Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

Complete answers must be provided. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose

The National Children's Study (NCS) is a national, longitudinal study that will be investigating the separate and combined effects of environmental exposures (chemical, biological, physical, psychosocial) and gene-environment interactions on pregnancy outcomes, child health and development, and precursors of adult disease. This application describes a substudy that will inform the development of the final protocol of the NCS. The substudy will be determining the parameters that will yield useful and reproducible data from placenta and cord blood being collected as part of the Vanguard Study. This study is critical for establishing not only the "gold standard" but also the minimally acceptable conditions appropriate for collection and sample preservation.

Participants

The placenta for this substudy will be collected from women who are enrolled in the NCS Vanguard Study at one of the seven original Vanguard sites, of which Duplin County, NC is one. Each of the seven Vanguards will collect 30 placentas for the purposes of this study.

Procedures (methods)

The placental specimens will be collected from the villous tissue with maternal decidua and chorionic plate absent. A maternal decidual flap will be created for removing the villous specimens in an attempt to leave the maternal decidua surface intact for morphology assessment. Immediately following the gross pathology assessment, specimens will be collected, preserved, and shipped to the University of Rochester Placental Processing Site (PPC) where the samples will be examined for an accurate assessment of morphology/pathology.

A.4.2. **Purpose and Rationale.** Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Background

The NCS protocol calls for the collection and evaluation of placentas. The University of Rochester's Placental Processing Site (PPC) has been storing the placentas collected by the NCS Vanguard Centers since the Vanguard Study launched in January 2009. The PPC has noted variability among the samples received, including variability in processing time of the tissue from the presentation of the placenta at the end of the labor to final acquisition of the tissue specimens by the PPC. The PPC has also noticed variability in the way the tissue is handled and processed by the Vanguard hospitals prior to shipment. The NCS protocol requires the placental specimens to be kept chilled. However, some the specimens have been frozen or fixed in formalin prior to receipt by the PPC. This variability in processing has raised questions how the different methods of specimen processing might be affecting the results of the specimen analysis.

Significance and Scientific Rationale

Each of the Vanguard Centers participating in the National Children's Study are following a protocol developed by NICHD to ensure that data are collected similarly across all the sites. Collection of placentas, however, has varied across the sites, with some hospitals preparing the placental tissue samples according to the hospital's standard procedure instead of the procedure outlined by the NCS protocol. This substudy will be evaluating placentas from each of the Vanguard Centers to determine not only the best method of collecting and processing placentas, but also the minimally acceptable conditions for doing so. Among other things, this study will help to standardize the collection process to assure that all specimens collected in the future as part of the NCS will be useful for the proposed stem cell banking, morphology/pathology, genetic and environmental assessments.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified. Researchers are reminded that additional approvals may be needed from relevant "gatekeepers" to access subjects (e.g., school principals, facility directors, hospital or healthcare system administrators).

This study involves collecting and evaluating 210 placentas from the deliveries of women enrolled in the NCS Vanguard Study. Thirty placentas will be collected from each of the seven original Vanguard sites, of which Duplin County is one.

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

The placentas will be collected from women enrolled in the NCS who deliver their babies during approximately an 8-month period beginning in early 2011 at one of the seven original Vanguard sites.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

The placentas will be processed at the Vanguard site hospitals (total of 30 at each of the 7 Vanguard sites). The specimens will be shipped to the NCS Placenta Processing Center (PPC) at the University of Rochester (UR) and to the Stem Cell Bank (SCB) at the University of California Davis (UCDavis). The placental specimens will only be collected from the villous tissue with the maternal decidua and chorionic plate absent. A maternal decidual flap will be created for removing the villous specimens in an attempt to leave the maternal decidua surface intact for morphology assessment. Immediately following gross pathology assessment, specimens will be collected and stored and shipped as described below.

A data sheet will be provided that must be completed in addition to any NCS information usually collected. The Vanguard sites must also document any concerns about the collection of each specimen.

Process:

- *Umbilical Cord*: Vanguard Site Staff will facilitate collection of a segment of umbilical cord at least 4 cm in length, clamped at either end with plastic cord clamps, ideally prior to delivery of the placenta or as soon after delivery of the placenta as possible.
- *Cord Blood*: In deliveries in which a cord blood bag is obtained, a 19-gauge needle and syringe will be used to draw off 10 mL of cord blood through the side port of the bag and aliquot immediately into a 10-mL tube.
- *Placenta*: Delivery staff will create a flap and remove multiple specimens of villous tissue within 10 minutes of birth (final time to be determined) avoiding any regions grossly noted to be infarcted. A 2x2x2-cm block of placenta will be removed and for stem cell processing. (i.e., 300 mg in RNAlater (two tubes 150 mg each); 2x2x2 cm each will be placed in 50 ml acid washed conical tubes and then kept on ice until frozen at -80c (all

times noted), and the remaining tissue will then be stored at 4°C until the fresh specimens are shipped with the data sheet and survey sheet to PPC to arrive within 24 hrs as per the standard NCS shipping protocol.

- *Shipment*: The specimens from the placenta will be sampled only from the maternal side creating a decidual flap of tissue with the villous tissue removed without disturbing the fetal chorionic plate. Once the specimens have been removed, the sponges in the collection kit will be used to absorb the blood that pooled where the tissue was removed. The blood will be allowed to soak into the sponge until a dry bed is achieved and then the placenta will be stored at 4 C or sent to pathology if required. Once pathology has completed its review, the placenta will be sent chilled with temperature monitors and the two tubes of tissue in RNAlater to the PPC. The NCS specimens will be collected under identical conditions and with identical techniques to evaluate the effects of storage.

Cord blood and placental specimens will be processed to isolate, expand, characterize, and cryopreserve stem and progenitor cell populations. Protocols for tissue handling, cell isolation and serum- and xenofree culture of stem cells from these sources following good laboratory and tissue practices have been developed. For cord blood samples, a small aliquot (<1 mL) will be removed for analysis in the Sysmex Automated Hematology Analyzer to document lymphocyte differentials and progenitor cell counts, and for flow cytometric analysis of HSC surface marker expression (e.g. CD34, CD45, CD133). From the remainder of the cord blood, mononuclear cells will be isolated by ficollgradient centrifugation and cryopreserved. For solid tissues, single-cell suspensions generated from dissected portions of placenta and amnion are cultured for 3-4 weeks and 2-3 passages to produce approximately 10s low-passage MSC) from each small sample for cryopreservation / banking. Following cryopreservation, neonatal MSC can be passaged multiple times. Expanded MSC cultures will be characterized using flow cytometry by positive signals for surface CD73, CD90 and CD105, in the absence of other stem and lineage markers. Additionally, we will perform growth curves and CFU-F assays prior to cryopreservation. We will process, test, and archive samples and generate data on study yields, specimen adequacy, feasibility, and costs.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

This study will not directly benefit individuals or communities but offers a societal benefit by helping the NCS to determine the parameters for successful specimen collection and processing.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects