the p47-deficient form of CGD established by our own group have also been reported. In that clinical trial, conducted by Malech *et al.* (Malech, Maples *et al.*, 1997), it was demonstrated that autologous hematopoietic progenitors can be transduced with a retroviral vector containing the missing gene. These progenitor (stem) cells can then be safely infused back into the patient. Following this procedure, a small number of genetically corrected neutrophils could be seen in the peripheral blood even out to 6 months without the use of conditioning.

Recently, gene therapy has been shown to be effective in a clinical trial involving patients with Severe Combined Immunodeficiency (SCID), further cementing its effectiveness (Cavazzana-Calvo, Hacein-Bey *et al.*, 2000). Unfortunately, in this trial, three of the successfully treated 10 patients have developed a T-cell leukemia resulting in the death of one of these children. In the first two cases, it was determined that as a result of insertional mutagenesis, there was an interaction between the transgene, which encodes the IL2 receptor common gamma chain and in and of itself provides a selective advantage to transduced cells, and the LMO2 gene (a known oncogene seen in T cell acute lymphoblastic leukemia [ALL]). This resulted in an increased production of the LMO2 protein leading to the development of a leukemic clone (Hacein-Bey-Abina, von Kalle *et al.*, 2003; Hacein-Bey-Abina, Von Kalle *et al.*, 2003). The third patient was found to have four insertional mutagenic integrations, including one near LMO2. The interactions between the transgene and these insertions have not been further characterized and it is not clear which if not all of these insertions are playing a role in the leukemogenesis.

In mouse models of tumorigenesis, of five instances of insertional mutagenesis within the LMO2 gene, two have also had a dysregulation of the IL2R common gamma chain gene (Dave, Jenkins *et al.*. 2004). The risk of insertional mutagenesis, while significant, lies in part with the underlying disease and the transgene itself. This has also been shown in murine studies looking at other transgenes, comparing non-proliferating to selectively advantageous products and has even been used to characterize possible oncogenes (Du, Spence *et al.*, 2005; Modlich, Kustikova *et al.*, 2005). Further, this risk of insertional mutagenesis is not one hundred percent even when the statistical likelihood of inserting into a gene is higher (Schroder, Shinn et al. 2002; Wu, Li *et al.*, 2003) and, in fact, the actual denominator is not known. A total of 24 patients to date have been transplanted successfully with genetically modified cells. Of these 24, three whose underlying diagnosis was SCID developed a serious adverse event in the study by Fischer's group in France. There have been no other insertions within the LMO2 gene causing increased production of the protein detected in the seven other patients within the aforementioned French gene therapy SCID trial, or from patients in a similar trial conducted in England. (ASGT presentations, St. Louis, MO, 2005, Cavazzana-Calvo and Thrasher)

On the other hand, gp91 phox confers no selective advantage to transduced cells, and perhaps in part explains the lack of clinical efficacy in patients that have received genetically modified cells without the benefit of conditioning. Limited engraftment without an ensuing selective proliferation does not lead to sufficient reconstitution resulting in phenotypic correction. However, at a recent public meeting, a German and Swiss collaborative group reported success in treating two adults with CGD using genetically modified cells after busulfan preconditioning (ASGT, St. Louis, MO, 2005 Grez *et al.*,). They also presented data showing a predominance of insertions within three genes, one of which is the MDS/EVI-1 complex. To date, no adverse effects have been seen with follow up of greater than one year in the first patient.

We propose treating patients with X-linked CGD and resistant infections by using autologous, genetically modified cells expressing the gp91 phox transgene and moderate dose busulfan preconditioning. The vector is an MFGS-based vector and the transgene is expressed off the Long Terminal Repeat (LTR). Specific details referencing the vector and its production are given in Attachment 1 (p. 51). This same vector and the actual supernatant to be used here are from the same production used in the prior gene therapy trial (NIH #95-I-0134) for X-linked CGD and have been extensively characterized.

