products. This makes possible the generation of stocks of replication deficient recombinant retrovirus such as MFGS-gp91^{phox}, free of replication competent retrovirus. The specific strategy employed for the expression of viral gene product in 293-SPA cells differs in an important way from that employed for the construction of first and second generation packaging cell lines. In those cases, expression of the *gag-pol* and *env* proteins is accomplished via a single Mo-MuLV based proviral transcriptional unit which carries a deletion of the viral packaging sequences, termed ψ sequences, and employs heterologous DNA sequences, rather than the normal 3' LTR, for the polyadenylation and termination of transcription. Both the deletion of the ψ sequences and the elimination of the 3'LTR dramatically reduce the possibility that RNA sequences encoding viral gene products could be encapsidated and transferred to recipient cells. The design of 293-SPA cells, however, further reduces the possibility of the transfer of viral protein coding sequences, since two separate proviral derived transcriptional units are used to express the viral gene products (one capable of expressing only the *gag-pol* product and one capable of expressing only the *env* gene product). A full description of the construction and features of the 293-SPA line are found in a manuscript by Davis et al (1997) Hum. Gene Ther. 8:1459 Figure 4 shows the number of cross over events with estimated frequency which would have to take place to produce replication competent retrovirus in this vector/packaging cell combinations and illustrates the safety features engineered into this system.

Engineering of the MFGS-GP91^{phox} Producer Cell Clones:

The 239 cell parent cell line was obtained from the American Type Cell Collection repository. The same CRIP plasmid elements present in ψ-crip were transfected into 293 cells and a clone (293-SPA) was isolated with single copy each of the *gag-pol* encoding plasmid and the *env* encoding plasmid. Plasmid DNA containing the MFGS-gp91^{phox} recombinant retroviral vector was produced in Dr. Malech's laboratory at NIH and transfected into 293-SPA to obtain high titer producer clones. The complete DNA sequence of the entire retroviral vector was determined before proceeding with the producer cell isolation. Five clones were selected which appeared to produce high titer of vector as determined by using NIH 3T3 cells as a target. Further testing of the clones in the laboratory of Dr. Malech confirmed that one clone, 293-SPA-gp91-155, produced extremely high titers of vector capable of routinely transducing human CD34+ cells at greater than 60% efficiency. When used to transduce CD34+ cells from a patient with X-linked CGD, the transduction efficiency was greater than 70% (as determined by flow cytometry analysis using a monoclonal antibody which detects gp91^{phox} expressed at the cell surface). Furthermore, neutrophils derived in culture from the transduced X-linked CGD CD34+ PBHP demonstrated full correction of superoxide-generating capacity when compared to equivalent cultures of CD34+ PBHP from normal volunteers using both the DHR flow cytometry assay or a chemiluminescence assay of superoxide production by the transduced cells.

Cell Banks and Large Scale Lots of Recombinant Virus Vector Supernate: Production and Quality Control Testing

MFGS-gp91^{phox} Vector Production:

The MFGS-gp91^{phox} cGMP clinical grade vector to be used in this trial was produced by Magenta, a subsidiary of Microbiological Associates in Rockville, Maryland under contract from the National Institute of Allergy and Infectious Diseases, NIH. This cGMP grade MFGS-gp91^{phox} was produced from clone 293-SPA-gp91-155. The production method is described below for production of MFGS-gp91^{phox} clinical vector.

Production of vector was performed in 225 cm^2 plastic tissue culture flasks. Flasks were seeded with 8 x 10^4 producer cells/cm^2 and expanded over 6 days to confluence (about a 10 fold increase in cell number) in standard culture medium (Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum, DMEM FBS). DMEM FBS will be removed and the medium in the flask replaced with 50 ml of X-VIVO 10 supplemented with 1% human serum albumin for a 10 hour vector collection. The vector supernate from the first harvest was held at 4EC. After harvest the medium was replaced with DMEM 10% FBS for 14 hours and then a second cycle of medium change and vector harvest occurred. At the end of the third cycle, the harvested medium from all three harvests from all flasks were pooled as one lot, filtered, frozen and stored till use in Cryocyte (Baxter) flexible plastic containers in

100 ml aliquots. Producer cells from the production run and an aliquot of the production lot of supernate were subjected to testing for sterility, absence of replication competent retrovirus and transduction potency.

Microbiological Associates of Rockville, Maryland, the parent corporation of the subsidiary, Magenta, has a long history of experience with a broad array of safety testing of biologicals for many clients in the pharmaceutical industry. In fact the bulk of the sterility, karyotype documentation, and biological replication competent retrovirus testing for production of the Master and Working Cell Banks, and the cGMP production lots of clinical MFGS-$p47^{phox}$ supernate used in the first phase of this trial was performed under contract by Microbiological Associates. The Magenta subunit of Microbiological Associates was formed to introduce cGMP vector production capability. Magenta has produced cGMP lots of both retrovirus vectors and adenovirus vectors used in a variety of clinical trials. They have in place a vector production facility, which satisfies FDA requirements for such facilities. Together with the Magenta staff (and based on our own experience with Cell Genesys and the testing employed in previous production of MFGS-$p47^{phox}$ cGMP vector) we have determined a series of protocols and safety tests designed to achieve FDA allowance of the use of the 293-SPA-gp91-155 clone for production of clinical grade MFGS-$gp91^{phox}$ vector for use in clinical trial outlined in this modified protocol.
A seed vial of very low passage of clone 293-SPA-gp91-155 was expanded by Magenta and shown to be free of mycoplasma or other microorganisms in sterility testing. It was also shown to be free of replication competent virus using *Mus dunni* co-culture followed by a PG4 S^+L^- assay. A 100 vial Master Cell Bank (MCB) was expanded from this tested seed stock and frozen as 10^7 293-SPA-gp91-155 cells per vial. The MCB was kept in frozen storage at Magenta. The testing program for this MCB includes studies specifically required to validate human cell producer lines.

