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

STUDY NUMBER: CONTINUATION: page 16 of 16 pages

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or other authorized people.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Dr. Elizabeth Kang, Building 10, CRC Room 6W-3750, Telephone: 301-402-7567. Other researchers you may call are: Dr. Harry L. Malech, at 301-480-6916; Dianne Hilligoss, CRNP at 301-594-5945; Effie Nomicos, RN at 301-402-5964; Akua Kwatemaa, RN at 301-451-7820. You may also call the Clinical Center Patient Representative at 301 -496-2626.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/Legal Representative _____ Date _____

B. Parent's Permission for Minor Patient.
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

C. Child's Verbal Assent (If Applicable)
The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM DECEMBER 10, 2004 THROUGH DECEMBER 10, 2005.

Signature of Investigator _____ Date _____ Signature of Witness _____ Date _____

MEDICAL RECORD	MINOR PATIENT'S ASSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Attach to NIH-2514-2, Consent to Participate in a Clinical Research Study

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: PRINCIPAL INVESTIGATOR: Elizabeth Kang, MD

STUDY TITLE: **Autologous Transplantation of Genetically Modified Cells for the Treatment of X-linked Chronic Granulomatous Disease**

Latest IRB Review:
Latest Amendment Approved:

Minor patient

This assent form is to help our young patients better understand why this study is being done and what tests and other treatments will be performed. No matter what your age, we will also give you a copy of the longer and more detailed consent form that your parents/guardians need to read, understand, and sign. If you are interested and able to do so, you are invited to read that longer, more detailed consent form. We will also talk to you about what is in that form and explain it to you. If you have any questions about any of the tests or treatments, we are very willing to answer them, so please ask any of the doctors or nurses at any time.

You do not have to take part in this study if you do not want to. You can stop any time during the study by telling your nurse or doctor. We will continue to take care of you at the NIH no matter what you decide.

WHAT IS THIS STUDY ABOUT?

You have some problem with the way your body fights infections. You have developed this problem because you have a gene that does not work well. Genes are the building blocks that make your body grow and function a certain way. People with Chronic Granulomatous Disease (or CGD) have a hard time fighting germs that cause infections because one of their genes does not work correctly. We would like to understand CGD better and treat this condition with a treatment called "gene therapy". We will do all your testing at our hospital, and then we will admit you to give you this treatment.

WHAT ARE THE TEST AND TREATMENTS THAT WILL BE DONE ON THIS STUDY?

Blood testing:

We would like to examine you and take blood from your arm and study the blood in the laboratory. The amount of blood taken will be about three tablespoons or less at any one visit. This will require sticking a needle in your arm for just a minute to draw some blood out. Needle sticks do cause some pain and sometimes it may be necessary to stick you more than once. There are medicines for children that can be placed on the skin to make it hurt as little as possible. Tell your doctor or nurse if you would like this medicine. Sometimes drawing blood causes a bruise, which will go away in a few days. We will put a band-aid on it. If you have any questions, please talk to your doctor or nurse.

Procedures:

Your doctor will ask about your medical history and do a physical exam on you and we will do a scan (chest X-ray or CT scan) when you first come to NIH for your treatment. If you had these scans before coming to NIH, we will get a copy.

Scans are special X-rays that do not hurt. A CT scan is a special X-ray that is taken while you lie flat and still on a moving table.

I.V. Lines:

If you do not already have one, you may have an I.V. (a large thin plastic tube) placed into a vein in your upper chest or neck. This I.V. will help us to give you the treatments including medicines and to draw blood so that we do not have to stick you in your arms so much.

Busulfan and Gene Therapy While You are in the Hospital:

You will then be given special medicine, called busulfan, through the I.V. The busulfan prepares your body to receive back the cells that were collected and treated for you in the blood bank. You will be given this medicine each morning for 2 days. Sometimes patients have side effects from busulfan, like nausea, vomiting, hair loss, and itchy skin. We will treat most of these side effects to help keep you comfortable. On the third day, the doctor will give you back the cells we had already collected and were specially treated with the gene therapy. There may also be side effects with the cells, too, like having an upset stomach, chills, itchy skin, dizziness, fever, and problems with breathing. If any of these occur, we will treat the side effects. During days after receiving the gene therapy, we will check your temperature, pulse, breathing, and blood pressure often (vital signs), and will take small blood samples from your I.V. Once your blood tests show that you have a good number of white blood cells (the type that are able to fight infections), and your vital signs are normal, you will be allowed to go home.

You will also be asked to come back for follow-up visits, exams, questioning, and future blood sampling as a part of this study. It is very important that these visits, exams, questionnaires, and blood samplings are completed for us to monitor your safety.

SOME SPECIAL TESTS WILL BE DONE THAT YOUR PARENTS OR GUARDIANS WILL BE TOLD ABOUT:

Before you begin this study, we will test your blood for certain blood viruses like AIDS. These tests are done for everyone who has a problem with the immune system. For this test, a small amount of blood (about 2 teaspoons) will be taken from a vein at the same time you are getting blood drawn for other tests. The results of all of your blood tests will be shared with you and your parents or guardian.

I have had this study explained to me in a way that I understand, and I have had the chance to ask questions. I agree to take part in this study.

Signature of Minor Patient:_____Date:_____

Signature of Investigator:_____Date:_____

APPENDIX M SUBMISSION FOR GENE THERAPY PROTOCOL ENTITLED:

"Autologous Transplantation of Genetically modified Cells for the Treatment of X-linked Chronic Granulomatous Disease", Elizabeth Kang, MD, Principal Investigator; Harry L. Malech, MD, Associate Investigator

Appendix M-I-A. Requirements for Protocol Submission

The following documentation required per this section of Appendix M is included in this submission:

I. **Submission Requirements – Human Gene Transfer Experiments**

General Considerations: This submission represents proposal for use of previously approved FDA BB-IND-6100. It is important here to emphasize features that remain the same as with the Original Submission of the NIH 1995 gene therapy protocol as approved for patient enrollment at that time by the RAC and FDA. What remains the same is that patients with Chronic Granulomatous Disease (CGD) will have CD34+ hematopoietic progenitors harvested from peripheral blood for culture and transduction *ex vivo* with replication incompetent murine retrovirus encoding normal human sequence for the phagocyte NADPH oxidase subunit defective in that patient's genetic form of CGD. The autologous gene-corrected CD34+ cells will be returned to the patient's blood by vein and monitoring will occur to detect the appearance of functionally-corrected oxidase-positive neutrophils by the same methods used in the original protocol. The exact same retrovirus backbone (MFGS) and the exact same packaging plasmid elements (the two CRIP plasmids: 5'LTR-gag-pol-3'SV40polyA and 5'LTR-AMenv-3'SV40polyA) are employed in the amended Protocol. Thus, the disease treated (Chronic Granulomatous Disease), the indication for treatment (prevention or treatment of life-threatening infection in CGD by providing a means of autologous production of functionally normal oxidase-positive neutrophils), the retrovirus vector backbone and packaging elements, and the genetically altered CD34+ cell product administered to the patient will remain the same as the original Protocol. The one difference includes the use of two days of busulfan given to the patient prior to the infusion of the genetically modified cells. Our post-treatment patient monitoring plan will be more comprehensive to account for the subablative side effects expected from busulfan conditioning. .

1. **A cover letter** on institutional letterhead, signed by the Principal Investigator(s), that: (1) acknowledges that the documentation submitted to NIH OBA complies with the requirements set forth in Appendix M-I-A, *Requirements for Protocol Submission*; (2) identifies the Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) at the proposed clinical trial site(s) responsible for local review and approval of the protocol; and (3) acknowledges that no research participant will be enrolled (per definition of enrollment in Section I-E-7) until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); IBC approval (from the clinical trial site) has been obtained; IRB approval has been obtained; and all applicable regulatory authorizations have been obtained.

2. **The scientific abstract** (included as page iii of the protocol)

3. **The non-technical (lay language) abstract** (included as page iv of the protocol)

4. **The proposed clinical protocol**, including appendices, attachments, and tables.

5. **Responses to Appendices M-II through M-V** (included in this section), *Description of the Proposal, Informed Consent, Privacy and Confidentiality, and Special Issues.* Responses to Appendices M-II through M-V are provided both as this appendix to the clinical protocol and as indicated in the responses to each of the Appendix M responses are incorporated in the clinical protocol.

6. **The proposed informed consent document** (is included in this submission and is described in the responses to Appendix M-III, *Informed Consent*).

7. **Curriculum vitae of the principal investigator(s)** (attached as two page biographical sketches).

Appendix M-II. Description of the Proposal

Responses to this appendix are provided below. For more discussion and literature references, please note the citations in these responses to portions of the attached proposed clinical protocol.

II-A. Objectives and Rationale of the Proposed Research

The objectives and rationale remain the same as in the Original Submission 1995, but are also outlined again on Page 2 and 3 of the amended Protocol.

II-A-1. Use of Recombinant DNA for Therapeutic Purposes

a. *"Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?"*

This question is addressed in the "Background/Introduction" section of the Protocol in the description of CGD on Pages 11-12. Briefly, CGD can be cured by bone marrow transplant; there is a direct correlation between oxidase activity (hydrogen peroxide production) in neutrophils or number of oxidase normal neutrophils and susceptibility to infection; gene therapy of "knockout" mice with either the $gp91^{phox}$ deficient X-linked CGD or the $p47^{phox}$ deficient autosomal recessive CGD results in increased host defense against infection; and in the 5 patients treated in the first phase of a previous NIH gene therapy protocol, and currently followed, resulted in the prolonged appearance in circulation of small numbers of functionally corrected oxidase-positive neutrophils.

b. *"Describe the natural history and the range of expression of the disease selected for treatment. What objective and/or quantitative measures of disease activity are available? In your view, are the usual effects of the disease predicable enough to allow for meaningful assessment of the results of gene therapy?"*

These questions addressed in the "Background/Introduction" section of the Protocol in the description of CGD on Pages 11-12. Briefly, CGD is caused by an inherited defect in neutrophil microbicidal oxidase production (superoxide/hydrogen peroxide) and affects about 4-5 per million individuals. CGD is caused by genetic defects in any of the genes encoding any of four subunits of the phagocyte NADPH oxidase ("phox"), with over 95% of cases resulting from defects in either the $gp91^{phox}$ or $p47^{phox}$ subunits. The subjects to be enrolled in this protocol are males (3-55 years of age and >20 kg in weight) with documented X- linked (gp91 phox deficient) CGD. Disease activity (susceptibility to infection) is directly correlated with both the percent of circulating oxidase-normal neutrophils and their individual level of oxidase activity, where levels of 5% or greater oxidase-normal neutrophils result a marked decrease in susceptibility to infection (normal phenotype). The oxidase activity of circulating neutrophils is easy to measure by flow cytometry and this oxidase activity directly correlates with disease activity.

c. *"Is the protocol designed to prevent all manifestations of the disease, to halt progression of the disease after symptoms have begun to appear, or to reverse manifestations of disease in seriously ill victims?"*

The goals of the Protocol are discussed in the "Study Objectives" on Page 17 and continuing to Page 23, "Primary and Secondary Endpoints". Briefly, the goal is to provide the patient with a means of producing gene-corrected autologous oxidase-normal neutrophils from his/her own marrow on a long-term (but not necessarily permanent) basis in order to provide increased host defense against an active or recurrent life-threatening infection. We do not believe that the current protocol will necessarily result in a permanent cure of CGD, but may provide prolonged benefit for several months that could be critical in preventing or curing an otherwise fatal infection.

d. *"What alternative therapies exist? In what groups are these therapies effective? What are their relative advantages/disadvantages as compared with the proposed gene therapy?"*
This is covered in the Protocol on Page 12, and discussed as "Current Therapies". A more detailed description is provided on Page 42 ("Alternate Therapies"). In brief, daily prophylactic oral antibiotics and three times weekly injections of interferon gamma reduce the frequency of infection, but mortality from infection remains at 2% per year (higher in younger patients). Allogeneic granulocyte transfusions are used to treat acute infection, but carry the risk of exposure to large numbers of donor blood products and use is limited by development of HLA alloimmunization. Bone marrow transplant can cure CGD, but continues to have significant morbidity, and is primarily limited to those with a fully matched sibling donor. For those without a sibling matched donor, matched unrelated donor transplantation may be considered but carries an even higher risk of either graft versus host disease and/or rejection. While observation of patients having undergone allogeneic transplantation appears to confirm that replacement of their hematopoietic system reduces or eliminates their CGD-related infections/inflammatory events, statistical support remains lacking.
The major disadvantage of autologous HSC gene therapy is that there may be insufficient numbers of stem cells corrected to improve the clinical status of the patients.

II-A-2. Transfer of DNA for Other Purposes
II-A-2-a.

"Into what cells will the recombinant DNA be transferred? Why is the transfer of recombinant DNA necessary for the proposed research? What questions can be answered by using recombinant DNA?"

Recombinant DNA will be transferred *ex vivo* by retroviral transduction into autologous peripheral mobilized CD34+ cells. The transfer of recombinant DNA is necessary for the proposed research because the gene encoding for the gp91 phox of CGD patients has a mutation, which prevents the production of oxidase. Since the patients with CGD produce neutrophils that lack production of oxidase, gene therapy must be directed at the progenitor cells, which give rise to these cells so as to provide a life long source of normal cells,.

The following questions can be answered by using recombinant DNA:

1. Can peripheral blood CD34+ cells, transduced *ex vivo* with MFGS-gp91 by the methods used, be re-administered safely to humans with CGD?
2. What levels of provirus integration, gp91 expression and gp91 function are achieved in hematopoietic lineages using our technique of gene transfer?
3. What is the duration of expression of gp91 following gene transfer by our method?
4. Are there changes in immune status, medication requirements, infections, growth, chronic medical conditions, and general quality of life as compared to pre-treatment baseline?

