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# NHGRI IRB

# Human Subjects Research

# Protocol Template

Project title: **Insights into Microbiome and Environmental Contributions to Sickle Cell Disease and Leg Ulcers Study (INSIGHTS Study)**

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**List of Abbreviations** *(as applicable)*

AE Adverse Event/Adverse Experience

CFR Code of Federal Regulations

CLIA Clinical Laboratory Improvement Amendment of 1988

COI Conflict of Interest

CRADA Cooperative Research and Development Agreement

DAC Data Access Committee

dbGaP Database of Genotypes and Phenotypes

DUC Data Use Certificate

DHHS Department of Health and Human Services

DSMB Data Safety and Monitoring Board

FWA Federal Wide Assurance

GCP Good Clinical Practice

GINA Genetic Information Nondiscrimination Act

GWAS Genome-Wide Association Study

HIPAA Health Insurance Portability and Accountability Act

ICF Informed Consent Form

IRB Institutional Review Board

MTA Material Transfer Agreement

N Number (typically refers to number of subjects/sample size)

NHGRI National Human Genome Research Institute, NIH

NGS Next Generation Sequencing

NIH National Institutes of Health

OHRP Office for Human Research Protections

OHSRP Office of Human Subjects Research Program

PHI Protected Health Information

PI Principal Investigator

PK Pharmacokinetics

QA Quality Assurance

QC Quality Control

SAE Serious Adverse Event/Serious Adverse Experience

SOP Standard Operating Procedure

UP Unanticipated Problem

UPnonAE Unanticipated Problem that is not an Adverse Event

Instructions: Insert your responses for each section. Be sure to address each point in all sections that are relevant. (It is not necessary to itemize each point or to specify “not applicable.”)

Please use a readable font, like Times 12-point type, to facilitate legibility. We encourage investigators to limit the overall length of protocols to 20 single-spaced pages or fewer (not including the 3 preceding pages, appendices, and other attachments).

1. **Precis:** *(In 400 words or fewer, describe the study objectives, population, design, and outcome measures*)

Leg ulcers are a serious and debilitating complication of sickle cell disease (SCD). This study will explore factors, namely microbial, genomic, and environmental (social and physical), that may influence the onset and progression of leg ulcer formation and delayed healing in individuals living with SCD. There is variation in the incidence and duration of SCD leg ulcers. They are often very painful, resistant to treatment, and recurrent in nature. The etiology of SCD-associated leg ulcers is unclear, and we hypothesize that predisposition to developing leg ulcers is multifactorial. This multisite study is an exploratory study of the microbiome and environment of individuals living with sickle cell disease leg ulcers. The study’s objective is to identify triggers that may be integral in leg ulcer onset and progression. The central goal of this study is to obtain an improved understanding of the participants’ clinical phenotype, leg ulcer microbiome and the psychosocial and environmental factors that may impact this complication. To achieve these goals, we will characterize the leg skin microbiome of SCD patients living with and without leg ulcers within the United States. In addition to assessing the microbiome, we will collect and analyze psychosocial and physical environmental data of individuals with SCD without leg ulcers and with leg ulcers.

In addition, we will examine the psychosocial impact of leg ulcers on individuals with SCD by conducting a qualitative phase to explore the individual experiences to understand the physical function, stigma, and self-esteem associated with those with active, recurrent, or single-occurrence presentations of leg ulcers. To accomplish this objective, an in-depth, semi-structured interview methodology will be employed. This will afford participants the chance to expound upon existing questions and will provide us the opportunity to understand the complexity of participants’ experiences and quality of life.

1. **Objectives and specific aims.** *(List the objectives concisely; whenever possible, state objectives as hypotheses.)*

The objectives of this study are to:

* Employ genomic approaches to characterize the skin microbiome in individuals living with SCD with and without leg ulcers
* Employ social science research measures to identify psychosocial and physical environmental factors that impact quality of life in individuals living with SCD with and without leg ulcers

Specific Aims:

* To characterize the microbiome of leg ulcers in SCD.
* To characterize the phenotypic variation in SCD
* To identify similarities and differences of the microbial signatures of chronic diabetic foot ulcers and SCD leg ulcers.
* To investigate physical and psychosocial environmental indicators that impact quality of life in individuals living with SCD with and without leg ulcers.
* To determine if an association exists between the microbiome (microbial diversity) of SCD leg ulcers and the quality of life (psychosocial and physical environment) indicators of individuals with SCD leg ulcers.
* To determine if an association exists between the microbiome (microbial diversity) of SCD leg ulcers and clinical phenotype of individuals with SCD leg ulcers.
* To determine how leg ulcers influence one’s physical function and one’s perceptions of stigma and self-esteem.

Hypotheses

**The overarching hypothesis is that there are combinations of factors: microbial, genetic modifiers, environment, and social determinants that likely impact leg ulcer formation and healing in patients with sickle cell disease**. We will test this hypothesis by using genomic and social science approaches to investigate differences in the microbiome in individuals living with leg ulcer.

**The hypothesis for the qualitative phase is that leg ulcers have an adverse effect on individuals.** We will test this hypothesis using semi-structured interviews to investigate how psychosocial factors may influence the quality of life in individuals with SCD leg ulcers.

**1a) We hypothesize that an altered skin microbiome predisposes a subset of SCD patients to developing leg ulcers.**

We will characterize the microbiome of leg ulcers in SCD patients employing genomic approaches. We will enroll up to 150 participants within the microbiome cohort of the study. One hundred fifty (n= 150) participants in total with (n=50) leg ulcers and without (n=50) leg ulcers, and those with no previous history of leg ulcers (n=50). We will sample ulcerated and non-ulcerated skin in individuals with leg ulcers. Participants without leg ulcers will sample non-ulcerated skin. Up to seventy five participants will be randomly invited to participate in a second sample collection in the microbiome cohort of the study.

**2) We hypothesize that the microbiome of diabetic foot ulcers and sickle cell leg ulcers are highly similar.** We will compare the microbiome of published diabetic foot ulcers data with leg ulcer microbiome data from this study to determine if specific microbial signatures are responsible for the delayed healing across multiple leg ulcer types [[1](#_ENREF_1)].

**3a) We hypothesize that quality of life and psychosocial factors (i.e., depression, stress, pain, stigma, discrimination, social function, health behaviors, and social support) modulate leg ulcer formation and healing processes.**

**3b) We hypothesize that environmental factors (i.e. exposure to pets, mold) modulate leg ulcer formation and healing processes.**

**3c) We hypothesize that telomere length is associated with having leg ulcers or not**

**3e) We hypothesize that telomere length is associated with measures of stress (self report and cortisol).**

We will explore these indicators to determine if there is an association between social and environmental factors and leg ulcer occurrence. We will also test interactions between these factors to predict formation and healing. To test these hypotheses, we will compare social measure ratings and physical environment with the experience of SCD patients living with and without leg ulcers.

1. **Brief Rationale and Background**: *Write a* ***brief*** *[****no more than 5 pages in length****]* *summary of the clinical background, limits of current knowledge, and significance of this protocol. For background on drugs and devices, cite animal studies, prior experience in humans, and discuss potential toxicities. Include* ***up to 20*** *key references.*

The ultimate goal of the proposed pilot research is to integrate research on microbiome, genomic, clinical, environmental, and psychosocial factors to understand sickle cell disease and the formation and healing process of individuals living with leg ulcers. To our knowledge, this is the first program of research to explore how these factors modulate leg ulcer formation and healing among SCD patients, as well as how these processes utilize an experimental design that compares individuals with and without leg ulcers to those with diabetic foot ulcers. The proposed project investigates these four distinct areas of research—genomic, clinical, environmental, and psychosocial—to offer an integrative theory as to why SCD patients develop leg ulcers and how to intervene to reduce severity and facilitate healing.

The first area of research is on clinical phenotype, which demonstrates that high levels of fetal hemoglobin (HbF) are linked to reductions in leg ulcer formation in SCD patients [[2](#_ENREF_2)]. The second is research on microbes, which based on research on diabetic foot ulcers, is a promising causal mechanism that may help account for the progression of leg ulcers and a delayed healing process among SCD patients. To be more specific, current research on diabetic foot ulcers

The third is research on environmental factors, which shows that contextual aspects of the environment, such as lack of proper footwear and high levels of residential mold, modulate leg ulceration risk [[3](#_ENREF_3)]. The fourth area of research draws from the social science research measures to ascertain individuals’ quality of life, stress, and psychological coping resources that may alter the immune system (i.e., wound healing) and the microbiome. This would suggest that psychosocial factors likely also modulate the progression of leg ulcers and the healing process among SCD patients.

This study deals with a disparity population where an understanding of SCD and leg ulcer formation and healing have not been extensively explored. Sickle cell disease (SCD) is a genetically inherited blood disorder that affects 90,000-100,000 Americans primarily of African descent in the United States and millions individuals worldwide are affected with the disease including individuals from sub-Saharan Africa, Saudi Arabia, India and Mediterranean countries such as Turkey, Greece and Italy. worldwide [[4](#_ENREF_4)]. This pilot project will provide data to explore the merit of this research. The goal is to conduct a large international study, which will include multiple geographic locations, ancestral and cultural populations with diverse social and physical environments living with leg ulcers in diverse regions of the world where SCD is a major health concern.

SCD is caused by a single point mutation in the 6th codon of the β-globin gene resulting in abnormal β. This produces red blood cells that are sickle-shaped and impaired in their function. In particular, hemolysis and erythrocyte membrane damage are hallmarks of the disease [[5](#_ENREF_5)]. Furthermore, SCD is characterized primarily by severe anemia and vaso-occlusion. SCD symptoms include severe pain (often referred to as the “painful crisis”), often recurrent and requiring frequent hospitalizations, hemolytic anemia, and end-organ damage that results in premature death.