Attachment 1a.

Manufacturer's Master Cell Bank 293-SPA-MFGS-gp91-155-MCB Lot # 2037-0022 (09/30/97)

TEST	METHOD	SPECIFICATION	TEST RESULT
Identity	Isoenzyme	Human origin	Human origin
Sterility	21CFR610.12	Negative	Negative
Mycoplasma	FDA Points to Consider	Negative	Negative
Human Viruses: Epstein Barr Virus Cytomegalovirus Hepatitis B Virus HTLV 1/2 Adeno-Associated Virus Parvovirus B-19 HIV	 PCR PCR PCR PCR PCR PCR Co-cultivation assay	 Negative Negative Negative Negative Negative Negative Negative	 Negative Negative Negative Negative Negative Negative Negative
Adventitious Viruses	In Vivo Assay In Vitro Assay	Negative Negative	Negative Negative
Bovine Viruses (Test serum lot)	Bovine Virus Sensitive Cells	Negative	Negative
Porcine Viruses (Test trypsin lot)	Porcine Virus Sensitive Cells	Negative	Negative
Replication-Competent Retroviruses	Mus Dunni Amplification Feline PG4 S+L- Assay of Production Supernate	Negative	Negative
Replication-Competent Retroviruses	Mus Dunni Co-cultivation Feline PG4 S+L- Assay of Cells	Negative	Negative
Viability	Trypan Blue	∃ 30%	∃ 90%
Producer Cell Vector Sequences	Southern Hybridization	∃ 1 copy	∃ 1 copy
gp91phox ORF	DNA Sequence	Present	Present
MFG-S Safety Features	DNA Sequence	Present	Present
Packaging Sequences Copy Number	Southern Hybridization	pCRIPenv$^-$: ∃ 1 copy pCRIPAMgag$^-$: ∃ 1 copy	pCRIPenv$^-$: ∃ 1 copy pCRIPAMgag$^-$:∃ 1 copy
Virus Titer - Vector copy # in K562 Cells	Southern Hybridization and/or flow cytometry	∃ 0.1 copy or ∃ 10% of cells express gp91	∃ 10% of cells express gp91phox
gp91phox Expression in Transduced Patient CD34+ Cells	Chemiluminescence Assay of Oxidase Activity	∃ 5% of normal	∃ 5% of normal
gp91phox Expression in Transduced Patient CD34+ Cells	DHR flow cytometry	∃ 5% of normal	∃ 5% of normal
gp91phox Expression in K562 Cells Engineered to Contain the	Nitroblue tetrazolium dye assay	∃ 5% of cells positive	∃ 5% of cells positive

Complementary Oxidase Factors			

Attachment 1b.

Transducing Particles 293-SPA-MFGS-gp91-155-MCB-Retrovirus (RV) Lot # 1059-0001 Supernate and the End of Production Cells (02/24/98)

TEST	METHOD	SPECIFICATION	TEST RESULT
Sterility (Supernate and End of Production Cells)	21CFR610.12	Negative	Negative
Mycoplasma (Cells)	FDA Points to Consider	Negative	Negative
Virus Titer - Vector copy # in K562 cells (Supernate)	Southern Hybridization and/or flow cytometry assay with anti-gp91phox monoclonal antibody	∃ 0.1 copies or ∃ 10% of cells express the gp91phox protein	∃ 10% of cells express the gp91phox protein
Replication-Competent Retroviruses (Supernate)	Mus Dunni Amplification Feline PG4 S+L- Assay of Production Supernate	Negative	Negative
Replication-Competent Retroviruses (End of production cells)	Mus Dunni Co-cultivation Feline PG4 S+L- Assay of Cells	Negative	Negative
gp91phox Expression in Transduced Patient CD34+ Cells (Supernate)	Chemiluminescence Assay of Oxidase Activity	∃ 5% of normal	∃ 5% of normal
gp91phox Expression in Transduced Patient CD34+ Cells (Supernate)	DHR flow cytometry	∃ 5% of normal	∃ 5% of normal
gp91phox Expression in K562 Cells Engineered to Contain the Complementary Oxidase Factors (Supernate)	Nitroblue tetrazolium dye assay	∃ 5% of cells positive	∃ 5% of cells positive
Endotoxin (supernate)	Limulus (LAL) Assay	negative	negative

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: PRINCIPAL INVESTIGATOR: Elizabeth Kang, M.D.

STUDY TITLE: **AUTOLOGOUS TRANSPLANTATION OF GENETICALLY MODIFIED CELLS FOR THE TREATMENT OF X-LINKED CHRONIC GRANULOMATOUS DISEASE**

Latest IRB Review: Initial Submission
Latest Amendment Approved:

Standard

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

For the benefit of parents or legal guardians giving consent for minor children to participate in this study, "you" is defined as "your child" and "your" is defined as "your child's" in the remainder of this document.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

You are invited to participate in an experimental protocol to test the effectiveness and safety of gene therapy for Chronic Granulomatous Disease (CGD). Gene therapy is an experimental treatment designed to help increase the number of cells in your blood to fight infections in a more normal way. The following consent document gives background information on your disorder, explains the specific reason for doing gene therapy, the entire process, and the research goals for the study we are proposing. Gene therapy is complicated, but we hope the information we

provide here is clear enough to help you decide two very important things: First, whether gene therapy seems to be the right choice for you, and secondly, whether you agree to the experimental research protocol we are offering.