II-A-2-b. *What alternative methodologies exist? What are their relative advantages and disadvantages as compared to the use of recombinant DNA?*

There are no alternative methodologies to the use of recombinant DNA for the proposed investigation.

II-B. Research Design, Anticipated Risks and Benefits

A detailed description of the Study Design encompasses Pages 19-22 of the Protocol. A detailed description of anticipated Risks and Benefits encompasses Pages 30 of the Protocol.

II-B-1. Structure and Characteristics of the Biological System

a. *"What is the structure of the cloned DNA that will be used?"*

In the protocol, we described gene therapy of the $p47^{phox}$ deficient autosomal recessive form of - CGD. The replication incompetent amphotropic envelope retrovirus vector MFGS-$p47^{phox}$ containing the sequence for the normal human $p47^{phox}$ cDNA open reading frame was used to transfer this therapeutic gene into *ex vivo* cultured autologous peripheral blood CD34+ hematopoietic progenitors in a three day transduction regimen. The MFGS vector with its safety features was described in detail and complete sequence data and restriction map data was provided in an Original Submission for the NIH Protocol 95-I-0134. The amphotropic packaging line ψ-CRIP with its two packaging elements (5'LTR-gag-pol-3'SV40polyA and 5'LTR-AMenv-3'SV40polyA) were also described in detail in the Original Submission in 1995. However, the Protocol in 1995 was amended to also treat patients with the $gp91^{phox}$ deficient form of CGD (X-CGD). This required replacing the open reading frame of normal human $p47^{phox}$ with the open reading frame of normal human $gp91^{phox}$ open reading frame in the same MFGS vector to create the therapeutic vector, MFGS-$gp91^{phox}$. Like $p47^{phox}$, $gp91^{phox}$ is a subunit of the phagocyte NADPH oxidase that has no biological activity outside of the context of expression of the other three phagocyte specific oxidase subunits required for assembly and activity of the oxidase and there are no novel safety issues that did not apply to expression of the $p47^{phox}$ gene in hematopoietic cells following transduction. This exact same vector will be used in this Protocol for treatment of $p91^{phox}$ deficient CGD. A novel feature we describe in the former and this new protocol is a new packaging cell line, 293-SPA which is derived from the 293 human embryonic kidney cell line into which has been permanently transfected the exact same two packaging elements used to create the ψ-CRIP packaging line. This "CRIP" 293 line designated 293-SPA is used to produce amphotropic envelope packaged MFGS-$gp91^{phox}$ that will be used for gene therapy of $gp91^{phox}$ deficient X-linked CGD in this Protocol.

a-(1). *"Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector. Provide complete nucleotide sequence analysis or a detailed restriction map of the total construct."*

Retrovirus vector construct and design, and packaging line construct and design are discussed in detail in the Protocol as Attachment 1. A restriction enzyme map and complete sequence of the MFGS-$gp91^{phox}$ plasmid is provided in the Appendix 1b. The two CRIP packaging elements present in the packaging lines are the same as that described in the Original Submission for NIH Protocol 95-I-0134 (see Figure 4 of Appendix I).

a-(2). *"What regulatory elements does the construct contain...? From what source are these elements derived? Summarize what is currently known about* the regulatory character of each element."

The MFGS sequence/regulatory elements and the CRIP packaging sequence/regulatory elements are described in Appendix 1 of the Protocol.

a-(3). *"Describe the steps used to derive the DNA construct."*

The MFGS-$gp91^{phox}$ and the CRIP elements were described in the Original Submission of NIH Protocol 95-I-0134 and will not be repeated in entirety here. Briefly, using hybridization detection with known $gp91^{phox}$ partial sequence, full length cDNA clones of human $gp91^{phox}$ were derived from a lambda phage library constructed from poly-A positive mRNA derived from the human HL-60 promyelocyte cell line that had been induced to differentiated to mature myeloid cells using DMSO.

PCR methodology was used to introduce a BamHI restriction site just after the natural stop codon of the open reading frame of $gp91^{phox}$. As $gp91^{phox}$ has a natural NcoI restriction site both at the ATG start codon and internal to the open reading frame, partial digestion of the resultant construct with limiting amount of NcoI enzyme together with digestion with BamHI enzyme yielded DNA fragments of about 1715 bp (full $gp91^{phox}$ open reading frame with no flanking sequence) that were purified from an agarose gel following electrophoresis separation. This fragment was cloned into the MFGS plasmid that had been previously cut with NcoI and BamH1 (the cloning sites) to allow directional insertion of the $gp91^{phox}$ fragment to yield the MFGS-$gp91^{phox}$ plasmid. The pMFGS-$gp91^{phox}$ plasmid was completely sequenced prior to its use in transfecting the 293-SPA packaging line for derivation of the producer clone used to provide infective replication incompetent retrovirus for this protocol. This sequence obtained from analysis of that pMFGS-$gp91^{phox}$ plasmid is shown in Appendix 1 with the restriction enzyme map of this sequence.

b. *"What is the structure of the material that will be administered to the patient?"*
Washed autologous CD34+ peripheral blood stem cells (PBSC) that have been cultured and transduced *ex vivo* are the final therapeutic product that will be administered to the patient In preclinical studies of CD34+ PBSC transduced *ex vivo* with the clinical grade certified MFGS-$gp91^{phox}$ vector that will be used in this current Protocol, the cells were found to contain unrearranged MFGS-$gp91^{phox}$ with all MFGS safety features preserved present in genomic DNA.

b-(1). " *Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells: (i) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests? (ii) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (for example, VL30 RNA, other nucleic acids, or proteins) or contaminating viruses (both replication-competent or replication-defective) or other organisms in the cells or serum used for preparation of the virus stock including any contaminants that may have biological effects? (iii) If co-cultivation is employed, what kinds of cells are being used for co-cultivation? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials? Specifically, what tests are being conducted to assess the material to be returned to the patient for the presence of live or killed donor cells or other non-vector materials (for example, VL30 sequences) originating from those cells? (iv) If methods other than those covered by Appendices M-II-B-1 through M-II-B-3, Research Design, Anticipated Risks and Benefits, are used to introduce new genetic information into target cells, what steps are being taken to detect and eliminate any contaminating materials? What are possible sources of contamination? What is the sensitivity of tests used to monitor contamination?* "
A detailed description of the derivation of the producer cell lines, which produce the amphotropic MFGS-$gp91^{phox}$ is provided in the Protocol in Attachment 1. The preparation, validation and safety testing of producer cell banks, and the description of vector production and safety testing and validation of clinical lots of retrovirus vector is described in detail in the Protocol. In Attachment 1a, summarized in tabular form, the preparation of producer cell banks, production of clinical lots of retrovirus, validation and safety testing of cell banks, and validation and safety testing of clinical lots of retrovirus. The procedures to be followed for *ex vivo* culture and transduction of the autologous CD34+ cells are described in detail in the Protocol in Attachments 1a and 1b.In brief, producer lines and lots of virus supernate have been shown to be free of replication competent retrovirus using co-cultivation biological assays that are the most sensitive indicators of such contamination currently available. The lines and clinical lots of supernate are also sterile, free of endotoxin and have tested