The term sickle cell disease refers to different genotypes that define this complex clinical syndrome in which HbS is the most abundant species [[6](#_ENREF_6)]. There are varying degrees of disease severity within the spectrum of SCD. The most severe phenotypes are in individuals that have two HbS genes (HbSS) commonly known as sickle cell anemia or a double heterozygosity for beta thalassemia0 and HbS (HbSB0). The least severe phenotypes comprise individuals that either have sickle cell with hemoglobin C (HbSC) disease or hemoglobin S-β+-thalassemia.

Stem cell transplantations can cure the disease but are still experimental and predominately in research settings available to very few patients [[7-10](#_ENREF_7)]. To manage SCD, clinicians rely on medication (hydroxyurea to increase fetal hemoglobin), pain medication, blood transfusions, and antibiotics to alleviate many of the clinical complications of SCD [[5](#_ENREF_5), [11](#_ENREF_11)]. These complications are numerous and may include stroke, renal dysfunction, infections (primarily bacterial), acute chest syndrome, and leg ulcers to name a few. In the United States, SCD is the main hemoglobinopathy that causes leg ulcers but individuals suffering from other hemolytic disorders, such as Thalassemia intermedia or hereditary spherocytosis, have been reported to also suffer from this complication [[12](#_ENREF_12), [13](#_ENREF_13)].

Leg ulcers are commonly the result of chronic and complex conditions such as diabetes and SCD. SCD-associated leg ulcers are breaks in the skin and subcutaneous tissues typically occurring at the ankles, where there is less subcutaneous fat, thin skin, and possibly decreased blood flow but are also infrequently observed on the anterior tibial area, the dorsum of the feet, and the Achilles tendon [[14](#_ENREF_14), [15](#_ENREF_15)]. Ulcers are extremely painful and sometimes difficult to heal, and recur often. In sickle cell patients, leg ulcers are common. Research studies have documented varying rates according to geographic location from 1.5 to 13.5% in Africa, 2.5% in the U.S. to over 29% in Jamaica [[15](#_ENREF_15), [16](#_ENREF_16)]. Socioeconomic status has been implicated as one of the contributing factors [[3](#_ENREF_3)]. Leg ulcers usually appear between the ages of 10 to 50 years and, in some reviews are more prevalent in men than women [54], others did not [14], including Associate Investigator Caterina Minniti’s own study.[19]. Although the pathogenesis of leg ulcer formation remains unclear, investigators speculate that trauma, infection, and inflammation may be responsible for causing SCD leg ulcerations [[15](#_ENREF_15), [17](#_ENREF_17)]. It is a complex disorder with multiple complications and the etiology of leg ulcers remains unknown warranting further investigation into the underlying mechanism(s) responsible for this condition.

A number of treatment options are available to SCD patients with leg ulcers, but none of them are very successful. These include topical interventions, such as wet to dry dressings, manual or surgical debridement, skin grafting, topical antibiotics, oral zinc supplementation, bed rest and the use of compression socks, Unna boots, and systemic interventions, such as chronic transfusions [[15](#_ENREF_15), [18](#_ENREF_18)]. As with many disorders, patient compliance to the treatment plan is often difficult to enforce. Moreover, educational interventions are often needed not only for the patient but also for the physicians and nurses who take care of SCD patients with chronic ulcers.

SCD leg ulcers are often resistant to therapy, recurrent, and responsible for causing social isolation, stress, stigma and depression [[19](#_ENREF_19)]. Adults living with SCD undergo many painful episodes throughout their lifetime, which impacts their quality of life [[20-23](#_ENREF_20)]. However, the lack of viable coping mechanisms and other interventions results in an increase in anxiety and stress levels [[21](#_ENREF_21), [24](#_ENREF_24), [25](#_ENREF_25)]. In the case of leg ulcers, it is common that the individual is unable to work and has to remain on bed rest for the duration of the leg ulcer.

Studies in Jamaica have found that social and economic factors influence leg ulceration risk. Specifically, a lack of proper footwear and socioeconomic status can increase one’s risk [[13](#_ENREF_13)]. Most of the research on the psychosocial impact of SCD on patients rarely focuses on the complications associated with SCD [[26](#_ENREF_26), [27](#_ENREF_27)]. In general, there has been limited research on leg ulcers in sickle cell disease patients. It is recognized that the social and physical environment of individuals with SCD may have an impact on health outcomes [[21](#_ENREF_21)]. Therefore, it is of great interest to the research team to characterize the microbiome of SCD leg ulcers and identify social and environmental factors that impact leg ulcer formation and healing.

There are several reports from the 1980s that highlight, using culture-based studies, an increase in the abundance of specific bacterial microflora [[28-30](#_ENREF_28)]. Interestingly, *Staphylococcus*, *Pseudomonas,* and *Streptococcus* were also commonly identified in leg ulcer cultures. In these studies, individuals with infections responded well to topical antibiotic treatment. This would suggest that microbes, if not responsible, are associated with the progression of this condition and likely impede the healing process similar to the speculated role of microbes in non-healing diabetic foot ulcers. A recent study characterized the microbiome of diabetic foot ulcers in 52 patients using two approaches, high-throughput sequencing and culturing. The most abundant sequenced genera in the diabetic foot ulcers were *Staphylococcus* (49/52 samples), *Streptococcus* (15/52), and *Lactococcus* (38/52) [[1](#_ENREF_1)]. Although culturing was only conducted in the HbSS leg ulcer studies from the 1980s, similar bacterial isolates were also recovered in the diabetic microbial foot survey. Gardner et al. suggest that in order to reveal informative relationships between microbiome changes and disease state (i.e. leg ulcers), microbiome studies must also consider the clinical metadata for each individual patient [[1](#_ENREF_1)]. To be more specific, the Gardner et al. study found that the microbiome colonizing diabetic foot ulcers (DFUs) is associated with clinical factors. Thus, the 52 participants with foot ulcers were also evaluated for the following clinical factors: hemoglobin A1c, oxygenation, ulcer duration, ulcer depth, ulcer surgace area, and necrotic tissue. The final results indicated that the following variables were significantly associated with the microbiome of DFUs: ulcer depth, ulcer duration, and poor glycemic control (i.e. higher hemoglobin A1c). The results indicated that poor glycemic control, higher ulcer duration and deeper ulcers gave higher species levels that were colonizing the DFU [1].

Researchers no longer rely solely on culturing for microbial identification but instead use sophisticated sequencing technologies to characterize the full diversity of microbial communities throughout the human body and the environment. Of interest is an understanding of the microbial diversity in a healthy individual and in disease. Microbiome research will shift the ways scientists and physicians think about human health and disease translating the findings into new interventions in clinical practice and public health [[31](#_ENREF_31)]. As with many studies in genetics and genomics, itis clear that genetics alone does not play a role in disease onset and progression. Instead multiple interactions between genetics, microbes, and the environment also influence disease severity and complication.

It is widely known that stress alters the immune system [[32](#_ENREF_32)]. For example, individuals under a significant amount of stress are more susceptible to infections. In the case of leg ulcers, wound healing is mediated by the immune system requiring cytokines and other immunological factors [[33](#_ENREF_33)]. Stress and racial discrimination also affects an individual’s mental health status and, perturbs the immune system [[34](#_ENREF_34)]. SCD patients (and those with leg ulcers) experience stigma because of their condition. We are interested in understanding whether psychological stress, racial discrimination, the microbiome, and other factors impair the wound healing process in SCD and diabetic patients by altering the immune system. It is known that poor healing increases the risk for wound infections, lengthens hospital stays, and disrupts daily activities and employment status [[33](#_ENREF_33)]. We speculate that all of these factors may work together to predispose certain populations of patients to developing leg ulcers. Assessing the complete environment of an individual will be key to understanding how and why leg ulcers develop in SCD patients.

We will explore the associations between leg ulcers formation, stress and telomere length of the participants in the study. We will analyze the data with the leg ulcer cohort and non-leg ulcer cohort as our controls. Telomere length is an inflammatory marker and indicator for morbidity in chronic disease. It has been studied in the sickle cell disease population in only one study [[35](#_ENREF_35)]. The authors found that individuals living with SCD have longer telomeres in comparison to healthy controls. They postulate that the longer telomeres in patients with SCD are related to unregulated telomerase activity secondary to the chronic systemic inflammation, and activated leucocytes. Thus, we would like to confirm these findings in our study population.

We will also investigate whether telomere length is associated with incident of severe pain. We will compare across cohorts with significant pain episodes versus those with limited/no pain episodes. It has been shown in pain research an association between pain and telomere integrity[[36](#_ENREF_36)].

Moreover, numerous studies have shown the importance of environment in modulating many diseases and disorders. It is known that multiple factors i.e. host age and sex, environment (physical and social), immune system regulation, genetic variation of the host, and hygiene all contribute to variation in this case, the human microbiome. Genomic researchers can no longer ignore the importance of a holistic approach when studying the impact of these factors in human health and disease. We have proposed a study that will rely on an interdisciplinary approach to elucidate the role of the physical and social environment and the microbiome in maintaining and/or altering the switch between health and disease states. This study will incorporate multiple social science measures and will also examine how the microbiome shifts in response to an individual’s health status. The results of this study will inform how researchers design microbiome and other non-microbiome related studies in the future facilitating discussions and collaborations among both basic and social scientists.