Purpose of this Study:

You have been diagnosed with having an inherited abnormality in your immune system called CGD or Chronic Granulomatous Disease. By taking part in this study, we will be able to determine if giving you gene therapy will correct the abnormality in your immune system so that you are better equipped to fight infections. A gene is a piece of a DNA molecule encoding the hereditary blueprint for producing a protein enzyme or a protein building block of a living cell. In this study, a segment of DNA, a gene, encoding a normal version of your defective CGD gene, will be inserted into the hereditary code of some of your blood cells.

Over the past several months, we have collected your cells (called stem cells) through a process called apheresis. A normal gene will be inserted into these stem cells, which hopefully will correct them into working better afterwards. You will receive a special type of medication (busulfan) allowing these corrected cells to replace abnormal cells in your bone marrow. This medication is designed to create space in your marrow, allowing the corrected cells to multiply and continue to produce normal infection-fighting white cells.

The method we are testing in this study will also provide information helpful in the development of gene therapy for other diseases affecting blood cells. Our long-term goal is to develop gene therapy that will cure CGD. We do not expect this present protocol to cure your CGD. However, it is possible that this gene therapy may partially treat your CGD by helping some of your neutrophils (infection-fighting white blood cells) to make what they are now lacking, hydrogen peroxide. This may result in an increased ability in your body's defense against infection. The information we gain from this study will help to determine what needs to be done to increase the survival time of these cells in the body and increase the number of corrected cells given through the process of gene therapy treatment.

What is Chronic Granulomatous Disease (CGD)?

Blood is made up of several components, such as red and white blood cells to name a few. Certain white blood cells are called neutrophils, which move in the blood system and function as germ killers. CGD is one group of inherited disorders affecting neutrophil function. In patients who have CGD, these neutrophils lack the enzyme machinery, called "oxidase". Oxidase normally makes hydrogen peroxide, which is necessary for killing germs, like bacteria and fungi. Without hydrogen peroxide, neutrophils cannot kill some germs and this is why repeated infections occur. Patients with CGD have an inherited defect in a small portion of their gene that normally guides the building of the oxidase enzyme machinery. There are four different genetic types of CGD. These correspond to defects in any of four CGD genes, which direct the production of the four building blocks of this oxidase. The most common type of CGD (2/3 of cases) is the X-linked form of CGD (affects **only** males) in which the gene coding for one building block of the oxidase called gp91phox does not work normally. Standard medical treatment for patients with CGD may include gamma interferon shots and/or medications such as antibiotics, antifungals and antivirals. Management of acute, much sicker episodes may include use of steroids and granulocyte (neutrophil) infusion therapy. CGD patients often undergo surgical procedures for abscesses (pus pockets) or other infected areas.

This protocol is designed to test gene therapy procedures following a moderate dose of the medicine busulfan.

What is Busulfan and How Does It Work?

The medication you will receive before you have your cells returned is called busulfan. Busulfan is a chemotherapy drug that has been given to many people for the treatment of leukemia, as well as in preparation for stem cell and bone marrow transplantation. In order to "clean the bone marrow" or make room for the stem cells in your bone marrow space to begin producing the corrected cells, we will treat you with a moderate dose of busulfan as a single agent. Busulfan ideally removes the abnormal myeloid cells and makes space for the stem cells (or graft) to produce new, properly functioning myeloid cells (particularly neutrophils). When given in high doses, it can kill almost all of the cells in the bone marrow. In lower doses, it tends to kill only a portion of the stem cells. For this reason, and from experience that we have from previous use, we have decided to use a moderate dose as the best dose for stem cell transplant in CGD patients. Busulfan does, however, have other possible side effects. As with many other chemotherapy-type drugs, you may have some nausea, vomiting, hair loss (alopecia), or venoocclusive disease (small obstructions in the blood vessels of your liver). Rarely, at high doses it can cause seizures (convulsions). As a precaution, you will be given a drug called Dilantin® to prevent any possible seizures from occurring. Also, since chemotherapy can suppress or destroy stem cell development for a short period of time, you will have blood drawn to check you for anemia (decrease in production of red cells) thrombocytopenia (decrease in the number of platelets, which are the cells that help with blood clotting) and leukopenia (low white blood cells, which help to fight infection). In extreme cases, a blood transfusion may be needed. Finally, a very rare but important complication, usually associated with receiving many doses of busulfan, is the development of an interstitial fibrosis of the lungs. Interstitial fibrosis means that hardened scarring can form in the tissues of your lungs. This can sometimes be treated with steroids and as stated, occurs rarely, but is important to know about.

What Are Stem Cells?

Stem cells are the basic building blocks from where blood cells arise. They divide to maintain their numbers throughout a person's lifetime and also mature to produce all types of blood cells, including lymphocytes. Stem cells are found in the bone marrow of people of all ages and in the umbilical cord blood of newborn babies. After a person gets injections of a medication called G-CSF ("granulocyte colony stimulating factor"), stem cells are released from bone marrow and travel through the blood vessels for a few days. Stem cells exist in small numbers and can be frozen and thawed while remaining alive. They can also be grown in test tubes, culture dishes or flexible plastic containers similar to those used for blood products. If they are handled in germ-free conditions with carefully controlled methods, such as in a clinical blood bank, they can be safely returned to the same person who donated them.

Before entering this protocol, you have already been given G-CSF, had stem cells collected, purified, and frozen under blood bank controlled methods.

What Is Stem Cell Gene Transfer?

This procedure is what is meant by the term "gene therapy". These corrected stem cells, which have been previously collected and processed in the blood bank, are then given back to you, often after being given some form of chemotherapy, like busulfan. This chemotherapy serves to make room in the bone marrow for the corrected stem cells. In this way you might be provided with a permanent, self-renewing supply of your own corrected cells. Because these cells are not from another person, problems with tissue matching, rejection, and Graft Versus Host Disease (GVHD) would be avoided.