negative using certified tests capable of detecting a variety of viruses, mycoplasma and adventitial agents following protocols conforming to sensitivity and accuracy required by the FDA. Materials such as bovine serum or porcine trypsin used for preparation of master cell banks have been tested and certified free of adventitial agents including BSE in the case of bovine serum. As in the Original Submission 1995, the medium used to produce clinical lots of retrovirus and to culture the CD34+ cells is X-VIVO 10 medium free of animal proteins or serum. The CD34+ cells are only exposed to frozen stocks of filtered and safety tested retrovirus vector supernate free of producer cells. The transduced autologous CD34+ cells will be administered after washing at the end of the transduction period. A 24 hour negative sterility culture will be available and the cells will be Gram's Stain and endotoxin negative, but testing for replication competent retrovirus and mycoplasma will not be available until some time after the product has been administered.

b-(2). " *Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?* "
The same procedure will be followed as in the Original NIH Protocol 95-I-0134 for selection of the CD34+ cells from the apheresis product using immunomagnetic beads and a murine monoclonal antibody anti-CD34. The culture medium used for the *ex vivo* culture and transduction is detailed in Attachment 2, "Retroviral Gene Transfer for Treatment of X-linked Chronic Granulomatous Disease". Extensive washing of the cells at the end of the culture and transduction process should remove the growth factors from the cells administered to the patient. The human recombinant fibronectin fragment will be clinical grade material produced under GMP conditions and tested to be sterile, substantially endotoxin free, and chemically pure. It will be used to coat the plastic culture bags by electrostatic adherence. The bags will be extensively rinsed before the cells and medium are added, in addition as indicated above the cells will be extensively washed. These maneuvers should result in a transduced CD34+ cell product administered to the patients that are free of any contaminating recombinant human fibronectin fragment.

II-B-2. Pre-clinical Studies, Including Risk-Assessment Studies

Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the model(s) chosen is/are most appropriate.
Only cell culture studies using cell lines or *ex vivo* cultures using CD34+ cells from patients with CGD were available to validate the gene transfer systems and to demonstrate that transduction with retrovirus encoding the oxidase subunit cDNA could provide functional correction of the CGD defect. Now in addition to culture studies, there are the results of both this clinical trial of gene therapy for CGD in human subjects and the strong positive results of gene therapy of two groups of knock-out CGD mice. The results of our CGD clinical trial of a single cycle of gene therapy to treat 5 patients with $p47^{phox}$ demonstrated that the procedures were safe and without side effects, and that small numbers of fully functional oxidase positive gene corrected neutrophils could be detected for a prolonged period in these patients. Furthermore, the results of gene therapy in both the $p47^{phox}$ deficient and $gp91^{phox}$ deficient mice demonstrated that not only can prolonged functional correction of the oxidase in circulating neutrophils be achieved by gene therapy, but also this is associated with increased resistance to infection challenge. Furthermore, there were no significant effects on hematopoiesis or appearance of malignancies or other unexpected side effects in these animals. These observations regarding gene therapy for CGD in humans and mice have been published (October 1997), providing a strong rationale and basis for this Protocol

II-B-2-a. Delivery System

a-(1). *"What cells are the intended target cells of recombinant DNA? What target cells are to be treated ex vivo and returned to the patient, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the target cells will incorporate the DNA?"*

With the availability of our results from humans studies to date with the past CGD Gene therapy trial and the CGD knockout mouse gene therapy studies noted above, it is now possible to state that at least long term reconstituting hematopoietic progenitor cells can be effectively targeted by gene therapy techniques employed in this Protocol. It can also be stated that there does not appear to be any adverse effect of expression in cells of other lineages (e.g. erythroid), which may also be derived from transduced CD34+ progenitors. It is not known whether true stem cells (permanently repopulating cells) can be targeted by this protocol, but benefit will likely accrue to our CGD patients in the form of resistance to infection even with prolonged, but not permanent production of functionally normal gene corrected neutrophils. Since replication incompetent murine retrovirus is being used as the gene transfer vehicle and the cell selection system and culture conditions ensure that only CD34+ progenitors are the only available proliferating cell type that can integrate the retrovirus vector, it is highly unlikely that cell types other than those derived from the *ex vivo* cultured CD34+ hematopoietic stem cells will be targeted. The transduction of CD34+ cells is assessed by functional assays assessing correction of oxidase activity in cells and colonies differentiated from these cells in culture. Following return of the transduced CD34+ cells to the patient the functional correction can be assessed in circulating neutrophils using a flow cytometric assay of oxidase activity. PCR based assays can be used to assess gene transfer independent of functional correction.

a-(2). *" Is the delivery system efficient? What percentage of the target cells contains the added DNA?"*

In the previous clinical gene therapy trial for patients with $p47^{phox}$ deficient CGD, the *ex vivo* transduction rate in CD34+ cells for the 5 patients averaged 10-20% and this led to a transient average peak of 1 in 5000 circulating neutrophils in the circulation *in vivo* demonstrating full functional correction of oxidase positive neutrophils. Using the clinical lot of MFGS-$gp91^{phox}$ vector to transduce CD34+ PBSC in a scale-up clinical sized test transduction (not for readministration to a patient) in fibronectin fragment coated bags, the transduction rate was over 70% representing a marked enhancement from previous experience. In related studies this level of transduction with the MFGS-$gp91^{phox}$ vector seen with CD34+ cells derived from a patient with $gp91^{phox}$ deficient X-CGD resulted in neutrophils derived from these transduced CD34+ cells with functional correction of oxidase activity indistinguishable from normal.

a-(3). " *How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?*"

As noted in the answer to **II-1-b** above, Southern blot and PCR analysis with detailed sequencing of the PCR fragment of the provirus insert in genomic DNA from CD34+ cells transduced with MFGS-$gp91^{phox}$ demonstrates that the vector sequence is integrated, unrearranged and retains all of the MFGS safety features.

a-(4). " *How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?"*

Using the clinical grade retrovirus supernatant MFGS-$p47^{phox}$ vector in NIH Protocol 95-I-0134 clinical trial we found that copy number of the bulk transduced CD34+ PBSC was about 0.13 and the number of colony forming cells giving rise to colonies containing functionally corrected neutrophils was about 15% Thus, copy number of the transduced CD34+ population was approximately equal to

the proportion of cells/colonies which demonstrate functional correction, i.e. expression of functional transduced protein product. This suggested that the copy number is approximately one copy per transduced cell and that most integrated transduced gene results in production of functionally active protein. Our experience with MFGS-gp91^{phox} clinical grade vector used in a pre-clinical scale up of our transduction regimen targeting CD34+ cells gave similar results, except that the average number of colonies in a colony assay consistently exceeds 70%. In colony assays with the CD34+ cells transduced with MFGS-gp91^{phox}, gene function persisted undiminished for the full period of time that the cells could be maintained in culture (about 30 days) indicating stability of the transduced gene using this vector. In the clinical trial of gene therapy, the MFGS-p47^{phox} vector resulted in detection of functionally corrected neutrophils in the circulation for 3 to 6 months indicating sufficient stability of genomic insertion and expression required for the present phase of this clinical trial. Given that the MFGS-gp91^{phox} performs as well or better in all *ex vivo* studies as the MFGS-p47^{phox} vector and given that these are identical constructs except for the fact that each encodes a different phagocyte oxidase subunit cDNA, it is expected that the MFGS-gp91^{phox} will show similar stability of insertion and transduced gene expression as was seen in the clinical trial with MFGS-p47^{phox}.