In this study, we will characterize the microbiome of leg ulcers in the recruited cohort of participants while surveying them for environmental triggers, which may put them at an increased risk for developing leg ulcers. Environment will include health behaviors, and social, physical, and psychosocial factors that function in concert with genetics to influence disease progression/delayed healing. Because of our expectations that the vast majority of our participants will be of African American and/or African descent, the importance of discrimination and race will be explored in this study. We will also compare the leg ulcer microbiome data to published diabetic foot ulcer microbiome data. As a result, comparing these two datasets will likely identify shared or distinct microbial signatures between the two ulcer types improving treatment options, developing new interventions (i.e. utilization of multiple antimicrobials that act in synergy) for those living with this complication while also identifying effective coping strategies that may help patients better manage leg ulcers and the disease [[37](#_ENREF_37)]. Expanding upon the existing study, this qualitative phase seeks to comprehensively examine the psychosocial impact of leg ulcers in a subset of the INSIGHTS sample population through a qualitative analysis. Leg ulcers are a complication of sickle cell disease (SCD) that have been reported to be debilitating and complex. Previous studies on this complication are focused specifically on the clinical severity, treatment and interventional options, and vasculopathy associated with ulceration [[38-41](#_ENREF_38)]. Because the prevalence of leg ulceration in the United States is relatively low (~2.5% of those with SCD), there is a dearth of literature that examines the social effects of leg ulceration. To our knowledge, this is the first qualitative study that examines the psychological impact of leg ulcers on individuals with SCD and this study seeks to address this gap in knowledge.

The main objective of this qualitative phase is to assess self-esteem, feelings of stigma, and perceptions of physical function in individuals with leg ulcers. We anticipate that our study participants will experience a psychosocial burden of increased perceived stigma and lower self-esteem as a result of the complication because there is a substantial amount of literature that reports psychosocial and physical challenges related to having SCD (e.g. disease state with no ulcers) [[42-46](#_ENREF_42)]. It is likely that there will be similar trends among our participants with ulcers, particularly because of the physical and social complexities associated with leg ulcers.

In this phase, we will use qualitative analysis to gain a comprehensive understanding of the psychosocial impact of leg ulceration and of the lived experiences of individuals with leg ulcers. We intend to capture the individual’s realities that would otherwise remain inaccessible (e.g. subjective experiences) and examine the constraints of everyday life [[47](#_ENREF_47)]. Conducting in-depth, semi-structured interviews, will afford us the chance to study questions that cannot be quantitatively examined or measured [[47](#_ENREF_47)]. These interviews will be conducted either in-person or via telephone. We will interview individuals that have had active, recurrent, or single-occurrence presentations. The results of this analysis will contribute to knowledge around understanding the everyday experiences of those with leg ulcers.

**Protocol Progress and Key Findings:**

* As of December 31, 2015, the study has recruited 64 participants. Of those participants, 22 have leg ulcers and are considered our cases cohort, while 42 are those without leg ulcers, and 25 of those 42 participants have no pervious history of leg ulcer.

* The recruitment rate was slowed significantly in 2015 because associate investigator, Dr. Caterina Minniti, relocated to Albert Einstein Medical College and Montefiore Medical Center, and was no longer available to regularly conduct the clinical exams of the participants. Only 8 new participants were recruited since the last continuing review which was in February 2015.
* As of January2016 the protocol’s research nurse practitioner received NIH credentials as a nurse practitioner which will allow her to independently conduct the exams and we will resume study recruitment and data collection at a rate comparable with before. It is our intent to open a second recruitment and data collection site in Bronx, New York, at Montefiore Medical Center, to enhance our collaboration with Associate Investigator Caterina Minniti and the INSIGHTS study will increase our accrual goal by 100 participants.
* Our data collection to date includes microbiome skin samples on 21 of the leg ulcer participants and 35 of the non-leg ulcer group.
* Microbiome Preliminary Data: In collaboration with Elizabeth Grice, Ph.D. at the University of Pennsylvania and postdoctoral fellow, Keisha Findley, Ph.D., sequenced 16S rRNA bacterial sequences from the skin of the first 12 (five with active ulcers) study participants using the Illumina MiSeq Technology. Approximately 1.3 million sequences were taxonomically classified using the online molecular ecology tool, mothur, and the RDP classifier and training set (version 9). The relative abundance of bacteria on the ankles of SCD participants with and without leg ulcers is distinct. Those with leg ulcers have lower bacterial diversity and an abundance of the skin commensal, Staphylococcus, while those without leg ulcers display higher bacterial diversity. This finding supports the claim that microbial diversity is higher in a healthy state and is conversely, lower in a disease state. Additionally, we conducted Principle Coordinate Analysis (PCoA) using thetayc distances comparing bacterial community structure (similarity) in participants with and without leg ulcers. This analysis confirmed that two communities differ, and we speculate that this variation could potentially explain the differences in the skin microbiome of individuals with SCD who develop leg ulcers over their life course. We are currently processing microbiome samples as they are collected and will complete the sequence analysis when the data is available. Data on the first 12 participants is presented in Appendix T.
* We have also collected data on clinical phenotypes, biological markers, environmental and psychosocial factors in order to further compare the two cohorts. (See Appendix T).
* We have completed 5 pilot qualitative interviews for the qualitative phase of the study.
* We have sequenced of the microbiome of the first 56 participants. This data will be analyzed in 2016 with the research team.
* We planning RNA expression analysis on the first 56 participants.

We have completed the sequencing of the microbiome of the first 56 participants and RNA Expression analysis for the first 62 participants. This data will be analyzed in 2016 with the research team and new collaborators.

A scientific poster was presented at the NHGRI 2014 Scientific Symposium on the microbiome sequencing of the first 12 participants by postdoctoral fellow Keisha Findley and received a best poster award. The study currently has no publications. The first publication will focus on the microbiome.

**The Conceptual Framework (see Appendix A for more information)]**

The study’s framework explores through genomic and psychosocial data the interactions that likely influence formation and healing of leg ulcers and will guide our approach in understanding the etiology of leg ulceration, which is currently unknown.

1. **Description of study design** *(Brief description of what study design has been selected)*

Overview

This is a descriptive study of individuals living with sickle cell diseases with and without leg ulcers (accrual goal 300 participants). Leg ulcers are not observed in all individuals with sickle cell disease and we are therefore interested in understanding why certain individuals develop leg ulcers using tools to explore the skin microbiome and social and environmental indicators. We will seek to include age-matched patients without leg ulcers for the microbiome phase of the study. We will recruit and sample from a total of 150 male or female adult participants with ulcers (n=50), currently without leg ulcers (n=50). We will further recruit and sample 50 participants who have no previous history of leg ulcers (n=50). A total of up to 75 study participants will be sampled longitudinally during the study. The second sampling will occur at least one month after the initial sampling. This will assess longitudinal stability of the microbial community in the patient cohort. Additionally, we will compare a previously published microbiome dataset from diabetic foot ulcers to SCD leg ulcers to identify microbial signatures (similarities or differences) that exist in the microbial communities present in the different ulcers, which may be important in the healing process.

We will conduct a cross-sectional study to investigate, stress, social function, health behaviors, and quality of life indicators for each participant with the goal of identifying environmental (i.e. social, physical, and psychosocial) factors that may impact sickle cell disease and the formation and healing of leg ulcers. We will recruit an additional 150 adult SCD patients who will not have their microbiome analyzed but who will participate in all other components of the study. The total sample size for the INSIGHTS study will be 300 participants.

In addition a qualitative phase analysis of research participants with sickle cell disease and active, recurrent, or single-occurrence leg ulcers will occur. We will sample from participants who have already enrolled in the INSIGHTS study and we will also recruit participants to meet our accrual (N=20). Participants will complete an in-depth, semi-structured interview with a researcher to assess individuals’ perceptions of their physical function, stigma, and self-esteem. Interviews will be audio recorded and transcribed verbatim and analysis will be conducted by two members of the research team.

1. **Description of procedures:** *(what will be done, when will it be done, where will it be done, duration of participant involvement. Specify which procedures are being done for research only, and which are being done both for research + clinical care.* Please include a flow-sheet or chart that depicts the subject's participation in the protocol from recruitment and consent to completion of the study procedures).

**Pre-Screening**

Potential study participants will be initially screened over the phone by the research coordinator (or research nurse) using the inclusion and exclusion criteria as a guide prior to enrollment in the study (Appendix K). Those eligible will be invited to participate in the study. Study participants will be consented, and sampled at either the NIH Clinical Center or Montefiore Medical Center (MMC) and will be seen by a physician, nurse practitioner, research nurse, and/or a wound care nurse.

**Screening for the qualitative phase:**

There will be two categories of individuals that participate in the complementary qualitative phase: those that have already completed the INSIGHTS study and those that have not. The screening process for both groups will differ slightly. Individuals that have already participated have already been screened and thus, will undergo screening to have their interviews taped (Appendix R). Individuals that have not completed INSIGHTS will be screened over the phone in a similar way to the existing INSIGHTS study by the research nurse or associate investigator. If the individual indicates that he/she would like to participate in the qualitative phase, the individual will also be screened using the inclusion criteria prior to enrollment. Those eligible will be invited to participate in the survey.

**Microbiome Study Participants**

For those research participants living outside of the Washington D.C. area (DC, MD, VA metropolitan region) travel to the NIH Clinical Center and overnight lodging for an outpatient stay will be provided to recruited participants who are completing both the microbiome sampling and survey. For those research participants living in the New York City metropolitan area, travel (local transportation) to MMC will be provided for the participants completing the study. Research participants will be asked not to shower/take a bath or use skin moisturizers for 24 hours prior to sample collection.