New genes are "transferred" into stem cells outside the body in the controlled cell culture conditions mentioned above. Correct copies of the gene are contained in extremely small packages or "vectors". Although the components of a vector are made from naturally infectious viruses called retroviruses, the vector used for gene transfer has been modified so that it can no longer reproduce itself or cause an infection. When added to stem cells in culture, the vector particles attach to stem cells and deposit the gene they carry into the cells. When the stem cell accepts a transferred gene, it divides and all its descendents retain and express the transferred gene.

In order to be a good treatment for CGD, corrected stem cells would have to:
- re-new themselves by dividing, so they would last for years or even a lifetime,
- be able to produce the normal protein missing -GP91
- be able to kill bacteria and fungi

The technique of "retroviral-mediated gene transfer" is still experimental. Around the world over 1,000 adults and children with a variety of diseases have received their own or other peoples' cells that have undergone gene transfer with vectors made from retroviruses. One widely reported event, which occurred in 1999, was the death of a of young gene transfer patient, Jesse Gelsinger. Gelsinger was treated with a viral vector called "adenovirus" which is not related to the retrovirus gene delivery system used in this CGD gene transfer trial.

The major limitation of gene transfer is that human stem cells are rare and they do not accept transferred genes efficiently with the vectors now available. Thus, the number of corrected cells has been too low in most previous trials to make any difference in the clinical condition of patients. However, recently, a clear benefit has been achieved in a clinical trial for patients with CGD. Also, another clinical trial for patients with Severe Combined Immuno-Deficiency (SCID) using gene therapy has been observed to provide "cure" for some subjects.

Is Retroviral Gene Transfer Safe?

Researchers have wondered whether a transferred gene might sometimes cause harm to the cell and whether these cells may grow out of control and harm the patient. This has happened to three children in a French study. These cells grew out of control and developed into a leukemia (a type of blood cancer) that needed to be treated. There is also a chance that the components of retroviruses that introduce the transferred gene could get together with natural retroviruses in the body and cause an infectious virus to emerge, called a "replication competent retrovirus" or RCR. Therefore, patients are routinely tested for RCR for many years after gene transfer, and no cases of humans developing retrovirus infections after gene therapy have been reported.

Retroviral gene transfer to stem cells is a new technique, so the procedure may have unknown risks in CGD. Unforeseen problems may occur, and serious illness or death may result

What is the Clinical Experience with CGD and Gene Therapy?

In an earlier version of this protocol, five young adult patients with the autosomal recessive p47phox defective form of CGD were treated with a single cycle of gene therapy using an approach that is very similar to this current modified protocol. Beginning at three weeks following gene therapy in all five patients a very small proportion of neutrophils in their blood (about 1 in 2000 to 1 in 25,000) appeared to be gene corrected and capable of producing normal amounts of hydrogen peroxide. The corrected neutrophils were found in the blood for 2 to 6 months in the different patients. After that period of time, these corrected neutrophils disappeared from their blood. Thus, the effect of the single cycle of gene therapy was temporary and only provided a very small number of corrected neutrophils. We do not know if

this resulted in any benefit to these patients. These five CGD patients received their gene therapy in the period of July to December 1995. There were no complications from the gene therapy. Follow up testing to date indicates no unexpected side effects and all patients are stable. We will provide to you any additional current information about those results, the status of those patients, and the current experience with this protocol.

Who is Eligible for This Study?

You may participate if you:

- Are a male diagnosed with X-linked CGD.
- Have a minimum of 5.0 X 10^6 CD34+ cells per kg body weight (collected and cryopreserved, prior to enrollment) available for transduction.
- Weigh 20 kg or more
- Have an ongoing infection that is unresponsive to the standard treatments currently available.
- Have a multidrug resistant organism (infection) determined by lab results.
- Do not have a suitable sibling who is HLA-matched for stem cell or bone marrow donation.
- Are between the ages of 3-55 years.

Who is Not Eligible for This Study?

You may not participate if you:

- Weigh less than 20 kg.
- Are in another type of gene therapy trial while participating in this trial.
- Have unstable blood pressure that requires special I.V. medication to support your blood pressure.
- Require ventilatory assistance (need the help of a breathing machine) with high levels of oxygen.
- Are not using an approved method of contraception, such as a barrier method (such as a condom) with or without spermicide.
- Have an HLA-matched suitable sibling for stem cell or bone marrow donation.
- Are under age 3 or over age 55.
- Are intolerant of busulfan.
- You have a condition which is defined as being a Grade 4 toxicity where a Grade 4 toxicity means something that is serious enough that you need to be admitted to a hospital or get immediate medical attention to prevent death.

We will enroll a total of five participants into this protocol.

Description of Study Procedures:

The following will briefly describe each of the steps in this protocol:

- **Prior to enrollment** we will review the most recent studies of your immune system. If no testing has been done within 2 months, we will ask you to have more recent studies completed under a separate protocol with its own consent form. You will remain on your pre-gene therapy standard of therapy regimen under the care of your primary physician until the date of admission. (Your physician will also resume care post-gene therapy under the guidance of

our research team.) Total blood volume expected for these immune system studies are expected to be no more than 95 mL (a little more than 3 tablespoons) in adults and about 60 mL (2 tablespoons) for pediatric patients.