II-B-2-b. Gene Transfer and Expression

b-(1). *" What animal and cultured cell models were used in laboratory studies to assess the in vivo and in vitro efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment? "* These issues are extensively addressed in the NIH Protocol 95-I-0134. Most relevant is that the clinical studies in this protocol to date using this MFGS vector system have been published ((Malech, Horwitz et al. 1998)). Furthermore, the preclinical studies targeting the same CD34+ PBSC that are the target of this study and/or animal studies using the MFGS vector system in general and the MFGS-gp91^{phox} vectors in particular are discussed in the Protocol on page 14. In brief, we have extensively examined the transduction efficiency of both the MFGS-p47^{phox} and MFGS-gp91^{phox} vectors in culture systems targeting both cell lines (such as EBV-B lymphocyte cell lines established from patients, or our new system of the engineered human K562 erythroleukemia cell lines genetically altered to produce different groups of the phagocyte NADPH oxidase subunits) and the CD34+ PBSC that are the actual planned target of the gene therapy. The MFGS-gp91^{phox} vector performs in culture systems two to three fold better than our MFGS-p47^{phox} vector system on human CD34+ PBSC, both from the point of gene transfer and functional correction of oxidase activity. It is reasonable to expect a significant improvement in outcome in the clinical trial results using the clinical MFGS-gp91^{phox} vector

b-(2). *"What is the minimal level of gene transfer and/or expression estimated to be necessary for the gene transfer protocol to be successful in humans? How was this determined?"*
This question is addressed in the Protocol on Page 23 under "Study Endpoints". Briefly, from observations in the X-linked form of CGD and in gene therapy of mice it appears that gene therapy that resulted in a permanent 3-5% of neutrophils with normal oxidase function would likely cure CGD. However, much lower levels of gene corrected neutrophils on even a temporary basis would likely provide some benefit in the form of increased defense against infection.

b-(3). *"Explain in detail all results from animal and cultured cell model experiments which assess effectiveness of the delivery system in achieving the minimally required level of gene transfer and expression."*
This was already addressed in the answers to II-B-2-b- (1) and (2) immediately above.

b-(4). *" To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?"*

Although long considered only a theoretical risk when using replication incompetent vectors, insertional mutagenesis is now a significant issue given the events in the XSCID clinical trial conducted by Fischer's group in France. Here, as has been reported extensively(Hacein-Bey-Abina, Le Deist et al. 2002; Hacein-Bey-Abina, von Kalle et al. 2003; Hacein-Bey-Abina, Von Kalle et al. 2003), three patients have developed a leukemia associated with overexpression of the LMO2 oncogene. In two of the cases this has occurred as the result of insertion of the common gamma chain expressing transgene near the promoter region of the LMO2 gene. Insertions near other oncogenes have also been found in the third case of insertional leukemogenesis in the French XSCID trial, but the role of any of these and which, including the LMO2 gene, has the dominant role is not yet clear. It is important to note that the common gamma chain itself leads to a proliferative advantage in transduced cells. Additionally, animal studies have shown a propensity for interaction between the common gamma chain gene and LMO2(Dave, Jenkins et al. 2004).

However unlike the situation involved when transducing cells with the common gamma chain, the insertion of the GP91 phox gene does not confer a survival advantage to transduced cells. There is no known function of $gp91^{phox}$ protein except for its' role as subunits of the oxidase. Even in that context these peptides require both the presence of the complementary subunits (found only in cells normally expressing oxidase activity) and also require a specific signal stimulus for assembly and manifestation of any enzymatic activity. It is unlikely that transcription of these genes or expression of the peptide would have any separate action on cellular gene expression, whether next to the transduced vector in the genome or at a distance. Further, two patients with chronic granulomatous disease, treated by a collaborative group in Germany and Switzerland using a different type of oncoretroviral vector (the Spleen focus forming virus or SFFV) have not shown any leukemogenic events despite achieving high marking levels and an oligoclonal insertion pattern. ((Ott, Schmidt et al. 2006)). Follow-up though is currently only out to 2 years and one patient recently died, but this does not appear to be due to an insertional event, but rather due to poor expression of the Gp91 protein from the vector used.

Still, there does exist some risk for a mutational event resulting from insertion of the vector into the genome, but we consider the possible benefits sufficient to justify using this technique in these patients who have a life threatening illness and do not have a sibling-matched donor. As always the risk of insertional mutagenesis is increased in the presence of replication competent vectors. We have incorporated highly sensitive biological testing to rule out the presence of replication competent retrovirus in our producer lines, the clinical vector supernate lots, and in the transduced cells returned to the patient. These issues are discussed in the Protocol on Page 26, under k, Page 28 under 6.2 d., and under 6.3 Long-Term Evaluations Beyond Month 42. Furthermore, the MFGS system does not incorporate any internal promoters or selection marker genes such as neomycin eliminating concerns about expression of non-therapeutic genes. We have also established rigorous monitoring of the patients, including the use of LAM PCR to detect any events, should they occur early on.

b-(5). *"In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?"*

The answers to these questions have been discussed in the previous sections of Appendix M. It was noted that the ratio of genomic copy number of vector insert to proportion of transduced CD34+ PBSC demonstrating correction of functional oxidase activity is very similar suggesting that most insertional events result in a construct that yields translated functional product capable of correction of the CGD abnormality in superoxide/hydrogen peroxide production. Furthermore antibody detection assays (flow cytometry for the transmembrane $gp91^{phox}$) indicate that transduction leads to production of protein that is similar in amount to that seen in normal neutrophils. In addition, quantitative functional assays of superoxide or hydrogen peroxide production (chemiluminescence assay of superoxide production and the very sensitive dihydrorhodamine flow cytometry assay)

indicate that correction of function is similar to normal cells (i.e. functional correction is 100% in neutrophils derived from transduced CD34+ PBSC). Finally, in gene corrected neutrophils derived from transduced CGD CD34+ cells, the oxidase demonstrates normal regulation (i.e. activity is not detectable until the cells are stimulated with a particulate or soluble stimulus).

b-(6). *"Is the gene expressed in cells other than the target cells? If so, to what extent."*
Briefly, the use of an *ex vivo* system in which highly enriched CD34+ PBSC are the only cell present capable of being transduced and the extensive testing to reduce and likely eliminate the risk of presence of replication competent retrovirus ensures that only hematopoietic stem cells/progenitor cells are transduced. However, it is expected that the transduced gene could be expressed in all blood lineages derived from CD34+ cells and not just phagocytic cells. As oxidase activity requires the expression of all the oxidase subunits in the same cell, oxidase activity would be limited to those differentiated progeny of transduced CD34+ cells that normally express the complementary oxidase subunits. In culture systems, there is no evidence that transduction expression of $gp91^{phox}$ alters the character or number of erythroid colonies. Furthermore, there is no evidence that treatment of mice with gene therapy with the $gp91^{phox}$ as therapeutic genes delivered in a retrovirus vector system results in any alterations in hematopoietic function other than correction of oxidase activity in circulating neutrophils.