**Day One:** Participants with and without leg ulcers will have samples collected for the microbiome study. Approximately 150 research participants will be sampled, 50 with leg ulcers, 50 without leg ulcers, and 50 with no previous history of leg ulcers. The participant’s medical history will be collected and clinical evaluation will be conducted (Appendix E and G). A clinical evaluation conducted by any medical provider within 45 days from study visit will be acceptable for completing the physical exam form (Appendix E).We will also obtain routine clinical blood labs and a research sample on each of the study participants ( n=300) for clinical and phenotype data and future genomic analysis based upon an amendment of this protocol.

**Day Two:** If not completed on day one, participants will return to complete questionnaires. These measures will obtain data on various clinical, psychosocial, and physical environmental indicators. It will take two 60-minute sessions to complete the survey.Some of the surveys (Appendix E3) will be self-administered.

**Microbiome Return Sampling Participants**

A subset (75 total) of the microbiome participants with and without leg ulcers will return for an additional sampling at least one month after the initial sampling to assess the longitudinal stability of the microbiome. We will select the 75 return participants according to the following criteria: (1) Proximity to the Washington D.C. Metropolitan area and the NYC Montefiore Medical Center (2) Presence of clinically interesting leg ulcers, which will be determined by the clinical members of the research team (research nurse and medical advisor).

For the initial 56 participants, samples were processed at the NIH and sequenced at the University of Pennsylvania according to the Material Transfer Agreement that has been established (Appendix L). Samples obtained at MMC will be shipped to NIH prior to processing and sequencing at either the NIH or University of Pennsylvania. A Material Transfer Agreement has been executed with MMC.

**Non-Microbiome Study Participants**

One-day visit to the Clinical Center or MMC the participant’s medical history, demographic data will be collected and clinical evaluation will be conducted. (Appendix E, E1, E2, and E3). We will also obtain routine clinical blood labs and a research sample on each of the 150 Non-Microbiome study participants for clinical and phenotype data and future genomic analysis based upon an amendment of this protocol.

**Day 1 and 2: Cortisol Collection**

Hair cortisol

Three cm or less of human hair will be collected. We will investigate hair cortisol levels in all research participants that consent to hair collection in order to measure long-term cortisol levels.

Salivary cortisol

Cortisol, a steroid hormone, is produced in the adrenal cortex and is released in response to stress. It increases blood sugar, suppresses the immune system and aids in the metabolism of fat. Furthermore, cortisol levels fluctuate in response to emotional or physical stress, illness, and injury. Cortisol can be measured using serum, urine, saliva, and hair. For hair cortisol, hair grows 1 cm/month, and cortisol is constantly deposited in the hair shaft. Hence, cortisol levels in the hair can provide information glucocorticoid exposure over time (55). Segmental scalp hair analysis is useful in determining periodic levels of cortisol ranging days to years (56). We hypothesize that patients with leg ulcers will have a high level of cortisol in scalp hair and in saliva.

We will collaborate with the Scientific Director of NICHD, Dr. Constantine A. Stratakis. See Appendix O and P for a detailed description of procedures for saliva and hair cortisol collection.

Flowchart of Study Procedures (see Appendix B)

**5.1 Medical information (*what information will be collected, any sensitive information, how long will it be stored, are there future anticipated uses?*)**

The research team will obtain a detailed medical history and personally identifiable demographics information (i.e. SCD status, past hospital visits, history SCD clinical complications, familial health history, etc.) (Appendix E, E1, E2, and E3) on each study participant, wound care assessment and wound care for those with leg ulcers, lab/blood/saliva work for clinical metadata and for future genetic testing/genomic sequencing, including physical and social environmental survey data which will all be used to understand how these factors may affect wound healing and the onset of this clinical complication.

We will store the biological specimens (e.g. blood, saliva, urine, hair samples) indefinitely. We will store the clinical evaluation and medical history and data from the social science measures indefinitely. Research samples will be shipped from MMC to NIH for storage.

* 1. **Diagnostic studies *(include use of radiation or sedation.)***

N/A

**5.3 Biological specimens (*How much will be collected, what disease categories will be studied, will cell lines be created, how long will samples be stored, are there future anticipated uses?*)**

**Characterizing the leg ulcer microbiome in SCD**

At time of sampling, a wound culture will be collected once the sample for the microbiome has been secured. The following procedure for wound care will be administered (microbiome sampling to be completed by the wound care nurse specialist, research nurse, Clinical Associate Investigator.. Sample collection steps:

• Cleanse ulcer with normal saline & gauze removing any debris from wound bed

* Perform Levine’s technique to collect microbiota and wound culture (as

mentioned above, see Appendix C)

\*\* In participants with leg ulcers, non-ulcerated skin will be sampled ≥ 2 cm

from the outside of the wound.

• Apply vaseline based ointment to moisturize under wrap

• Cover ulcer with Mepilex white foam

• Apply 2 layer compression wrap to leg (depending on the condition of the ulcer)

• Medical advisor will make a clinical decision to order oral analgesics, or place a topical local analgesic above the leg ulcer if needed

During sampling, the wound care/research nurse will also record the duration of the ulcer, whether patients are unilaterally or bilaterally affected, size and shape, site of ulceration, history of leg ulcers (current and past), current medication, necrotic tissue, and any odors associated with the ulcer (Appendix E).

Wound Measurement: The surface area of the study ulcer will be measured and photographed with defined lighting, distance, exposure and camera. At each visit the wound care nurse will manually measure and record the diameter of the ulcer and the depth. We will also record the pH of the wound.

A total of 75 study participants will be sampled longitudinally during the study. The second sampling will occur at least one month after the initial sampling.

Laboratory studies will be obtained and up to 80 mL of blood will be collected from all 300 participants. Please see Appendix D for list of labs/blood workup. Hemoglobin F levels will be measured for each participant currently living with and without leg ulcers in the study.

A blood sample (2.5 mL Paxgene tube and EDTA tube) and salivary sample from all 300 participants will be collected and stored indefinitely in the Clinical Center for potential future use. We will prepare DNA for future research use from all participants. We will obtain consent to conduct DNA sequencing at the time of original consent for the study. We will not perform DNA sequencing analysis of any of the participants in Phase I of the study. In Phase II of this pilot study we will add additional genomic sequencing researchers to the protocol to conduct studies that will identify the role of genetic modifiers in patients with and without leg ulcers. Specifically, we may conduct exome, whole genome, or targeted sequencing in a subset of the participants to study the genetic factors responsible for variation in leg ulceration in our patient population.

If a research participant is in the Clinical Center or Montefiore Medical Center for another study and consented to this protocol we may use blood/lab data obtained from the other protocol collected on the same visit for this study. We will also discuss with the participant the option to return at another date to complete the blood/lab work if needed.

**Cortisol Collection**

Hair cortisol collection

In order to evaluate long-term cortisol levels, we will ask all 300 participants to provide a small sample of hair, 3 cm or less. The amount of hair is approximately the width of a pencil, and will be trimmed from as close to the scalp as possible.

All samples including negative controls will be processed at the NIH. The Catch-All™ Sample Collection Swabs will be used in the clinic for microbial isolation from patients with active leg ulcers and those without leg ulcers (Epicentre, Madison, WI). All samples will be collected according to Levine’s technique, see Appendix C [[48](#_ENREF_48), [49](#_ENREF_49)]. Individuals without leg ulcers will be swabbed in areas where leg ulcers are commonly observed. Individuals with leg ulcers will be swabbed at the center of the ulcer and non-ulcerated skin using different swabs for each sample. Additionally, some patients have multiple leg ulcers on both legs. For a subset of the patients, we will sample each leg ulcer. Swabs will be stored in lysis solution provided with the MasterPure™ Yeast DNA Purification Kit (Epicentre, Madision, WI) in a -80°C freezer.

To complete the DNA isolation step, we will begin by incubating the skin samples stored in yeast lysis buffer and lysozyme (20 mg/mL) for 1 hour with shaking at 37°C. Two to three 5 mm steel beads will be added to each sample/microcentrifuge tube to mechanically disrupt cells using a Tissuelyser (Qiagen, Valencia, CA) for 2 minutes at 30 Hz. We will utilize the Invitrogen PureLink Genomic DNA Kit (Invitrogen, Carlsbad, CA) for all subsequent steps required to extract microbial DNA from each sample.

To amplify bacterial DNA, we will use the V1\_27F and the V3\_534R primer pair. To amplify fungal DNA, we will use 18SF and 5.8S-1R plus barcode primers. We will use the following PCR conditions: 2 μl 10X AccuPrime Buffer II, 0.15 μl Accuprime Taq (Invitrogen, Carlsbad, CA), 0.04 μl V1\_27F/18SF (100 μM), 2 μl primer V3\_354R/5.8S-1R (2 μM), and 2 μl of isolated microbial genomic DNA. We will perform the PCR in duplicate for 30 cycles (bacterial) and 32 cycles (fungal). The duplicate amplicons will be combined and the DNA purified using the Agencourt AMPure XP-PCR Purification Kit (Beckman Coulter, Inc., Brea, CA). We will then quantify the purified DNA using the QuantIT dsDNA High-Sensitivity Assay Kit (Invitrogen, Carlsbad, CA). An average of ~8-10 ng total DNA of each of the individual amplicons will be pooled and purified with the MinElute PCR Purification Kit (Qiagen, Valencia, CA). Samples will be sequenced using the Illumina MiSeq Benchtop Sequencer platform under the direction of Dr. Elizabeth Grice. Lastly, we will compare the microbiome of diabetic foot ulcers to SCD leg ulcer types. These two datasets will be mined for any obvious microbial signatures (i.e. similarities or differences) that are specific or shared between the different leg ulcers.