- We will set up the CGD gene therapy with at least 5 million CD34 cells per kilogram of your weight. These CD34 cells have been obtained under other approved protocols and stored in blood bank conditions. If obtained on more than one apheresis collection, the cells will be pooled together.
- The cells will be specially prepared before they are given back to you. Before giving them back to you we will also make sure no bacteria, other germs or toxins are detected in the cells.
- You will be admitted to the NIH Clinical Center on the day prior to the start of the chemotherapy (busulfan). You will have a large I.V. placed in your body called a "central line", if you do not already have one. The I.V. will be inserted by the trained staff of the Intensive Care Unit or by the Interventional Radiology staff in the Department of Radiology. Numbing medicine or conscious sedation will be administered by either an anesthesiologist or other individual certified to administer conscious sedation, to prevent pain during the insertion of the line. You will then be given the busulfan through that IV once daily for two days. You will also receive (phenytoin) Dilantin® prior to each dose of busulfan in order to decrease any risk of seizures. After receiving the first dose of busulfan, we will draw a series of small blood samples, at certain time intervals, to measure the blood levels of the busulfan in your body over time (not to exceed 20 mL or 4 teaspoons).
- You will receive a second dose of busulfan on the **second** day, along with a second dose of (phenytoin) Dilantin®. No levels will be drawn on the second day.
- When the cells are ready and have passed all safety checks, they will be returned to you through the I.V. over 10 minutes on the **third** day. Vital signs will be taken often as a safety measure. You will be monitored for at least 3 days or until we are satisfied that you are stable and in good condition. Chemotherapy typically drops the number of your white blood cells for a certain period of time, for which you will be required to remain in the hospital, usually expected to be around 8-12 days after the last dose of busulfan. You will be allowed to go home when your white cells are sufficient enough in number to not place you at greater risk for infection. This will be determined after the review of daily lab draws.
- How long you remain close to the NIH Clinical Center will be determined by your lab values, your infection and your overall physical condition. The estimated amount of total blood to be drawn will not exceed 450 mL (15 tablespoons) over a 6-week time period for adults per the NIH Clinical Center Guidelines. Blood drawn for children will be limited by their weight and will not exceed 7 mL per kilo (about 1 ½ teaspoons per 2 pounds body weight) over a 6-week time period.
- After you receive the gene-transferred cells, we will want to keep track of all aspects of your health, including number of infections, need for antibiotics, missed school or work days, etc. You should continue to receive the same care as before, antibiotics, and other medicines determined by your doctors at home, who are encouraged to discuss any changes with us. We will treat you for your underlying infection while you are enrolled in this protocol. We will ask you and your local doctor for updates every 3 months, but you should get in touch with us sooner if anything unexpected or serious happens. We will ask you to sign release forms for your outside medical records to be sent to us by your local doctor(s) and the medical facilities where you receive care.
- For the first three months following gene therapy, we will call you once a week to check on your condition. We will also ask you to return to NIH each month for the first 3 months then every 6 months for 42 months after the gene

transfer. During these visits, (months 1, 2, 3, 6, 12, 18, 24, 30, 36, and 42) we will conduct a history and physical exam, and ask you to fill out a Quality of Life Questionnaire (except on Months 1 & 2) and draw a blood sample. These blood samples will be used to screen for any harmful effects of the treatment. At 3 months and thereafter we will determine how many T cells, B cells, and NK cells (the white blood cells that fight germs) you have and whether your cells contain newly inserted copies of the CGD gene.

- Further follow-up will be visits to your home doctor's office. As stated above, at least every 6 months at NIH we will review your health, examine you and take a blood sample to check your immune system and make sure other blood values are within your normal ranges. For the in-between months (months 9, 15, 21, 27,33, and 39 after receiving gene therapy) someone from our team will telephone you to see how you are doing. At these times we will arrange for you to have blood tests collected locally and sent to NIH for immune function and other tests. You will be provided blood-sampling kits. You are encouraged to call us any time you have concerns, and we may ask you to come to NIH to have these concerns looked into.
- For your safety, 42 months after receiving gene therapy, we will ask you to come to the NIH every 6-12 months for a history and physical exam and blood work for at least 15 years. Long-term follow-up after gene therapy is part of any trial that uses a retroviral vector for gene therapy. These visits will not be conducted for research purposes but to make sure you are not experiencing any bad effects from the corrected cells. This is because there could be very late effects of gene therapy, and we want to screen you for any late complications and learn from them. The blood samples we collect will be used to look for abnormal cell expansion or leukemia, and part of the samples will be stored in case further testing is needed. If you should die, we will ask your family for permission to perform an autopsy (a medical examination after death with analysis of pieces of tissues). In such a case, it would be particularly important to examine small samples of your bone marrow, liver, and lungs, all places where the gene corrected blood cells grow or work to prevent infection. If an autopsy is done elsewhere, we will ask to obtain tissue specimens for examination for replication competent retrovirus (RCR), as well as studies of insertion sites of the gene therapy vector.
- We would want to know whether the new gene persisted in blood cells, whether it spread to other parts of the body or if it caused any problems.
- **If there are no signs of gene transfer or improvement in your immune function**, you would still be eligible to undergo a matched unrelated donor bone marrow transplant, however, eligibility for such a transplant can only be assured based on clinical realities. You are free to explore the option of a matched unrelated donor here, if available, or at other transplant centers. You may also be eligible for a future protocol at NIH or elsewhere that might work better for you. However, after your first infusion, no further gene transfer within this protocol will occur. Any future trial will be discussed with you in detail and would require you to sign a different consent form.

How May You Benefit from Gene Therapy?

If you have an active severe infection, or have had recent relapses of such an infection, there may be an immediate benefit to you from the gene therapy by helping your body produce normal neutrophils that make hydrogen peroxide to fight this infection. This may be the case even if both the number of corrected cells is small and the time in which they are detected is short. CGD patients with very severe infections are sometimes given neutrophil transfusions from normal blood donors. However, most individuals develop an immune reaction (like a severe allergic reaction) to these donor neutrophils leading to reactions to the transfusions. Many CGD patients can no longer receive neutrophil transfusions because of severe immune reactions. In addition, there are increased risks of contracting blood borne

diseases when receiving transfusions from large numbers of donors even with the most careful testing by the blood bank. Gene therapy is an alternate way of providing these infection-fighting normal neutrophils and avoiding the problem of multiple transfusions from multiple donors.