II-B-2-c. Retrovirus Delivery Systems

c-(1). *"What cell types have been infected with the retroviral vector preparations? Which cells, if any produce infectious particles?"*
Most of the studies with the MFGS-$gp91^{phox}$ retrovirus supernate preparations to be used in this clinical trial have been examined for the efficiency of transduction of CD34+ PBSC. These are also the target cells for this trial. This vector preparation efficiently transduces the CD34+ PBSC. See Attachment 1b. in the Protocol Transducing Particles 293-SPA-MFGS-gp91-155-MCB-Retrovirus (RV) As these are replication incompetent retrovirus vectors, no production of infectious virus has been detected from the human CD34+ PBSC following transduction.

c-(2). " *How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?"*
These issues were extensively discussed in the Original Submission 1995 NIH Protocol # 0134. There is extensive clinical experience with the use of the MFGS vector system, which has been demonstrated to have a high level of stability. Because no internal promotor or resistance element is included in these vector constructs, there is less opportunity for rearrangements. We have performed studies with CD34+ PBSC transduced with MFGS-$gp91^{phox}$ to demonstrate in the target cells that the provirus in the genome is not rearranged and most important of all, that by sequence analysis (using PCR amplification with detailed sequencing) the safety features of MFGS indicated in this Protocol submission are retained.

c-(3). " *What laboratory evidence is available concerning potential harmful effects of the transfer (e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses)? What steps will be taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?"*

Although the use of an oncoretroviral vector based gene therapy has resulted in neoplasia as described in section II-b-2-b(4),there have been no other reports of neoplasia in any other human clinical gene therapy trial using different oncoretroviral vectors including the MFGS vector. The MFGS system, similar to that of most of the other murine retrovirus vectors used in human clinical trials has been designed to eliminate to the greatest extent possible the production of any virus protein product or even small peptide portions of such viral products. In clinical trials using this vector we have not found, and there are not reports of others finding replication competent retrovirus generated in patients treated using *ex vivo* transduced cells. Generation of RCR is the major toxicity to be avoided and this is achieved through the extensive series of testing documenting lack of RCR in both the Master Cell Bank of the producer line and in the clinical lot of vector supernatant as outlined in the Protocol Attachment 1a and 1b.

c-(4). *"Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells? What is the sensitivity of these assays?"*
There is extensive published experience using the MFGS system (including the recent publication from the results of this clinical trial of gene therapy using the MFG-$p47^{phox}$ vector) and there is no evidence of production of replication competent virus using this system and therefore it is highly unlikely that there is any entrance of new vector related genetic material into untargeted cells or germ line. Furthermore, the use of *ex vivo* transduction only and the fact that no infectious retrovirus is directly administered should prevent vector sequence entering germ-line cells. One CGD patient receiving such gene therapy who expired from non-gene therapy complications of his disease and an associated allogeneic transplant was reported to OBA and the FDA in March of 2000. At autopsy gonadal tissue was analyzed for the presence of vector sequence using a highly sensitive TaqMan™ real time PCR analysis that is capable of detecting viral copy number of 0.01 and no vector sequence was detected. Similar analysis did demonstrate vector sequence in blood cells and bone marrow as expected. While evidence from one patient does not prove that germ line transfer cannot occur using the MFGS vector in an *ex vivo* approach to hematopoietic cell gene therapy, it provides some support for the theoretical basis for considering germ line transfer a highly unlikely outcome of this approach to gene therapy.

c-(5). *"Has a protocol similar to the one proposed for a clinical trial been conducted in non-human primates and/or other animals? What were the results? Specifically, is there evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?"*
As noted in previous questions above, 5 patients with the $p47^{phox}$ deficient form of CGD were treated with the MFGS-$p47^{phox}$ vector in NIH Protocol 95-I-0134. Testing of patient blood cell samples for a long period after the gene therapy revealed no evidence of generation of replication competent retrovirus There is also the previously mentioned German-Swiss clinical trial (Section II-b-2-b(4). In this study, the patients have shown clinically significant levels of marking, up to 40% of peripheral blood shows corrected cells, with some evidence of clinical benefit and no adverse events documented to date.

II-B-3. Clinical Procedures, Including Patient Monitoring
"Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study. Specifically:"

a. *"Will cells (e.g., bone marrow cells) be removed from patients and treated ex vivo? If so, described the type, number and intervals at which these cells will be removed."*
The treatment regimen and the method of analysis of outcome and patient monitoring are summarized in the description of Study Design on the Protocol Page 19 to Page 22. Specific

procedures of the treatment are described in detail beginning with the Study Design Flow Chart section of the Protocol on Page 20 through the top of Page 23. Briefly, patients will be treated with one cycle of gene therapy after receiving two days of moderate dose busulfan conditioning. Patients will be treated (on a separate protocol) with marrow growth factors to mobilize CD34+ cells to the peripheral blood during which patients will undergo apheresis procedures (processing 2.5 to 3 blood volumes per apheresis) on successive days to harvest a mononuclear fraction containing the CD34+ cells. Following immunomagnetic bead selection of the CD34+ cells should yield about 2-4 x 10^8 CD34+ cells (in a young adult) for each cycle of gene therapy. The CD34+ cells will be transduced *ex vivo* for three to four days, washed and then returned to the patient by vein in a single injection of about 50 ml of buffered isotonic solution over a 10-minute period.

b. *"Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes?"*
Patients will receive any treatment with busulfan at 5mg/kg intravenously (no radiation) to condition or otherwise reduce the resident bone marrow stem cell population to provide any survival advantage to the incoming transduced CD34+ PBSC. Busulfan is specifically toxic to stem cells (myelosuppressive) without being significantly immunosuppressive. This is stated explicitly in the Protocol on Page 15.

c. *"What treated cells will be given to patients? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?"*
These issues are discussed in the answer to II-3-a immediately above. Briefly, for the single treatment of gene therapy, a minimum transfusion dose of cell product of 5 million cells per kilo body weight *ex vivo* transduced autologous CD34+ PBSC will be administered by vein in about 30-50 ml of Plasmalyte with 1% human serum albumin over 10 minutes.

