/84/4 | | | | 0.625 |
| 12/83/5 | | | | 0.55 |
| 12/82/6 | | | | 0.505 |
| 12/81/7 | | | | 0.505 |
| | | | | |
| 11/89/0 | **0.84** | **0.98** | **>0.995** | |
| 11/88/1 | 0.585 | **0.80** | **0.91** | |
| 11/87/2 | 0.425 | 0.595 | 0.76 | **0.855** |
| 11/86/3 | | | | 0.69 |
| 11/85/4 | | | | 0.54 |
| 11/84/5 | | | | 0.425 |

Considering the previously cited study demonstrating 39% of SCD subjects were sexually active compared to 81% of controls, an assumption of differences on the order of magnitude of 11, 88, 1% (0, 1-2, 3+ partners, respectively) in the cases compared to the presumed distribution of 6.3%, 86.6%, 7.1% in controls is reasonable. As 80% power is achieved with 150 subjects per group in this distribution, this was chosen as the sample size to achieve study objectives and maintain feasibility within resources.

## 8. Data Management and Transfer

Data collected from the ACASI interview is stored on the local netbook or laptop computer used to conduct the ACASI. The RTI study manager and data analyst will work directly with the staff developing the computer assisted questionnaire program and will test the system to ensure that appropriate skip patterns are followed and that the standard response categories (e.g., don't' know, refused, not applicable) are consistent coded throughout the survey. The ACASI data files will be sent weekly by research assistants in Hemope, Hemorio, Hemominas Juiz de Fora and Hemominas Montes Claros to the lead study coordinator in Hemominas Belo Horizonte (HBH). This designated study coordinator in HBH is responsible for review and basic cleaning of data to identify missing or irregular data. This coordinator will then up load the files to the data coordinating center, RTI, through a secure, encrypted FTP site on the private REDS-III website using the same procedures defined for other REDS-III studies.

The HIV case report form and SCD case report form (medical record questionnaire) will be programmed into the data entry and storage system that has been developed for the REDS-III Brazil SCD Cohort study. This system utilizes a web-based interface for data entry. Data is then directly transferred to databases within the University of Sao Paulo for storage. Once per month data will be extracted from the USP database and securely uploaded to RTI for quality control checks. RTI will follow data management best practice guidelines means developing and implementing quality control processes from the survey develop stage through analysis. This will minimize data manipulation and allow for quicker evaluation of the data, identification of any data and data retrieval/remediation.

## 9. Required Approvals

This protocol and associated questionnaires and consents will be submitted to the REDS-III Executive Committee then the REDS-III International Advisory Committee (IAC) for review and approval. After approval from REDS-III Committees, the package will be submitted to the Observational Monitoring and Safety Board (OSMB). Upon OSMB approval, the team will draft the Office of Management and Budget (OMB) application and submit to OMB after approval by NHLBI. On parallel tracks, the protocol will be submitted to Brazilian National IRB (CONEP) and subsequently to local Brazilian and US IRBs. Timeline for various approvals are included in table 6.

Table 6: Required Approval for Aim D of REDSIII SCD Study

| Task | Date |
|---|---|
| Submit to EC for review | 1/14/2015 |
| Team joins EC call to discuss protocol | 1/23/2015 |
| EC approves protocol pending any required changes | 1/30/2015 |
| IAC reviews comments and approves | 2/6/2015 |
| Submit package to OSMB | 2/9/2015 |
| OSMB review | 2/27/2015 |
| Submit to CONEP | 4/15/2015 |
| Provide draft 1 OMB application | 4/15/2015 |
| Submit OMB application | 5/1/2015 |
| Submit to local Brazilian CEPs, pending CONEP approval | 8/30/2015 |
| Submit to BSRI IRB | 8/30/2015 |
| Anticipate all approvals final | 12/31/2015 |
| Study begins | 1/18/2016 |

## 10. Study Timeline

| | 2015 | | | | 2016 | | | | 2017 |
|---|---|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 |
| Programming and Testing of electronic CRF and ACASI | X | X | X | | | | | | |
| Manual of Procedures | | X | X | | | | | | |
| SMS | | X | X | | | | | | |
| Training | | | | X | | | | | |

| | | | | | | X | X | X | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Enrollment into protocol | | | | | | X | X | X | | |
| Confirmatory HIV testing | | | | | | X | X | X | | |
| Data Analysis | | | | | | | | | X | X |

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