To analyze and classify the bacterial sequence data, the online computational tool for microbial identification and analysis, Qiime (<http://qiime.org/>) will be utilized [[50](#_ENREF_50)]. The following steps will be employed to successfully complete the analysis of the sequence data: remove chimeras, filter and align sequences, taxonomically classify sequences, pick OTUS based on sequence similarity within the reads, pick representative sequences for each OTUs, and generate phylogenetic trees. Qiime will be used to perform the alpha and beta diversity analyses to determine the community richness (Chao1), and the community structure (Theta Index) and membership (Jaccard Index) present in the sample set.

A member of the research team may contact the leg ulcer participants to monitor the healing process every six months after initial sampling for an update on the status of their health condition. A brief medical history questionnaire will be used during the follow-up telephone call (Appendix M).

**5.4 Approved drugs being used for research (*Include dosage in study and range approved for clinical use.*)**

N/A

**5.5 Unapproved drugs/devises (*indicate if approved for other use, include dosages in study, and IND or IDE number, if applicable.*)**

N/A

**5.6 Specific results that will be given to participants or their health care providers (*Is laboratory CLIA certified? What are the plans to return DNA sequencing results?*)**

Microbiome analysis will not be returned to the participants or their health care providers. If specific clinical abnormal test results or genetic information are identified and felt to be urgently medically significant, we will confirm our findings within a CLIA certified laboratory, and will let participant know. By “urgently medically significant”, we mean that these changes have immediate health implications for the participant or their family, and that there is an intervention or treatment that would be helpful.

**5.7 Describe questionnaires or other psychological instruments and estimate how long they will take to complete, and whether they address sensitive topics (*Enclose copies.*)**

Survey items can be found in Appendix F and G. The measures and items will address: the health behaviors, social, physical, and psychosocial information of each individual. The goal is to identify environmental factors that may impact leg ulceration in SCD patients and Quality of Life indicators. The project research coordinator or postdoctoral fellow will collect the survey data.

**Data Collection and Protection of the Questionnaire and Psychological Instrument Data:**

Responses to the survey data will be collected online using a 4 digit numeric code assigned to each participant (Identification Number) using the University of Wisconsin Survey Center (UWSC) Qualtrics website. The code number will be linked to the NIH ID number for each respondent. The participants that complete the study at MMC will be given a unique coded identifier different from the NIH participants. The study ID number will be maintained in a locked file at NIH and linked to the 4 digit data collection ID used for survey data collection. The ID will be used to merge personally identifiable information that is stored on NIH servers if needed.  With this method, the Qualtrics servers and UWSC staff will never have access to the ID or personally identifiable information of participants (e.g. name, address (street, city, state), email address, name of health care provider, medical history, date and place of birth, identifiable information about a family member or any other personal information which is linked to the research participant).

Survey data collected will be compressed into an encrypted file and made available for download from a secure UWSC server to the research team. When the principal investigator has received the file, the file will be removed from the server. Sample files will be handled only by the UWSC project director and programmer for this study, and will not be connected to study data files at any time.

The qualitative phase will utilize in-depth, semi-structured interviews. An interview guide will be provided to the interviewer to provide a framework for discussion. Interviews will be recorded using an audiotape recorder and will be transcribed into a written form.

Participants that have previously participated in INSIGHTS will be assigned the same 4 digit numeric code as the one used in INSIGHTS. Participants that are newly enrolled will be assigned a new numeric code, which will be linked to their NIH ID number. The numeric codes will serve as identifiers to link participants with their recorded data.

**Measures:**

The study includes PROMIS® instruments for Patient Reported Outcomes Measurement Information System, and the Adult Sickle Cell Quality of Life Measurement Information System (ASCQ-Me) measures of patient–reported health status for physical, mental, and social well–being [[51](#_ENREF_51), [52](#_ENREF_52)]. Specifically, we will use: (1) PROMIS short form Global Health Scale; ASCQ-Me Short Form measures: (2) Emotional Distress; (3) Pain Episode Frequency and Severity; (4) Pain Interference; (5) Quality of Care for SCD; and (6) Sleep. We will also use the online PhenX toolkit, which includes, standard measures for complex diseases, phenotypic traits, and environmental exposures [[53](#_ENREF_53)]. The following PhenX measures will be employed: health-related behaviors (alcohol and tobacco use, dietary supplements, etc.,), physical environment, psychosocial (depression, self-esteem etc.) (see below for more information). Other measures retrieved from PhenX highlight stress and its impact on health and stigma/racial identity in individuals living with chronic (and mental health) disease [[54-56](#_ENREF_54)]. It will take two 60-minute sessionsto complete the survey.

List of measures:

**Clinical Assessment** (Appendix E)

Medical History Information/Physical Assessment (modified from C. Minniti Leg Ulcer Study Medical History Form)

**Health Related Behaviors** (Appendix F)

TheAlcohol lifetime useis a single question that assesses lifetime exposure to alcohol (i.e., at least one drink of any kind of alcohol in respondent's entire life.)[[1]](#footnote-2)

The Alcohol 30-day quantity and frequency asks the respondent two questions about quantity and frequency of alcohol consumption during the past 30 days.[[2]](#footnote-3)

The Tobacco smoking statusis a one to three item questionnaire that determines if the respondent has smoked at least 100 cigarettes in his or her lifetime, and as appropriate, the frequency [[3]](#footnote-4)

The Tobacco 30-day quantity and frequency questionnaire is used to calculate the respondent’s 30-day quantity and frequency use of cigarettes. There are three sets of question protocols: (1) a protocol for Every-Day Smokers, (2) a protocol for Some-Day Smokers, and (3) a protocol for Former Smokers.[[4]](#footnote-5)

The History of being breast-fed is a single question that asks the respondent whether his/her mother breastfed him/her.[[5]](#footnote-6)

The Dietary supplements use is a measure that quantifies intake from commonly consumed dietary supplements including vitamins, minerals, herbs or other botanicals, or a concentrate, metabolite, constituent, extract, or combination of these ingredients.[[6]](#footnote-7)

**General Health, Psychosocial and Physical Function**

The Patient-Reported Outcomes Measurement Information System (PROMIS) Global Health Scale is a measure that consists of seven items. This measure assesses the respondent’s pain, fatigue, physical function, anxiety/fear, depression/sadness, and satisfaction with social roles and activities.[[7]](#footnote-8)

Physical Function measure is a PROMIS scale comprised of 10-items that address the respondent’s ability to engage in physical daily activities.

Brief Illness Perception Questionnaire has nine items. This measure has adequate test-retest reliability, and its predictive validity have been correlated with self-efficacy.[[8]](#footnote-9)

Stigma Measure: is based on the 22-item Internalized stigma of mental illness (ISMI) scale which measures the respondent’s internalized stigma due to their SCD health condition.[[9]](#footnote-10)

ASCQ-Me Pain episode frequency and severity measure includes five questions regarding the frequency, timing, and severity of sickle cell pain events.[[10]](#footnote-11)

ASCQ-Me Pain interference measure includes five questions that assess how the respondent’s pain within the last seven days interferes with his or her daily activities.[[11]](#footnote-12)

ASCQ-Me Sleep measure consists of five questions that assess the respondent’s ability to sleep within the last seven days.[[12]](#footnote-13)

**Health Care and Social Experiences**

Quality of care for sickle cell disease is a 27-item Adult Sickle Cell Quality of Life Measurement Information System (ASCQ-Me) scale that documents health outcomes in adults with SCD.[[13]](#footnote-14)

Medical Mistrust Index contains 7 items. These measure mistrust of health care organizations and allow for examining the relationship between mistrust and health care service underutilization. [[14]](#footnote-15)

ASCQ-Me Emotional distress scale is a five-item scale that measures the respondent’s anxiety and depression about their health condition [[15]](#footnote-16)

Beck Depression Inventory (BDI) is a 21-question multiple-choice self-report inventory that measures the severity of depression.[[16]](#footnote-17)

Positive affect and negative affect schedule (PANAS) is a 60 item scale that measures the two broadest dimensions of emotional state (positive and negative affect). The PANAS also includes subscales used to measure more specific emotions within these broad groupings. We are using a 20-item subscale for the PANAS measure.[[17]](#footnote-18)

Pearlin self-mastery scale is an inventory composed of seven items assessing the degree to which people perceive themselves as masters of the events that befall them.[[18]](#footnote-19)

Perceived social support/conflict scale is a 30-item scale that measures both perceived positive and negative social interactions with the respondents spouse or partner, other members of the family, and friends.[[19]](#footnote-20)

Rosenberg self-esteem scale is a 10-item scale used to evaluate the respondent’s general self-worth, with 5 positive statements and 5 negative statements about his or her sense of self-respect and value.[[20]](#footnote-21)

Stress Measures: The following stress measures include 95-items that will be used to assess a respondent’s perception of ongoing and enduring sources of stress in his or her life conditions: Lifetime, Past 5 years, Job Dissatisfaction, No Control, Job Insecurity, Work Demands, Job-Nonjob Conflicts, Job Hazards, Financial Strain, Total Economic Problems, Everyday Discrimination, Vigilance Against Discrimination, Job Harassment, Treated Unfairly Job, Marital Stress, Marital Abuse, Child-related Stress, Total Problems for Children, Friend Criticism, Parental Stress, Parental Educational Involvement, Hunger, Violence, Total Victimization, and Disorder.[[21]](#footnote-22)

Cohen global perceived stress scale is a 10-item scale that measures the respondent’s level of perceived stress in the past month.[[22]](#footnote-23)

Centrality and Regard subscales (14-items) of the Multidimensional Inventory of Black Identity (MIBI) measures the respondent’s racial identity as part of the person's self-concept that is related to her or his membership within the Black race.[[23]](#footnote-24),

Brief resilience scale is a six-item scale that assesses resilience as the ability to bounce back or recover from stress and may provide unique and important information about people coping with health-related stressors.[[24]](#footnote-25)

Self-compassion scale is a 26-item scale that measures compassion to one’s self i.e. self-kindness, common humanity, and mindfulness in instances of perceived inadequacy, failure, or general suffering.[[25]](#footnote-26)

Social desirability scale contains 5-items that evaluate the respondent’s tendency to give socially desirable responses.[[26]](#footnote-27)

John Henryism scale for active coping is a 12-item measure how individuals who actively cope with psychosocial stressors in the face of low socioeconomic resources are more likely to exhibit higher blood pressure levels than those with greater socioeconomic resources. [[27]](#footnote-28)

Perceived stressful racial discrimination instrument is a 14-item measure that assesses discrimination from African-Americans and non-African Americans[[28]](#footnote-29).