1.4.2 Busulfan

The necessity for some form of conditioning has been proven by a myriad of animal studies. Levels of 5-10% peripherally marked cells encoding for marker genes such as neomycin resistance or GFP (green fluorescent protein) can be seen in large animals only after high levels of radiation conditioning have been used. (Kiem, Andrews et al. 1998; Tisdale, Hanazono et al. 1998; Kiem, McSweeney et al. 1999) Lowering the dose of radiation to reduce the toxicity leads to levels of engraftment that are insufficient for clinical benefit (Rosenzweig, MacVittie *et al.*, 1999; Kang, Hanazano et al. 2001). Additionally, in diseases where a proliferative advantage is not conferred by the corrective transgene, such as in the case of adenosine deaminase (ADA) deficient-SCID or XSCID, efficacy of gene therapy human trials without prior conditioning has been disappointing(Dunbar and Emmons 1994; Kohn, Weinberg *et al.*, 1995; Dunbar, Kohn *et al.*, 1998; Kohn, Bauer *et al.*, 1999; Liu, Kim *et al.*, 1999). Even in the ADA-SCID trials, the best success has been seen after the addition of busulfan preconditioning (Aiuti, Slavin *et al.*, 2002).

Busulfan is a well-known alkylating chemotherapeutic agent, which has been used in traditional allogeneic transplant regimens in combination with other drugs. It is FDA approved for use in the treatment of chronic myelogenous leukemia as well as for use in transplant conditioning regimens. It is specifically toxic to stem cells with very little to no significant immunosuppressive activity. Its efficacy may, in fact, stem from this myelosuppressive specificity. Interestingly, a second gene therapy trial for ADA-SCID, where melphalan (another chemotherapeutic agent) conditioning was used, has not had as high a level of correction as seen in those patients treated with busulfan (Thrasher, A. abstract presentation, ASGT St. Louis, MO 2005). Prior use of busulfan was, however, limited as multiple dosing and drug levels to assess the Area Under the Curve (AUC) were necessary given erratic absorption and therefore, somewhat unpredictable pharmacokinetics from the oral formulation. However, there is now an intravenous formulation available, which makes administration and dosing much more practical and predictable.

The dosing of busulfan at 4mg/kg total chosen for the Italian ADA-SCID trial was somewhat arbitrary and the low dose may have been feasible as the investigators treated patients who were not receiving pegylated ADA, the enzyme lacking in the patients and of which exogenous administration has been considered standard treatment. As a result, the corrected cells would have a small survival advantage when used in combination with busulfan and provide phenotypic correction. As well, these were all infants of age less than 3years, except for one in whom the engraftment was much less successful, but who also received a lower cell dose. There are ongoing animal studies looking at dosing in normal animals where the corrected cells would have no selective advantage at all and 8-12mg/kg (moderate-dose) preliminarily appears to be the threshold at which benefit is seen.

We have also used moderate-dose busulfan in an IRB-approved protocol (NIH # 04-I-0289) giving a total of 10mg/kg over two days to treat a unique pediatric patient in whom a nonmyeloablative allogeneic transplant using his sister as his donor had previously been tried. As a result of the immunosuppressive conditioning regimen, this patient had complete peripheral lymphoid engraftment after a number of Donor Lymphocyte Infusions (DLIs) were given. In contrast, the myeloid chimerism, which was initially at 40%, declined over a period of 2 months to less than 1%, despite the lymphocyte infusions. This patient thus continued to have difficulties due to abnormal neutrophil function. Given the mechanism of action of busulfan, it was opted to treat him with intravenous moderate-dose busulfan as a single agent, without any post-transplant immunosuppression and to use a $CD34^+$ selected graft from the same donor. The patient did not develop any lymphopenia during the transplant and currently demonstrates full engraftment by donor cells in both peripheral blood and bone marrow $CD34^+$ analysis. The entire transplant course was relatively uncomplicated and the patient did not require any antibiotics or transfusions. Thus, we know that 10mg/kg is well tolerated in a pediatric patient, but is sufficient to allow stem cell engraftment.

