

# SEVERE CHRONIC NEUTROPENIA TISSUE BANK PROTOCOL

## Introduction

The objective of this study is to learn more about the causes of severe chronic neutropenia through the study of blood, bone marrow, skin biopsies and buccal smears of people with diseases causing severe chronic neutropenia, autoimmune neutropenia, and idiopathic neutropenia, and their immediate relatives. The study compares the blood and bone marrow samples of people with severe chronic neutropenia in contrast to the samples of their immediate relatives or normal volunteers. We hypothesize that all of these conditions are caused by impairment of myeloid cell production and delivery to the bloodstream through accelerated turnover of developing cells in the bone marrow. We further hypothesize that the risk of leukemia in some, but not all, disorders causing severe chronic neutropenia is linked to the hyperproliferative response of primitive hematopoietic precursor cells which occurs consequent to the accelerated turnover of their maturing progeny. We will use the cells of the tissue repository and DNA, RNA, and proteins derived from these cells, to define the cellular, molecular and genetic mechanisms for these disorders.

## Objectives

The objective of this study is to learn more about the causes of severe chronic neutropenia through the study of blood, bone marrow, and other cells of people with this condition, and their immediate relatives. The study compares the blood and bone marrow samples of people with severe chronic neutropenia in contrast to the samples of their immediate relatives or normal volunteers. The study is looking at the genetic causes for severe chronic neutropenia and comparing the results of the subjects with neutropenia to the subjects without neutropenia. The information learned through this research may be of long-term value in understanding those conditions and developing new treatments.

## Subject Eligibility

### Inclusion criteria:

#### For Neutropenia Subjects:

- A confirmed diagnosis of severe chronic neutropenia as evidence by the following:
  - Documented absolute neutrophil count (ANC) of less than  $0.5 \times 10^9/L$  on at least three occasions.
  - Cyclic neutropenia subjects: It is necessary to monitor the ANC three times per week for a period of at least six weeks to establish the diagnosis of cyclic neutropenia.
  - For subjects with Barth syndrome, Shwachman-Diamond syndrome, Wiskott-Aldrich syndrome, Glycogen Storage Disease 1b, and Cohen's syndrome, a confirmed clinical or genetic diagnosis of the condition.

For Non-Neutropenic family members:

- Documentation of a family member with congenital or cyclic neutropenia.

For Normal Volunteers:

- Documented normal routine blood counts and differential.
- No chronic illness or medications.
- Greater than 18 years of age.

Exclusion Criteria:

For Neutropenic Subjects:

- Neutropenia known to be drug-induced.
- Thrombocytopenia (platelet count less than 50,000/cmm) or anemia (hemoglobin less than 8gm/dl) except in the case of Shwachman-Diamond syndrome or glycogen storage disease type 1b.
- Myelodysplastic syndrome, aplastic anemia, or other hematologic diseases without previous history of severe chronic neutropenia.
- Neutropenia due to known immune diseases such as rheumatoid arthritis, Felty's syndrome and systemic lupus.
- Previous chemotherapy for cancer (within the last 5 years).

For Non-Neutropenic Family Members:

- There are no exclusions for family members, all members may give blood.

Normal Volunteers:

- Smoking.
- Heavy drinking.
- Abnormal CBC.

## **Enrollment Procedures**

Neutropenic or non-neutropenic subjects will be screened for inclusion/exclusion criteria. The consent form is reviewed with each subject and if all the subject's questions have been answered and they agree to participate, the subject will sign the consent form. After signing the Consent Form, they are asked questions about their current health to assist us in filling out the registration form, which also includes information such as the subject's name and demographic information.

The registration form requires: the name, telephone/fax numbers and address of the referring physician, the signed consent, the name, demographic information, diagnoses for the subject, information on the treatment history for the subject, including growth factor cytokine treatment, and records of the subject's blood counts and other hematological tests. In addition, information is requested regarding neutropenia, leukemia, other blood disorders for immediate family members and relatives and whether the individuals listed are living or deceased.

## Collection Procedures

### Blood:

CBC and the research tissue bank sample should be drawn on the same day at the time of a routine blood draw.