**Physical Environment (Appendix G)**

Current environmental tobacco smoke exposure is composed of four questions that asks about smoking at the respondent’s home and at work. If smoking occurs at the home, a household roster is utilized to document, which person smokes and the number of cigarettes smoked per day. The respondent is also asked if he/she smells smoke at work and if so, the number of hours per day the smoke is present.[[29]](#footnote-30)-[[30]](#footnote-31)

Characteristics of current residence asks 18 questions about the type of home, age, time lived in the residence, garage, pets in the home, and water damage.[[31]](#footnote-32)-[[32]](#footnote-33)

The Religiosity measure is 13-item scale that asks questions about the respondent’s religion/spirituality (Appendix H).

Additional demographic analysis information (Appendix I)

**PII Demographics (Appendix E3)** xxxii

-Birthplace

-Birthplace of parents

-Birthplace of grand parents

-Years living in U.S.

-Household roster-relationships

-Race

-Ethnicity

-Current educational attainment

-Current employment status

-Current marital status

-Health insurance coverage

-Annual family income

**5.8 Genetic counseling (*By whom, would counseling happen in person, will understanding be assessed?*)**

Genetic counseling will be made available in Phase I (microbiome sampling and social science and clinical measure data collection) if requested or recommended by the research team.

For Phase II genomic analysis, if we identify genomic findings that are deemed important to return the findings to the participant we will provide an opportunity for the participant to obtain counseling from a genetic counselor.

**5.9 Description of criteria for withdrawal from study.**

This study is completely voluntary and the participant may choose to withdraw at any time.

1. **Description of Study Population:** 
   1. **Estimated number of participants, enrollment ceiling, and anticipated enrollment by year.**

We will enroll up to one hundred fifty (n=150) participants in the microbiome sampling cohort. Fifty (n=50) participants with leg ulcers, fifty participants (n=50) without leg ulcers, and fifty participants (n=50) who never had a leg ulcer within the United States. In addition to the 150 SCD leg ulcer microbiome patients, we will recruit up to an additional 150 SCD patients that will complete the clinical evaluation, (including blood sample) and survey instruments for a total of up to 300 participants at both sites, NIH (n=200) and MMC (n=100).

We anticipate closing enrollment within **two years** of the study going into the field, with a total of 300 participants. Of the total participants we will resample the microbiome of up to 75 individuals from each of the 3 initial sampling groups: SCD with, without and never had SCD leg ulcers.

* 1. **Description and justification of clinical inclusion/exclusion criteria.** (*affected individuals, family members, controls? Define clinical criteria: Will this determination be made by review of prior records or will a screening evaluation be performed? Justify population choice in regards to age, gender, ethnicity, primary language spoken, prisoners, pregnant women, fetuses, people with impaired decision-making ability, healthy volunteers, lab personnel*)

Prior to enrollment, all participants will be screened on the phone by the research coordinator or research nurse using the following criteria below and the pre-screener questionnaire. New patients, who have never been seen at the NIH or MMC, will also be requested to send medical records as part of the screening phase.

**Inclusion criteria:**

Each subject must meet all of the following inclusion criteria during the screening process in order to participate in the study:

* All subjects must have a diagnosis of sickle cell disease (HbSS, HgSC, HbSB 0 or HBSB+)
* Be at least 18 years old.
* Provide written informed consent.
* For the Qualitative phase: must have a recurrent, active, or single-occurrence presentation of a leg ulcer(s).

**Exclusion criteria:**

Any subject that meets any of the following criteria during baseline evaluation will be excluded from the study:

* Pediatric population (<18 years old)
* Participants for microbiome study (only) who have received oral and/or topical antibiotics or antifungals < 2 weeks prior to enrolling in the study for leg ulcers (for those with leg ulcers only)
* Subjects presenting with clinically diagnosed bacterial infection (i.e. clinical appearance, clinical judgment, fever, redness around ulcer, purulent drainage etc.) at the site of ulceration (This can only be diagnosed clinically by the research nurse during sampling and is only applicable to those with leg ulcers only).
  1. **Location of study (*specify NIH Clinical Center facilities and/or other off-site locations*).**

All sampling, surveys, and processing of samples will take place at the NIH Clinical Center or at the second site, MMC. All samples will be stored at NIH.

* 1. **Description of recruitment strategies (*How participants will be identified; note efforts to include under-represented minorities; include copies of recruitment advertisements and letters.*)**

The research coordinator or research nurse will screen (using the inclusion and exclusion criteria) all individuals prior to enrollment and will consider self-referrals as well as physician referrals for participation in the study. To ensure we recruit an adequate number of participants with and without leg ulcers, we will rely on multiple recruiting methods, which will include posting flyers, social media advertisements (Twitter posting tweets and Facebook entries), and webinars, emails/phone calls directly contacting hematologists and adult SCD patient advocacy groups across the U.S. An example of the recruitment flyer is in Appendix J. The recruitment brochures and email letters are also attached (Appendix J). Example tweets and Facebook entries can be found in (Appendix S\_). We will also be mailing patient newsletter to update participants on the study.

As for the qualitative phase, the research nurse or associate investigator will screen (using inclusion and exclusion criteria) all individuals prior to enrollment and will consider self-referrals and physician referrals. We anticipate that most of the study population will consist of individuals who have completed the INSIGHTS study, but we will recruit participants if needed. Recruitment will include referrals, email/phone calls to hematologists and advocacy groups, and advertisements.

* 1. **For existing sample/data sets, note whether samples were originally collected for research or clinical practice. If obtained for research, include a description of the original purpose of study and prior plans for sample storage. Was consent obtained that would be applicable to this study? (*Include copy of original consent forms.*)**

N/A

* 1. **Description of any financial compensation. If participant withdraws early, describe whether compensation will be modified.**

We will compensate the study participants $100 for the microbiome sampling and $100 for survey completion, for a max of $200. Travel and one night local lodging will be provided for subjects completing the microbiome and survey. For those only completing the survey 4 to 6 hours will be required for completion. A subset of the participants will be resampled for a longitudinal assessment of microbial stability. The participants returning for a second sampling of their microbiome will receive $100. Anyone who withdraws from the study early will only be compensated for the portion of study they have completed.

We will compensate study participants $50 for the completion of the qualitative phase. Travel will be provided. Participants will not receive lodging due to the length of the survey (e.g. 1-1.5 hours). Anyone who withdraws from the study prior to completion will not be compensated.

1. **Description of study statistical considerations and/or analytic plan:** *Write a* ***brief*** *[****no more than 3 pages in length****]* *description of how data will be used to answer hypotheses, sample size and power calculations, methods of analysis, criteria for significance, as applies to this protocol.)*

**Social and Environmental Data**

Sample sizes were selected to allow for detection of predicted effects at *p* < .05 and power = 0.80, using effect sizes obtained in past relevant research to estimate the magnitude of predicted effects. The calculations of effect size, sample requirements, and power follow Cohen [[57](#_ENREF_57)]. Power calculations were performed using G\*Power 3 [[58](#_ENREF_58)]. As a large variety of analytic approaches will be utilized to thoroughly test the hypotheses of the proposed studies, we focus here, given space considerations, on power for the most central statistical tests.

In this study, the primary goal will be to test for condition differences on psychological and physiological measures. Using a one-way ANOVA approach, the test would require 19 participants per condition to detect significant between-group differences. In practice, the analytic models will include relevant covariates, which will greatly increase power. Nevertheless, to be conservative, we have proposed 30 per condition.

The mean, median, standard deviation, range, and percentage of participants scoring the minimum and maximum for each survey measure will be calculated. We will examine the measures in a multivariate context, beginning with simple descriptive statistics (measures of central tendency, measures of variability, and measures of bivariate relationships). We will explore the relationships between the measures and health outcomes including leg ulcers. We will also consider basic demographics, (e.g. sex, age, income, education).