For both allogeneic transplantation and gene therapy, the size of the graft and the source of the cells are also important. In mouse models, a low level of engraftment can be achieved without the use of conditioning when substantially high (and clinically unfeasible) cell doses are infused into the recipient (Quesenberry, Ramshaw *et al.*, 1994). Further, in the aforementioned gene therapy trials, patients who received higher cell doses have engrafted better than those with less than $1x10^6$ cells/kg infused. In this study we will use stem cells collected by peripheral blood apheresis after mobilization with Granulocyte Colony Stimulating Factor (GCSF) obtained on a separate NIAID IRB-approved protocol (NIH # 94-I-0073). This is a standard method used for collecting cells from patients for autologous or allogeneic/ MUD transplantation.

As it has been shown that a larger cell dose has an increased likelihood of engraftment, the patients will undergo a minimum of two mobilizations and apheresis procedures so as to have a sufficiently large graft. This will also maximize the chance of infusing a graft capable of polyclonal reconstitution.

The actual transduction will take place using recombinant human cytokine and growth factor supplemented media in retronectin-coated bags. These methods have been optimized over a series of experiments at the Laboratory of Host Defenses (LHD) lab and others. Cytokines are necessary for the maintenance and cycling of hematopoietic progenitors, which is necessary for vector incorporation as well as maintaining progenitor and repopulating ability. The cytokines and growth factors to be used are stem cell factor (SCF), fetal liver tyrosine kinase 3-ligand (flt-3), megakaryocyte-derived growth factor (MDGF) and interleukin-3 (IL-3). These are cytokines/growth factors that are found normally in the human body and in some cases have been used in human clinical trials as experimental therapeutic agents themselves. The transductions will all be done in the Department of Transfusion using standard SOPs established for our previous gene therapy protocols with only a few minor changes described in the appended SOP (Attachment 2).

1.5 STUDY OBJECTIVES

1.5.1 Primary Objectives

1) To assess the safety and efficacy of using busulfan as a non-myeloablative conditioning regimen for the engraftment of genetically modified cells in patients with an ongoing infection who lack a sibling matched related donor.

2) To assess the efficacy of using an amphotropic pseudotyped vector expressing the gp91 phox protein in order to obtain phenotypic correction.

3) To eradicate an ongoing infection unresponsive to standard medical therapy.

1.5.2 Secondary Objectives

1) To determine the level of engraftment and correction needed for phenotypic cure.
2) To monitor the duration of correction relative to the initial level of engraftment and marking in order to further elucidate the patterns of hematopoiesis.
3) To monitor clonal contribution to hematopoiesis.

1.6 SCIENTIFIC AND CLINICAL JUSTIFICATION

CGD is one type of inherited immune disorder that results from a mutation in any one of the four genes that encodes the subunits of the phagocyte NADPH oxidase enzyme that converts molecular oxygen to superoxide and hydrogen peroxide. X-linked CGD patients are susceptible to having a higher incidence of recurrent bacterial and fungal infections and have a significantly reduced life expectancy. The patients being considered here are at high risk of death due to their underlying infection and have limited treatment options.

The standard modality used for cure of CGD is hematopoietic progenitor transplantation, however, this therapy is limited in a number of ways, primarily due to donor availability. The best results are obtained using HLA-matched sibling donors of whom these patients have none. Although the use of matched, unrelated donor transplantation (MUD) improves the chances of finding a suitable donor, the risks of graft rejection and/or GVHD are increased, and both related and unrelated transplants continue to have significant risk.

The clinical research program in the Genetic Immunotherapy Section of the LHD is actively developing new treatments for patients with various abnormalities of immune function. This protocol offers potential correction of X-linked CGD immunodeficiency through the administration of gene therapy after conditioning with moderate-dose busulfan in patients who lack a suitable HLA-matched sibling donor. Gene therapy offers a potential cure with less risk related to the actual procedure itself. In addition, even if the treatment is not life-long, it can act even temporarily to eradicate the infection in question. Finally, the use of gene therapy, if not successful, does not preclude the use of a MUD.

1.7 QUALIFICATION OF INVESTIGATORS

This protocol will be conducted by a multidisciplinary team encompassing expertise in immunology, infectious diseases, and hematology/oncology required for the diagnosis and care of patients with the abnormalities of immune function that are to be enrolled into this protocol. The Principal Investigator, Dr. Elizabeth Kang, and the care team are also knowledgeable in the conduct of good practice principles of clinical research and the regulatory requirements for the protection of human subjects. All investigators collaborating in this study have met the training requirements of the Office of Human Subjects Research. Copies of CV's to demonstrate the experience and qualification of all investigators will be updated and kept on file.