#### Non-treated neutropenic subjects ( $ANC < 2.0 \times 10^9/L$ )

- In children less than 8 years old the maximum blood sample taken will be 2 ml/kg (approximately 1 teaspoon per 5 pounds of body weight) in ACD (yellow top tubes) or EDTA tubes.
  - A minimum of 10 ml ACD (yellow top tubes) or EDTA tubes should be drawn from infants; more blood is preferable (less than 10 ml is not valuable).
- For subjects over 8 years of age or greater than 80 pounds and NOT taking Neupogen or  $ANC < 2.0 \times 10^9/L$ , samples should be 20-30 ml (approximately 1-2 tablespoons) in ACD (yellow top tubes) or EDTA tubes.
- The tubes need to be labeled with the subject's name, the kind of specimen, i.e., blood, and the date of collection.
- The samples will be shipped by FedEx at room temperature to Dr. Dale's lab (we will supply the FedEx account number upon request).

#### Treated neutropenic subjects or non-neutropenic family members with $ANC > 2.0 \times 10^9/L$ :

- In children less than 8 years old the maximum blood sample taken will be 2 ml/kg (approximately 1 teaspoon per 5 pounds of body weight) in ACD (yellow top tubes) or EDTA tubes.
  - A minimum of 10 ml ACD (yellow top tubes) or EDTA tubes should be drawn from infants; more blood is preferable (less than 10 ml is not valuable).
- For subjects on Neupogen with an  $ANC > 2.0 \times 10^9/L$ , samples should be 10-20 ml of blood in ACD (yellow top tubes) or EDTA tubes.
- The tubes need to be labeled with the subject's name, the kind of specimen, i.e., blood, and the date of collection.
- The samples will be shipped by FedEx at room temperature to Dr. Dale's lab (we will supply the FedEx account number upon request).

### Buccal Smear (if applicable)

A small plastic spatula or spoon is used to gently scrape cells from the inside of the mouth wall to obtain a few cells from the surface of the subject's cheek inside the mouth.

### Mouthwash Rinse Sample (if applicable)

The subject is asked to rinse their mouth with water to remove any food particles and then do not drink or eat for one hour. At the end of the hour they are asked to swish approximately three teaspoons of mouthwash or sterile water from side to side in their mouth, continue to swish for about 30-45 seconds and then spit into a sterile container.

## Bone Marrow Procedure (if applicable)

### Neutropenic Subjects:

The bone marrow sample for research will be drawn at the time of a routine bone marrow aspirate. An extra sample of bone marrow, up to one teaspoon (5 ml), will be removed. Samples are collected in preservative free heparin. The samples need to be labeled with the subject's name, kind of specimen, i.e., bone marrow, and date of collection. The samples will be shipped by FedEx at room temperature to Dr. Dale's lab (we will supply the FedEx account number upon request).

### Non-neutropenic Adults:

For adults having a bone marrow aspirate for research purposes only, a sample of bone marrow, up to one teaspoon (5 ml), will be removed and collected in preservative free heparin. Samples are collected in preservative free heparin. The samples need to be labeled with the subject's name, kind of specimen, i.e., bone marrow, and date of collection. The samples will be shipped by FedEx at room temperature to Dr. Dale's lab (we will supply the FedEx account number upon request).

## Skin Biopsy (if applicable)

For adults, the skin biopsy is done with a local anesthesia (usually lidocaine 1% up to 1 ml or 1/5 of a teaspoon) injected under the skin to prevent pain. A very small hole, about the size of a pencil lead (2 mm) is created.

## Banking Procedures

The cells and material from them (DNA, RNA and proteins) will be frozen and kept indefinitely for research including genetic testing. The samples will not be identified with the subject's name, but will have a code number, which can only be linked to the subject's personal health data (the information on the form sent with the sample) by one of the researchers listed on the consent form.

## Privacy and Confidentiality

All cell samples that are collected for this study will be studied at the University of Washington and in other research institutions only for the purpose of increasing understanding and improving the treatment of severe chronic neutropenia. No links between study codes and subject names will be shared with anyone other than the researchers listed in the consent form. These studies are done for research purposes and are not diagnostic tests. All information collected for the study will be strictly confidential. Records with identifying information will have this information removed and given a study number. These files will be kept in a locked filing cabinet. The code that links a subject's name to the study number will be kept in a password-protected computer file and will be kept indefinitely.