**Microbiome Data**

There is currently no existing microbiome data on leg ulcers in SCD patients. Since this is a pilot study, we have used data generated from the diabetic foot ulcer microbiome to conduct the power calculations (provided by Elizabeth Grice, Ph.D.). The target sample size is up to 150 (n=50 with leg ulcers, n=50 without leg ulcers, and n=50 with no previous history of leg ulcer). In the previous microbiome study of diabetic foot ulcers from 52 patients, the standard deviation of Shannon diversity was 0.87 [31]. If the true difference in experimental and control means is 1.0, we will need to study 13 experimental subjects (SCD patients with leg ulcers) and 13 control subjects (SCD patients without leg ulcers) to be able to reject the null hypothesis that population means of the experimental and control groups are equal with probability (power) 0.80. The type I error probably associated with this test of this null hypothesis is 0.05. Anticipated recruitment of 50 individuals with leg ulcers and 50 without leg ulcers will provide ample statistical power to complete the analytic plan for the microbiome data. We will also highlight the relative abundance of pathogenic microbial genera like *Staphylococcus*, *Streptococcus*, and anaerobes. In microbiome studies, relative abundance is used to classify taxon whether bacterial or fungal present in a given sample. Individuals without leg ulcers should contain a lower relative abundance of pathogenic organisms in comparison to individuals with leg ulcers. Therefore, high relative abundance of pathogenic organisms correlates with the presence of an ulcer with delayed healing while low relative abundance of pathogenic organisms suggests the absence of a leg ulcer.

Additional microbiome measures of diversity may be included:

**Number of Operational Taxonomic Units (OTUs):**

Standard deviation=15.0 (mean 30.3)

Detect difference of 15, need 17 participants each

**Relative abundance of Staphylococcus:**

Standard deviation = 0.33 (mean = 0.32)

Detect difference of 0.30, need 30 participants each

**Bacterial load (logs):**

Standard deviation = 0.37 (mean =7.11)

Detect difference of 0.50 logs, need 10 participants each

Analysis related to hypothesis 1: The first hypothesis suggests that specific microbes are predominant in leg ulcers of SCD patients. To identify these microbes, we will first characterize the microbiome of HbSS leg ulcers and will include individuals without leg ulcers. The presence of such isolates may impede the healing process, worsening the condition and increase healing time. To address this hypothesis, we will process and prepare samples for DNA extraction and sequencing from all study participants. Samples will then be sent off for sequencing using the MiSeq platform. We will first check for chimeras and if any are identified, remove from the sequence file. Then, we will align and taxonomically classify our sequences using online microbial ecology tools like Qiime (or mothur) and a 16S (bacterial) and/or ITS (fungal) reference database to characterize the microbial communities present on the foot of HbSS patients with and without leg ulcers [[50](#_ENREF_50), [59](#_ENREF_59)]. Using the same bioinformatics tools, we will assign the amplicon sequences to operational taxonomic units using a percent similarity cutoff to further bin/group sequences. This step is important for the analysis employing diversity-based metrics to highlight, community richness (Chao1), and microbial community structure (Theta Index) and membership (Jaccard Index). We will also infer phylogenetic relationships using the same tools (UniFrac) mentioned above. To visualize these relationships among microbial communities, we may also wish to conduct principal coordinates analyses (PCA) to show similarities or differences between two environments (i.e. disease vs. healthy states). If we decide to sequence the fungal community present in the leg ulcers, we will follow the procedure described above with minor modifications, using CD-HIT for sequence clustering [[60](#_ENREF_60)]. We will complete the same analyses described above for the participants resampled in the longitudinal study.

Blood work/lab will be performed for each participant, (n=300). In particular, HbF and stress hormone levels will be assessed. We will determine if in our patient cohort association between HbF level and leg ulcers. Additionally, we postulate that the levels of stress hormones will be higher in the leg ulcer patient population.

Analysis related to hypothesis 2: The second hypothesis will compare the microbiome of diabetic foot ulcers with SCD leg ulcers to determine whether the two ulcer types are highly similar. Specifically, we will compare the data analyzed in hypothesis 1 to leg ulcer microbiome data from a recent microbiome publication on diabetic foot ulcers [[1](#_ENREF_1)]. Identification of key bacterial and fungal microbes may help inform clinical making decisions for the most effective treatment options. We will use bioinformatics tools within Qiime and mothur to evaluate the two datasets to identify similarities or dissimilarities in the microbial communities present in the two leg ulcer types. We have the ability to generate figures within both programs to visually represent our data (i.e. venn diagrams, phylogenetic trees, or heatmaps).

For hypotheses 1 and 2, we will use the statistical software, R (http://www.r-project.org/), to represent visually the taxonomic classification of the sickle cell leg ulcer and diabetic foot ulcer data. UniFrac will require phylogenetic information generated in earlier steps of the analysis to compare both leg ulcer microbial community datasets [[61](#_ENREF_61)]. We will also employ Spearman’s correlation to identify significant abundance relationships accounting for variation in each sample.

Analysis related to hypothesis 3: The third hypothesis will examine whether health behaviors, and physical and social environmental factors in adult SCD patients likely influence the onset and progression of leg ulcer formation. It is known that some adult SCD patients experience depression, anxiety and stress, and experience work-related problems. These psychosocial issues increase especially when SCD patients experience a painful episode or deal with a complication of the disorder (i.e. leg ulcers, renal dysfunction, or acute chest syndrome).

All study participating students will complete a baseline survey of clinical, psychosocial and environmental factors hypothesized to be associated with the microbiome. Microbiome data from participants with leg ulcers (n=50) will be compared to participants without leg ulcers (n=50), participants with no previous history of leg ulcers (n=50), and participants who complete only the survey measures (n=150). The baseline survey will assess: (A) health related behaviors, which include physical activity, medical history, alcohol use, tobacco smoking status, use of dietary supplements and history of being breast-fed; (B) physical environmental factors, that is, questions identifying living conditions in the home that may heighten or diminish formation and healing of leg ulcers; (C) clinical indicators of well-being, such as depressive symptoms; (D) current quality of life, such as, the type and intensity of social stress, self-esteem, perceived self-efficacy, and general emotional well-being; (E) stigma, related to leg ulcers and racial group membership; and (F) resilience, which includes assessment of spirituality and social support. Validated measures exist for each of these constructs.

Taken together, this baseline survey will serve three functions. First, it will provide data on predictors that can then be associated with leg ulcer formation and healing. Second, it will provide data on variables that might moderate the effect of the presence or absence of leg ulcers in SCD patients. Lastly, the effects of completing this baseline measure will be experimentally assessed via sequence analysis by comparing the microbiome of SCD patients with and without leg ulcers to a third cohort of participants with diabetic leg ulcers (see study design).

**Qualitative Analysis**

Two members of the research team will code transcripts independently. To establish inter-coder reliability, the researchers will review the transcribed documents for validity and consistency. If there are discrepancies in the transcripts or coding patterns, the audiotaped data will be re-transcribed by both reviewers until the differences are rectified. The final dataset will be reviewed to identify the most salient experiences, perceptions, and concerns related to the questions. NVivo qualitative research software will be used to support the coding and analyses of the data.

1. **Description of potential benefits of study:**
   1. **Direct benefits to participants *(Include only those physical or psychosocial benefits that derive directly from an intervention being studied)***

There are no direct benefits to the participants.

* 1. **Collateral benefit to participants *(medical or genetic counseling care and other benefits associated with being a research subject at the NIH that are not directly related to the specific study intervention. Do not include financial compensation as a direct or collateral benefit.)***

Subjects may receive indirect benefits from the study, such as being seen by a physician with expertise in leg ulcerations in SCD patients and who may provide a plan of care. The second piece of the study will highlight environmental factors that may influence the onset and healing of leg ulceration in sickle cell disease patients. This may allow patients to better manage their disease and reduce or eliminate exposure to environmental factors that may predispose them to leg ulcers with the goal of improving health outcomes i.e. ability to work, and significantly reducing stress, depression, and anxiety.

* 1. **Benefits to society**

The data generated from this study may generate novel interventions to be employed by hematologists, patients, and family members on the management of leg ulcers and coping strategies. Additionally novel therapeutics for treatment may be identified.

1. **Description of likelihood and seriousness of harms and how safety will be maximized:** *(Include potential physical and psychosocial harm from both research-related and medically-indicated procedures, alternative interventions that might be advantageous to participants, and provisions for medical or other professional interventions in the event of adverse events.)*
   1. **Therapeutic interventions (*drugs/devices/gene transfer*)**

N/A

* 1. **Diagnostic interventions (*blood draws/imaging/biopsies*)**

*Blood Drawing:* There may be some physical discomfort when we collect blood with a needle and there is a small chance that the participant may develop a bruise, feel lightheaded, faint, or develop an infection at the needle site. Routine blood drawing protocol will be followed to minimize this risk.

*Microbiome sampling by swab method:* During the leg ulcer sampling, we will take several steps to prevent pain. A staff clinician will order oral pain medication (at his/her discretion) prior to sampling. If unsuccessful at mitigating pain, a topical local analgesic will be placed above the leg ulcer to manage the pain. This approach is commonly used at the Georgetown Wound Care Clinic and provides relief to the patient during sampling and wound care process (and does not appear to alter the skin microbiome).

*Cortisol collection:* Hair cortisol - Obtaining hair sample for the test is associated with minimal risk, similar to haircut in barbershop/salon. Salivary cortisol - Saliva collection involves no risk, but may be inconvenient.

* 1. **Radiation (*Provide documentation of approval from Radiation Safety Committee.*)**

N/A

* 1. **Sedation**

N/A

* 1. **Psychological harms (*misunderstanding, anxiety, self esteem, depression*)**

We do not anticipate psychological harm to the participant during the study. However, the psychological survey measures may cause feelings of anxiety or depression. For the survey data collection, the research coordinator or the postdoctoral fellow will collect the data. If at any time during the survey, the participant seems uneasy or displays any level of discomfort, we will temporarily stop the survey and provide counsel to the participant. The research coordinator and the postdoctoral fellow will both receive training from Dr. Valerie Purdie-Vaughns, a Social Psychologist and Assistant Professor at Columbia University and an associate investigator on the project, on how to handle unique circumstances in which a participant experiences discomfort during a study. Dr. Purdie-Vaughns will provide examples and walk through these with members of the team to ensure that they are well equipped to handle any situation they may encounter during the course of this study.