You may also benefit from this gene therapy because corrected neutrophils in your blood provide some protection (prophylaxis) against development of future infections. The greater the number of corrected neutrophils in your blood and the longer they remain in your body, the greater is this protective effect. Some immune system blood cells called monocytes are similar to neutrophils and produce hydrogen peroxide to kill germs. Unlike neutrophils, which are short lived, monocytes settle into your lungs, liver and other organs where they live a very long time. It is theoretically possible that gene corrected monocytes could provide infection protection in the liver and lungs for much longer than corrected neutrophils detected in blood. In addition, new information gained from these studies also might benefit society in general by contributing to the future development of improved methods of CGD gene therapy that provide long term protection against infection or even a cure.

An indirect benefit of this study is that it will increase our knowledge of human gene transfer. The results of this study will help researchers learn how to use gene transfer for other patients with CGD and related diseases.

Compensation:

There is no financial reimbursement for participation in this gene therapy study.

Risks and Discomforts:

Gene therapy is experimental. The retrovirus technology, culture system and other materials and procedures used in this trial are based on our own and other researchers' previous gene therapy studies. Adverse reactions may occur, and such reactions could lead to serious illness or even death.

Risk of Cancer: When a retroviral vector enters a normal cell, the DNA of the vector inserts itself into the normal DNA of that cell; this process is called integration. Most integration is expected to cause no harm to the cell or to the patient. However, there may be some regions of the normal human DNA where integration of the viral vector's DNA may result in activation of neighboring genes. For example, if a vector were integrated into cellular DNA near a gene encoding a growth factor, uncontrolled division of the cell could occur, resulting in cancer. This type of event occurred in one animal study, where vector integration gave rise to cancer in mice.

In 2002, a similar event was identified in three children who received a retroviral vector in the experimental gene therapy study for XSCID (another type of inherited immune dysfunction) conducted in France. While most of the children who participated in this clinical trial appear to have been cured of their disease, three children have developed leukemia (a form of cancer of the blood) approximately 30 months after receiving the gene therapy treatment. These patients had extensive testing done to determine the cause of the leukemia. A group of experts looked at all the test results, and concluded that the gene therapy caused the leukemias. One of the children responded to chemotherapy treatment of the leukemia and appears to be in remission with continued correction of his immune function from the gene therapy, but his long-term prognosis is unknown at this time. However, the second of the two children had a relapse of the leukemia after chemotherapy, underwent a matched unrelated donor bone marrow transplant, and then died as a result of this relapse of the leukemia. The third patient appears to be doing well after treatment with chemotherapy; however, it is too soon to draw conclusions on what will be the outcome. It is important to note that these three cases of leukemia occurred in the XSCID gene therapy French studies and we have not had any reported cases of leukemia in our U.S.

studies nor in similar studies conducted by others in the United Kingdom. The risk of another cancer, including leukemia, developing in the children already treated in France is unknown.

There is a risk of developing leukemia as a result of receiving gene therapy, which you should be aware of if you decide to enroll into this study. This is a serious risk because cancers of the blood can lead to death. We will need to watch for the development of cancer in you by doing regular check-ups and blood counts for your entire life. In the event that a cancer does develop in the genetically altered cells we return to you, we would provide treatment. Blood cancers may be treated with chemotherapy and/or bone marrow transplantation, which in your case would be with an unrelated donor if one can be found.

Risks of Using Retroviruses to Transfer New Genes to Blood Progenitors (Stem Cells):
Previous clinical protocols using recombinant retroviruses have not yet revealed any long term toxicities associated with gene transfer. However, in theory there are several areas of concern about the gene transfer process. The retrovirus used in this study to carry the normal gp91phox gene into your blood progenitor (stem) cells is produced by cell lines of human or mouse origin. Both the viruses and the cell lines have been altered to make it very unlikely that the virus particles produced by the lines are capable of multiplying in other cells. The gene therapy retrovirus particle is supposed to be able only to deliver a gene to a new cell, such as your cultured blood progenitors (stem cells), and then be incapable of multiplying in that new cell. Very sensitive tests have been devised to test for retroviruses capable of independent growth in a new cell. While these tests are extraordinarily sensitive, there always remains the theoretical possibility that such a virus might be present. The main concern about replication competent retrovirus (RCR) is that in a study of monkeys where replication competent virus was known to contaminate the retrovirus gene transfer solution, the animals subsequently developed cancers of the lymph nodes.

In this protocol, any retrovirus solutions added to your cultured progenitor (stem) cells have been tested extensively to be as certain as possible that RCR is not present. If not present in the retrovirus solution added to your progenitors, it is very unlikely that such an infectious retrovirus would then be produced in the cultures of your gene altered blood progenitor cells. Despite this, it would be best to test your corrected stem cells for the presence of RCR before these cells are returned to you. However, the special tests required for RCR testing take many weeks to be complete and the progenitor cells need to be reinfused by day four of the culture or the cells lose their ability to survive and grow in the body when reinfused. Because of this, we propose to start the testing for RCR on a sample of the gene therapy corrected progenitors and give you the cells before having the results of this testing. While theoretically some risk remains, we believe that the extensive testing of the retrovirus solutions before addition to your cells in culture greatly reduces and likely eliminates this risk.

Risks and Discomforts of Obtaining Blood Samples:
During this study, we expect that no more than about 95 ml (about 6 tablespoons) of blood will be drawn at each scheduled visit for adults. The volume will be proportionally less for children and be based according to their weight. The risks and discomfort from needle sticks for blood drawing may include pain and bruising at the site, a lump at the site, and possible lightheadedness or even fainting. A very small risk of infection exists. Accessing an indwelling (existing) venous catheter for blood samples may also cause discomfort and has a low, but potential risk of clotting or infection of the catheter. To minimize the discomfort of needle sticks, topical numbing cream can be used. Ask your doctor or nurse about this numbing cream. The amounts of blood obtained for studies will be monitored to prevent development or worsening of anemia (red blood cell counts that are too low).