d. *"How will it be determined that new gene sequences have been inserted into the patient's cells and if these sequences are being expressed? Are these cells limited to the intended target cell populations? How sensitive are these analyses."*
These issues are extensively discussed in the Protocol in the sections indicated in the comments to the introduction to Section II-B-3 above. Briefly, we will use both PCR assays to detect gene insert into genomic DNA of circulating blood cells and an extraordinarily sensitive and accurate flow cytometric assay of functional oxidase activity in circulating neutrophils. The PCR assay can detect as few as one vector copy in 10,000 cells. The dihydrorhodamine flow cytometric assay measures the oxidase activity in individual neutrophils and can easily detect one functionally normal gene corrected neutrophil per 100,000. Moreover the flow assay also provides an indication of the level of correction of oxidase activity on an individual cell basis. Because of the nature of the CGD genetic abnormality, oxidase activity cannot occur without production of functionally active gene product (the missing oxidase peptide subunit) transcribed and translated from the vector insert. While expression of the inserted cDNA is limited to cells derived from CD34+ cells, this can include hematopoietic cells (such as erythrocytes or platelets), which do not normally express oxidase subunits. However, the transduction derived oxidase subunit is inert and enzymatically inactive except in cells, which naturally make the complementary set of the other three oxidase subunits. This limits expression of oxidase activity (but not the transduced oxidase subunit peptide) to only cells that naturally express oxidase activity.

e. *"What studies will be conducted to assess the presence and effect of the contaminants."*
The use of animal serum-free and animal protein free medium and vector supernate for the *ex vivo* CD34+ PBSC culture and transduction should reduce the presence of contaminants leading to

immune or other unwanted reactions. Since murine monoclonal antibody is used for the CD34+ immunomagnetic bead selection we will continue to monitor for development of human antibodies against mouse Ig. Extensive washing of the transduced CD34+ cells before administration to the patient should greatly reduce the presence of the growth factors or vector present in the transduction medium. The patients will be observed overnight in the NIH Clinical Center Hospital after receiving the cell infusion. They will have vital signs, physical exam and laboratory studies, as outlined in Protocol Appendix B, checked prior to discharge. Early follow-up visits will occur monthly for the first 3 months of the study to monitor for any post-infusion toxicity that could be related to possible infused contaminants. These visits will include complete physical examination and laboratory studies, with CBC, differential count, sedimentation rate, and chemistry panel of 20 tests (see Protocol Appendix B). Patients and their families will be instructed to contact their local physicians and study principal investigators, Drs. Kang and Malech, if any changes in their status are noted, particularly during the first 3 months post-infusion. Prompt diagnostic and therapeutic measures will be instituted in the event of such contacts.

Infectious retrovirus that might be harbored within the gene-modified cells: RCR testing will be performed according to the schedule in protocol Appendix B. The method for RCR detection in samples of patient blood cells will be PCR detection of the amphotropic envelope sequence. If a patient dies and permission for autopsy is given, RCR testing and real-time PCR for provirus will be performed on tissue samples.

f. *"What are the clinical endpoints of the study? Are there objectives and quantitative measurements to assess the natural history of the disease? Will such measurements be used in patient follow-up? How will the patients be monitored to assess specific effects of the treatment on the disease? ...What is the sensitivity of the analyses? How frequently will follow-up studies be conducted? How long will patient follow-up continue?"*

The study objectives are precisely outlined on Page of 17 of the Protocol. This is further elaborated upon in the Protocol in the section on Patient Follow-up Post Transfusion on Page 22 and Safety Follow-up, also on Page 22. Additionally, the objectives are further outlined under Study Endpoints on Page 23. On Page 11, a clear connection between oxidase activity and percent oxidase normal neutrophils and susceptibility to or host defense against infection (i.e., disease activity in CGD) is established. This provides a rationale for using level of oxidase activity in circulating neutrophils as a precise and easily measured indicator of level of correction of CGD and a predictor of susceptibility to infection. As indicated above, we can precisely and accurately, and with extremely high sensitivity measure correction of oxidase activity in circulating neutrophils following gene therapy using the DHR flow cytometry assay.

g. *"What are the major beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of deleterious consequences from the disease if recombinant DNA transfer is not used."*

As discussed on Page 11 of the Protocol, the current mortality from CGD is that about 2-5 percent of patients per year with the severe form of CGD. Few patients survive to over 50 years of age. In the former clinical trial in which CGD patients received gene therapy, there were no adverse effects encountered from the treatment. Furthermore, the most physically taxing portion of the protocol for the patients was and is expected to be the apheresis process which, itself, is a routine and extensively performed clinical procedure that carries little risk. Thus, actual experience to date with the previous gene therapy trial suggests that these procedures were benign and well tolerated. Though there clearly remain a variety of theoretical hazards to this or any form of gene therapy (most concerning being the theoretical risks of insertional mutation from vector integration causing cancer), these hazards would appear to be less for patients with chronic granulomatous disease given the lack of a

proliferative advantage in the transduced cells and the results from our previous clinical trials where to date with more than 5 years of followup there has not been any evidence of insertional mutagenesis leading to neoplasia. However, *ex vivo* retroviral gene transfer is an experimental treatment that may produce unexpected reactions that could lead to serious illness or even death. The greatest problem in the previous clinical trial (NIH Protocol 95-I-0134) was that the number of corrected neutrophils was small and the effect, although prolonged (several months), was not permanent. This provides a rationale for considering the use of moderate dose busulfan conditioning in this study, which has been shown beneficial in other studies to increase the engraftment of corrected cells. Other risks of this gene therapy include development of antibodies to the therapeutic gene. The Protocol includes monitoring for this risk, but such antibody formation has not been seen in the previous gene therapy protocol for the treatment for X-linked CGD.

As with other gene therapy trials using retrovirus vectors, we incorporate all of the usual and required tests to rule out contamination with adventitial agents, replication competent retrovirus, mycoplasma or other contaminants at every manufacturing step. However, we also have made a particular effort to include some safety features into our gene therapy clinical trial that have not been a part of other trials of this type. First, we have eliminated animal serum and animal proteins from both the culture medium and the retrovirus vector supernate. Second, we are handling and culturing our CD34+ cells in a closed system of gas permeable plastic containers, which can be connected with FDA-approved sterile tube connector docking devices. This not only greatly reduces the chance of contamination, but makes the process more amenable to more widespread use should we be successful in developing a beneficial and efficient method of gene therapy.

h. *"If a treated patient dies, what special post-mortem studies will be performed?"*

This is stated explicitly in the Protocol beginning with the last paragraph on Page 31 . Briefly, an attempt will be made to obtain permission from a family member to have a post-mortem examination performed if the patient dies of any cause during the study or in the follow up period. The follow up period will be for a minimum of 15 years to life so that insertional mutagenesis can be conducted. Post-mortem studies will particularly include search for presence of replication competent retrovirus and the presence of transduced gene in lymph nodes, lungs, liver, gonad, spleen or bone marrow, and in peripheral blood.