* 1. **Risks to family relationships (*related to determination of genetic/disease status, parentage, adoption*)**

Identification of genetic variants that identify an individual or family at higher risk of a disease can be upsetting to the participant and family members as a result of participation in this study. If we return any genomic findings we will provide an opportunity for the individual and family members to meet with a genetic counselor.

Some genetic testing can also determine if people are directly related. If individuals learn that they are adopted or that their biological parent is someone other than their legal parent, we will not communicate such results with the participants unless it has direct medical implications for the individual or their family.

* 1. **Discrimination (*insurance, employment*)**

We understand that discrimination is a major concern for participants who undergo genetic testing. Therefore, we will inform participants and also report in the consent form that there are federal and state laws to protect them against genetic discrimination. Also, researchers who have access to the genetic information will take necessary measures to protect the participants from discrimination by their employer or insurance company.

1. **Description of how privacy and confidentiality of medical information/biological specimens will be maximized.**

**10.1 Will participant identifiers be attached to data, or will samples/data be coded or unlinked? (*Even if names are removed, how likely is potential identification?*)**

Once a participant is recruited into the study, the research nurse or research coordinator will assign each patient a 4-digit code that will be housed in a password-protected database only available to NHGRI key study personnel. Additionally, all samples/photos collected from patients will be coded.

**10.2 Description of any clinical/demographic information that will be included. (*age, ethnicity, sex, diagnosis, stage, treatment*)**

In the password protected file, we will keep the following information: current address, racial background, annual family income, age, ethnicity, gender, height, weight, birthplace, birthplace of parents/grandparents, years living in the U.S., household roster relationships, current educational attainment, current employment status, current marital status, health insurance coverage, and smoking and alcohol use. We will also collect information on each participant’s complete medical history: i.e. history of sickle cell disease complications, diagnosis (presence or absence of leg ulcer), past treatment(s), HbF level, duration of ulcer, whether individual is unilaterally or bilaterally affected, site of ulceration, history of leg ulcers, pain history, measures of necrotic tissue, wound tissue oxygenation, blood work, and any odors associated with leg ulcer(s). For a complete list of demographic and medical history questions administered in this study see Appendix G and E, respectively.

**10.3 How might this information make specific individuals or families identifiable?**

It is scientifically possible that genomic data collected may make specific individuals or families identifiable. Genomic data will be limited to the research team and dB Gap controlled-access database. Demographic, medical history and survey data will be maintained in protected files at NIH. However, we will ensure that the research team, primarily the research coordinator and the research nurse are the only individuals with access to this information. As stated above, the 4-digit code will be used to identify each study participant to maintain confidentiality.

**10.4 If research data will be coded, how will access to the “key” for the code be limited? Include description of security measures (*e.g., password-protected database, other*). List names or positions of persons with access to the "key" for the code.**

All study information will be kept in a password-protected file with limited access. The names indicated below will only have access to the protected file. The study Principal Investigator, Medical Advisor, and study NIH Associate Investigators will all have the access to the “key” for the code.

Please note that all key study personnel have met their respective institutions’ training requirements for human subjects’ research.

**10.5 Will pedigrees be published? Include description of measures to minimize the chance of identifying specific families.**

No pedigrees will be published. Four-digit codes will be used to de-identify study participants. Access to this information will be restricted to members of the research team.

**10.7 Will personally identifiable information be released to third parties?**

The participant’s current address and demographic information could make the individual, or family identifiable. No personally identifiable information (PII) will be made public and will not be included in any public databases (or released to third parties).

**10.8 Under what circumstances will data/samples be shared with other researchers or deposited in various repositories, biobanks, and/or databases voluntarily or as mandated by NIH policies (*e.g. dbGaP*)?**

Coded medical (phenotype) information, microbiome and genomic data will be put in a controlled-access database. Controlled-access data can only be obtained if a qualified researcher has been authorized by the appropriate Data Access Committee. The information in this Controlled-access database will be available only to researchers requesting access to conduct research on sickle cell disease and/or sickle cell disease leg ulcers.

**10.9 Describe any additional features to protect confidentiality.**

We will ensure that if data is shared with other researchers in the future, names and all other PII will be removed prior to the transfer of data. We will also maintain the code, which will be linked to each participant’s data/biological materials. The research coordinator and research nurse will be the only members of the research team that will have access to the PII.

We will ensure that each member of the team follows the guidelines established in the Genomic Data Sharing Policy for intramural researchers.

1. **Assessment of Risk/Benefit Ratio** *(Reasonableness of risks to participants in relation to the anticipated benefits of the study and in relation to the importance of the knowledge that may reasonably be expected to result.)*

We do not anticipate any risk(s) to the patient during either the sampling or survey. If there is a complaint during the study, we will address the concern before proceeding with the study. For individuals living with leg ulcers, it can be painful and debilitating, especially if it is a recurrent leg ulcer and resistant to most forms of therapy. Since this is the case in many SCD patients living with leg ulcers, it is reasonable risk to participants in relation to the importance of the new knowledge that may result.

1. **Unanticipated Problems: Collection, monitoring, analysis and reporting of adverse events and protocol deviations**
   1. **Describe all potential adverse events that can be anticipated and monitored for this protocol. If this is either a natural history or limited encounter protocol, explain this to the IRB and specify the occurrences that will be excluded from adverse event reporting. *(For natural history protocols, describe range of medical events independent of any protocol encounter that are known to occur in subjects who qualify for study enrollment. Natural history protocols will monitor, but not consider as reportable, occurrences that are purely a consequence of an underlying genetic or medical condition under study in a protocol. Furthermore, adverse events need not be ascertained in limited encounter protocols such as linkage studies or tissue array studies, in which NHGRI investigators are not providers of medical services.)***

Adverse events include injuries that occur from specimen collection for the study of the research samples including the genomic, microbiome and psychological measures. If the data collecton causes unintended distress to the participant.

An exception to this reporting will be a vaso-occlusive pain crisis and co-morbidities of sickle cell disease experienced by a subject, which occurs frequently and at unpredictable intervals in person with SCD, and may require hospitalization. These will be summarized and reported at the time of continuing review.

* 1. **Describe plan to monitor and report adverse events and protocol deviations, as outlined in SOP 16 (available at** [**https://federation.nih.gov/ohsr/nih/index.php**](https://federation.nih.gov/ohsr/nih/index.php)**).**

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations"). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, Unanticipated Adverse Device Effects and serious protocol deviations, will be reported to the IRB and CD (Clinical Director) as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

* 1. **Describe whether a Data Safety and Monitoring Board (DSMB) and/or any other additional monitoring measures will be used.**

Since this is not a clinical trials study, a DSMB will not be required for this study.

1. **Description of alternatives to participation** *(Other clinical or research interventions, if any, that participants should consider.)*

This study is completely voluntary and all participants have the option of not participating.

1. **Description of Consent Process**
   1. **Who will obtain consent *(PI, AIs)?***

Each study participant will receive an oral and written explanation of the consent form (attached with supporting materials). The study Principal Investigator, and Associate Investigators will all have the ability to consent and enroll participants.

* 1. **Setting where consent will be obtained *(location of in-person discussion, phone, mail).***

The consenting process will take place in person and at the NIH Clinical Center or off site at MMC.

* 1. **What information will be provided to participants? *(Include consent and/or assent forms, printed or web-based materials, phone scripts and any other related material.).***

The objectives of the study and the risk and benefits will be discussed with each study participant. Each research participant will be provided the appropriate consent form to read, review and sign prior to data collection (see attached materials Appendix N). The consent form will include the objective/purpose of the study, a confidentiality statement, any risks associated with the study, and the provision that states this study is completely voluntary. The participant can withdraw at any time and will receive compensation for the portion of the study they have completed.

A copy of the consent will be given to the participant and the research nurse will file the hard copy away in a locked cabinet in the Clinical Center.

* 1. **Protections for participants who may be vulnerable to coercion or undue influences *(pregnant women, fetuses, children, people with impaired decision-making ability).***

***For adults who may not be able to consent for themselves, the protocol should be consistent with NIH policy M87-4, available at*** [***http://cc-internal.cc.nih.gov/policies/PDF/M87-4.pdf***](http://cc-internal.cc.nih.gov/policies/PDF/M87-4.pdf)***. Specifically, all research protocols should state whether adults who are unable to provide initial informed consent are excluded or are eligible to enroll, and the conditions, if any, under which adults who lose the ability to provide on-going consent subsequent to giving initial consent, may continue to participate. If adults who are unable to consent are eligible for enrollment and/or continued participation, the protocol will describe the justification for their inclusion; how adults’ ability to provide initial and on-going consent will be assessed; that the permission of an appropriate surrogate will be obtained per this policy; the risks of the research and likelihood of benefit (if any) for adults unable to consent; the procedures for obtaining assent, and the procedures for respecting dissent; and any additional safeguards that will be used (e.g., consent monitoring).***

If the potential participant is unable to consent, they will be ineligible for enrollment in this study.

* 1. **Are there special circumstances regarding obtaining *consent? (Waived consent, opt-out, verbal consent, consent with speakers of other languages and translation of materials into other languages.)***

In instances when an eligible prospective subject’s preferred or primary language is not English and an approved protocol consent form is not available in the prospective subject’s preferred or primary language, an NIH-approved translated short form/oral consent process may be used, consistent with NIH policy 77.2 and SOP 12. The IRB-approved English language consent form will serve as the summary that will be provided to the participant by an interpreter.

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