Risks and Discomforts of Receiving Busulfan:

The most commonly listed side effects experienced by patients receiving busulfan therapy include nausea and vomiting, rash, itchiness of the skin, high blood sugar, low blood phosphorus and magnesium levels, increased potential for seizure, bone marrow suppression, soreness in the mouth, hair loss, infertility, veno-occlusive disease, potential for birth defects, pulmonary fibrosis (lung tissue scarring), and cancer. Risks and discomforts are generally more associated with patients receiving higher doses. The risk of seizure will be minimized by giving you phenytoin (Dilantin) 30 minutes prior to busulfan treatment.

Risk and Discomforts of Central Line Placement:
Central line placement catheter carries a small risk of bleeding, bruising or pain and a very low risk of accidental injury to the nearby artery or nerve. These risks and discomforts associated with placing and utilizing a central line are balanced by not having the pain and uncertainty of multiple I.V. insertions or blood draws in your arms. The risk of a having a central catheter is small, includes the potential for hematoma at the site of insertion. Very rarely pneumothorax (collapsed lung), may also occur. Using only trained and experienced staff for the line placement procedure, as discussed above, will minimize these risks.

Risks at the Time of Injecting Gene-transferred Cells:
Based on both published and unpublished results of clinical trials of gene therapy, the infusion of cultured CD34 cells that have undergone retroviral gene transfer has not caused any significant immediate reactions. There have been cases of allergic reactions, including temporary changes in blood pressure or breathing trouble, that may have been due to trace amounts of substances carried over with the cells from the culture. However, extensive washing of the cells before returning them seems to reduce these reactions, and such washing will be done in this study. Cells that have been in culture can potentially clump together or stick to blood vessels in the lungs, causing temporary shortness of breath, wheezing, low blood pressure, dizziness and fever. If this happens, the cell infusion will be stopped and treatment will be provided as needed with oxygen, antihistamines, steroids, other medications, and fluids.

Because the CD34 cells are maintained outside the body for several days, they could become contaminated with germs. We will minimize this risk by keeping the cells in sterile, sealed plastic bags. In addition, on the day before and on the final day the cells are given back, we will perform cultures for germs and other sensitive tests to detect contamination. Cells with any sign of contamination would not be infused. If during the cell infusion you were to develop chills, fever, low blood pressure or shortness of breath we would be concerned that germs we were unable to detect might be present. We would stop the infusion and give you appropriate antibiotics and any other necessary treatment to prevent infection.

Some patients receiving gene-transferred cells have developed immune responses to products that come in contact with the cells, such as mouse-derived antibodies for isolating CD34 cells or growth factors present in the cell culture fluid. Such immune reactions have been uncommon and have not been associated with clinical symptoms.

Are there any Special Safety Concerns for Hospital Staff Handling your Blood?
This study will require multiple blood draws from you and involve extensive handling of your blood cells by laboratory personnel who will practice Universal Precautions Your blood be initially tested for hepatitis viruses and the virus which cause AIDS. These tests will be done only if they have not been done within the last six months. These viral illnesses may have an impact on your health and also on the outcome of the study. You will be given the results of these screening tests regardless of whether you are enrolled in this study. Whenever testing is done for the AIDS virus as part of a research protocol, it is an NIH rule that a page containing specific wording be included in the consent form. This is included as the next to last page of this consent.

Risks of Conditioning and Transplant on Fertility:
Most patients are usually unable to have children after receiving chemotherapy drugs and transplants. Some of these patients consider having their eggs or sperm banked for future reproductive purposes before undergoing transplant therapy. This service is not offered as a part of the transplant here at NIH. Although this study does not use a chemotherapy (busulfan) that will totally ablate (wipe out) your bone marrow, it is not clear what the future effects will be on your ability to have children.

New Information about Gene Therapy Research:

Gene therapy is a rapidly developing field, and new advances are reported almost every day. It is important for you to have new information about your health. As long as we are able to get in touch with you, we will inform you of new developments in gene transfer that may impact your health or otherwise be important for you to know. If your address or phone number changes, be sure to let us know. Of course, we will stop contacting you and discontinue your participation in any part of this protocol at any time at your request. However, it is highly advised that you return as planned so that we may perform the scheduled physicals and safety monitoring.

Study Withdrawal:

You should be aware that your participation in this study in entirely voluntary, and that you may withdraw at any time; however, if you have been given the busulfan, we will strongly encourage that you receive the cells, as without them you will have a longer period of low white blood cell counts which will increase your risk of developing another infection and/or worsening your current infection. It is also highly advised that you return so that we may perform the scheduled physicals, blood samplings, and especially to monitor for the risk of leukemia and replication competent retroviruses (RCR).

Stored Samples and Future Research:

We may take extra blood and tissue samples and store them for future research. These samples will help us learn more about CGD or related conditions. In general, the research tests we perform are not like routine medical tests, and may not relate directly to your medical care, so we may not put future test results in your medical record. However, if you wish, someone on the study team will discuss the test results with you. We will not share these test results with your private doctor unless you ask or permit us to do so.

By agreeing to participate in this study, you do not waive any rights that you have regarding access to and disclosure of your records. For further information on those rights, please contact Dr. Elizabeth Kang.

Labeling of Stored Samples
We will label your stored samples with a code and not with your name so that only the study team can link to you. We will keep any information that can be traced back to you private to the extent permitted by law.