II-B-4. Public Health Considerations

"Describe potential benefits and hazards of the proposed therapy to persons other than the patients being treated. Specifically:"

The potential benefits to society in general is the development of an effective means of treating inherited diseases affecting bone marrow cell function using these gene therapy techniques. This could provide benefit to patients with a variety of disorders and not just CGD. Furthermore, this could reduce health care costs to society and the affected individuals. The risks to persons other than the patients being treated is very low since these the retrovirus vectors used are not derived from human pathogens and every effort is made to ensure that they are free of replication competent retrovirus or other contaminants. Furthermore, work with these vectors is done under Biosafety Level 2 containment and waste management protocols, and only transduced CD34+ cells free of infective vector is administered to the patient.

a. *"On what basis are potential public health benefits or hazards postulated?"*

The benefits indicated above do not require further elaboration. The murine retrovirus vector systems, free of contaminating replication competent retrovirus have been demonstrated over many years to be without significant risk to society or to workers handling these reagents. Thus, spread from the patient to others is extremely unlikely.

b. *"Is there a significant possibility that the added DNA will spread from the patient to other persons or the*

environment?"
This replication incompetent vector integrated in the genome of CD34+ cells in the patient is extremely unlikely to spread to other individuals.

c. *"What precautions will be taken against such spread?"*
There will be no isolation of patients or other specific cautions regarding patient interaction with other individuals. Health workers performing the IV infusion of patient cells will wear gloves. "Needle-less" infusion equipment will be used. No other precautions will be needed.

d. *"What measures will be undertaken to mitigate the risks, if any, to public health?"*
Vector materials used in the transduction procedures will be handled and disposed of following standard protocol for Biosafety 2 level biohazard. Since the biohazard risk is extremely low, no other measures will be taken. There are no anticipated risks to public health.

e. *"In light of possible risks to offspring, including vertical transmission, will birth control measures be recommended to patients? Are such concerns applicable to health care personnel?"*
As indicated in the Informed Consent Document and Page 25 of the Protocol, patients are instructed to use an approved form of birth control for the duration of the gene therapy protocol. Since the transductions are performed *ex vivo*, the cells are extensively washed before administration, and the virus vector supernate is free of replication competent retrovirus, it is highly unlikely that there will be any spread of vector sequence to cells other than the CD34+ cells transduced outside the body before administration. Despite this low risk, the use of birth control during the period especially proximal to administration of transduced cells further limits the possibility of risking transfer of the vector to offspring. There are no anticipated risks of transmission for health care personnel.

II-B-5. Qualifications of Investigators and Adequacy of Laboratory and Clinical Facilities
Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of recombinant DNA. Describe the laboratory and clinical facilities where the proposed study will be performed. Specifically:
All patient treatments and studies will be performed at the National Institutes of Health in the Clinical Center, Building 10 CRC (The inpatient service or outpatient clinics at the Clinical Center; Dr. Kang's and Dr. Malech's laboratory {for basic studies relating to vector and CD34+ cell biology}; in Dr. Fleisher's laboratory in Clinical Pathology {for flow cytometric assays}; or in the Department of Transfusion Medicine under the supervision of Dr. Leitman {for apheresis procedures, selection of CD34+ cells, and the clinical culture and transduction of CD34+ cells}). A variety of safety studies (for example the biological assays for detection of replication competent virus) are done at SAIC Frederick, MD under a National Institutes of Health contract supervised by Dr. Kang and Dr. Malech.

a." *What professional personnel (medical and nonmedical) will be involved in the proposed study and what is their relevant expertise? Provide a two-page curriculum vitae for each key professional person in biographical sketch format* (see Appendix M-I, *Submission Requirements--Human Gene Transfer Proposals*).

Key personnel for this project:

Principal Investigator and Co-Investigator:

Elizabeth M. Kang, MD Clinical Investigator, Laboratory of Host Defenses, NIAID; Expertise in: Bone marrow transplantation; hematopoietic stem cell biology; basic and clinical application of gene transfer to

hematopoietic stem cells.

Harry L. Malech, MD, Deputy Chief, Laboratory of Host Defenses, NIAID; Expertise in: The clinical care of patients with immune deficiencies; hematopoietic stem cell biology; basic and clinical application of gene transfer to hematopoietic stem cells.

Associate Investigators with Specialized Expertise Critical to the Project:

Charles S. Carter, Head Technologist, Cell Processing Laboratory, Department of Transfusion Medicine (DTM), Warren Grant Magnuson Clinical Center (CC); Expertise in: Clinical scale GLP cell processing production of transduced CD34+ PBSC.

Elizabeth J. Read, MD; Chief, Cell Processing Laboratory, DTM, CC; Expertise in: Hematology and blood banking practices; clinical scale GLP cell processing production of transduced CD34+ PBSC.

Other Medical Staff Personnel Involved with this Project:

Sub-Investigators:

Susan F. Leitman, MD; Chief, Blood Services Section DTM, CC

SukSee DeRavin, MD; Clinician, LHD, NIAID

Julia Friend, PA; LHD, NIAID

Akua (Nana) Kwatemaa, RN; Protocol Coordinator, LHD, NIAID

Effie Nomicos, RN; Protocol Coordinator, LHD, NIAID

Dianne Hilligoss, MSN, CRNP; Nurse Practitioner, LHD, NIAID

Susan Foster, RN; Protocol Coordinator LHD, NIAID

Mary Garofalo, RN; Protocol Coordinator, LHD, NIAID

b. " *At what hospital or clinic will the treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will patients occupy regular hospital beds or clinical research center beds? Where will patients reside during the follow-up period? What special arrangements will be made for the comfort and consideration of the patients? Will the research institution designate an ombudsman, patient care representative, or other individual to help protect the rights and welfare of the patient?*"

All clinical studies, treatments, procedures and follow up will take place in the National Institutes of Health Clinical Center located in Bethesda, MD. However, some blood samples may be obtained by the patient's local physician and shipped to NIH. Patients without active infection will be seen in an outpatient day hospital setting, while those patients with severe infections will be inpatients. All outpatient and inpatient facilities at the NIH clinical center are "research" beds or facilities and all treatments and studies at NIH are at no cost to the patient participant in a clinical trial. In addition, any blood studies for this trial done by the patient at home by his personal physician will be paid for by NIH. Patients who are outpatients will be housed during the required stay during any therapy or for follow up at no cost to the patient in either the Children's Inn on the NIH campus (for children) or in nearby hotel/apartment facilities (for adults). NIH has in place a variety of social services, which assure that the social needs of the patient and/or patient family during study participation are met. The NIH Clinical Center has a patient representative who together with his staff assure that patients' rights are protected and that patients can find help from an advocate at the Clinical Center who