Release of Medical Records:

In the course of applying for certain types of insurance (e.g., medical insurance, life insurance, disability insurance), people are often asked to sign forms that authorize insurance companies to obtain medical records. If you sign such a form in the future it is possible that the insurance company will present this form to the Clinical Center of the National Institutes of Health. In that event, the National Institutes of Health will comply with your request to provide the insurance company with your medical record. It is possible that the information contained in your medical record might affect (favourably or unfavourably) the willingness of the insurance company to sell you insurance.

Future Studies:

Other investigators may want to study your stored samples. If so, the NIH study team may send your samples to them, (without any information that can identify you). The study team may also share information such as your gender, age, health history, or ethnicity. In some cases, an Institutional Review Board (IRB) will review new research proposals that would like to use your samples. The IRB is a committee that oversees medical research studies to protect volunteers' rights and welfare.

Investigators will *only* use your samples for research. We will not sell them. Future research that uses your samples may lead to new products, but you will not receive payment for these products. Some future studies may need health information (such as smoking history or present health status) that we don't already have. If so, the NIH study team will contact you for this information.

Benefits
In general, future research that uses your samples will not help you, but it may help us learn what causes CGD or related conditions. This research may also help us learn how to prevent or treat the condition.

Risks
The greatest risk related to future studies using your stored samples is that someone may take information from your medical record without permission. The chances of this happening are very low. If this information becomes available, you may face discrimination when you apply for insurance or a job. You may also have similar problems if you share the information yourself or let us release your medical records.

Making Your Choice
If you agree to participate in this study, you agree to let us store your samples for future research. You also agree that we can contact you again in the future. No matter what you decide, you may still participate in other studies at NIH. However, your refusal to let us store your samples may lead to your withdrawal from this specific study. Even if you agree now to let us store your samples, you can change your mind later. If you do, please contact us and say that you do not want us to use your samples for future research.

Alternatives Treatments or Procedures:

The current standard therapy for managing CGD may include using a combination of prophylactic antibiotics, antifungals, steroids, gamma interferon injections and occasionally resorting to surgery and granulocyte transfusions for more acute (sicker) episodes.

The only available treatment other than the standard therapy above is a bone marrow transplant, (BMT), especially from a HLA-match sibling donor; however, in order to be eligible for this study it has already been determined that you

do not have an HLA matched sibling. Using an unrelated donor is also a possibility, and this alternative should be discussed with one of the primary investigators of the trial as well as your own home physician.

Because gene therapy is experimental and the long term consequences of such treatment are not known, federal agencies regulating such therapy discourage patient participation in more than one type of gene therapy trial at the same time. You cannot be enrolled in other protocols involving gene therapy at the same time that you are participating in this gene therapy protocol. However your enrollment in this gene therapy protocol may or may not prohibit you from participating in any future gene therapy protocol, whether it is for CGD or another disease

As part of your participation in this study, it will be necessary to test your blood for the presence of antibodies to the Human Immunodeficiency Virus (HIV), the virus that causes Acquired Immune Deficiency Syndrome (AIDS). In order to perform the test, a small amount of blood (approximately 2 teaspoons) will be withdrawn from one of your arms with a needle. You may experience some slight discomfort at the needle entry site and there may be some bruising. In addition, there is a very small risk of you fainting or of infection at the needle entry site. If your test results are found to be positive, or if you are otherwise diagnosed as having AIDS, you should be aware of the following Clinical Center HIV Testing Policy:

1. Your physician will notify you promptly of the HIV test results.

2. Your physician and/or the Clinical Center HIV counselor will offer you, and any current and/or ongoing sexual partner(s) (spouses are generally considered to be current or ongoing sexual partners) or needle-sharing partner(s) you identify, information on the meaning of the test results and how to prevent the spread of the infection.

3. Because the virus may be transmitted in several ways, it is important that you inform sexual and/or needle-sharing partner(s) that any, or all, of them may have been exposed to the HIV virus and encourage them to be tested. If you request it, staff at the Clinical Center will assist you in notifying your partner(s) and arrange counseling for them through an HIV counselor.

4. The results of your HIV test and/or documentation of the diagnosis of AIDS will become a part of your Clinical Center medical record and, as such, will be protected from unauthorized disclosure by the Federal Privacy Act of 1974. In general, access to your medical record will be restricted to those health care professionals directly involved in your care or in the conduct of ongoing biomedical research, and information is not usually released to other third parties without your permission or that of your designated representative. However, there are some particular routine uses of such information of which you should be aware.

   a. If you are unwilling or unable to notify your partner(s), the Clinical Center is responsible for attempting to contact and inform them of their possible exposure to the virus. Reasonable attempts will be made to protect your identity including withholding your name when notifying any partner(s) of their possible exposure. Some notification or counseling of current and/or ongoing partners may be carried out through arrangements with, or referral to, local public health agencies.

   b. A summary of your care at the Clinical Center will be sent to the physician who referred you here for treatment.

   c. The Clinical Center may report certain communicable diseases, such as HIV infection, to appropriate State and Federal government agencies.

      i. For Clinical Center patients who are Maryland residents, the Clinical Center reports by "Patient Unique Identifier Number" (rather than by name) newly obtained HIV-positive results from its laboratory to the Maryland Department of Health and Mental Hygiene. Patient Unique Identifier Number is: last four digits of social security number, birth month, birth day, birth year, race and gender.

      ii. For Clinical Center patients who are Maryland residents, the Clinical Center reports by name new cases of AIDS to the Maryland Department of Health and Mental Hygiene.

iii. For Clinical Center patients who are not Maryland residents, the Clinical Center reports HIV-positive results and/or AIDS to the patient's primary care/referring physician.

If you have any questions regarding the HIV testing or the information provided above, you are encouraged to discuss them with your physician and/or a Clinical Center HIV counselor: (301) 496-2381.