The Deputy Director and Chief of the Blood Services Section of the Department of Transfusion Medicine (DTM), Dr. Susan Leitman, the Chief of the Cell Processing Laboratory of the DTM, Dr. Elizabeth J. Read, are Associate Investigators on this protocol. They are experts in blood banking technology and specifically have extensive experience in the culture, transduction, and preparation of gene altered human hematopoietic stem cells for administration to patients. They will have direct involvement with and oversight of the entire cellular therapy manufacturing process.

1.8 CONFLICT OF INTEREST

Investigators conducting this protocol shall ensure that procedures are upheld to avoid real or apparent conflicts of interest within accordance to the guidelines of the institution for which they work. No reportable conflicts of interest have been identified by any of the investigators conducting this trial.

1.9 CONDUCT OF THE TRIAL

This protocol will be conducted according to all applicable laws, regulations, and policies of the NIAID and the NIH governing IRB approved protocols. The Principal Investigator will assure that no deviation from, or changes to, the protocol will take place without prior documented approval from the IRB, except when necessary to eliminate immediate hazards to the trial participants, or when the changes involve only logistical or administrative aspects of the trial (e.g., change in monitors). The Principal Investigator will promptly report to the IRB any changes in research activity and all unanticipated problems involving risk to human subjects, or others.

Prior to the implementation of this study, the protocol and the proposed patient consent form will be reviewed by an IRB operating under the Code of Federal Regulations (DHHS, 21 CFR 56) (see attached Patient Consent Form). A signed and dated letter stating that the IRB has approved the protocol will be submitted to the sponsor, the Clinical Director and the DSMB prior to start of the study.

In addition, the study will not proceed without prior approval by the National Institutes of Health Institutional Biosafety Committee (IBC), the Center for Biologics Evaluation of the Food and Drug Administration (FDA), and a notification of allowance by the Office of Biotechnology Activities (OBA) (which includes a review by one or more members of the Recombinant DNA Advisory Committee).

2.0 STUDY DESIGN

This is a pilot study to evaluate the safety and feasibility of gene therapy in patients with X-linked Chronic Granulomatous Disease (CGD) who have an ongoing, unresponsive infection and no available HLA matched sibling donor. The primary criterion for safety and efficacy will be: negative results of all safety studies (all safety criteria met and/or no problems identified); no reports of toxicity related to the infusion of busulfan conditioning greater than Grade 2 (CTC, Version3.0) (http://ctep.cancer.gov/forms/CTEP/CTCAEv3.pdf, Appendix A); no reports of toxicity related to the infusion product greater than Grade 2; the successful insertion of provirus into 5% or more of patient $CD34^+$ cells *ex vivo*; the detection of provirus in any mature blood lineage *in vivo*; and the appearance of any neutrophils in the peripheral blood which express normal gp91; clearance of the ongoing infection.

$CD34^+$ peripheral blood progenitor cells, using GCSF mobilization, will be collected under NIH Protocol #94-I-0073. The patients will undergo a minimum of two mobilizations and apheresis collection procedures. The first product will then be $CD34^+$selected, frozen, and later combined with the subsequent product(s), which may or may not be frozen prior to use. The cells will be thawed, if necessary, and transduced in retronectin-coated bags in media containing SCF, flt-3, thrombopoietin (TPO), and IL-3 with the MFGS gp91 expressing, amphotropic pseudotyped vector over a period of four days.

Patients will be enrolled as they meet the criteria for eligibility and preparations of the cell product have met all transduction requirements as outlined in the DTM SOP. There will be three months between the treatment of patients. Two days prior to the day of cell infusion, the patients will be admitted to the NIH Clinical Center and given busulfan, 5mg/kg daily, infused over 2 hours, for two days. One day after completion of the busulfan infusions, the cells will be collected from culture, washed, and infused into the patients.

The patients will be monitored for toxicities associated with the busulfan and/or the gene therapy including monitoring of the complete blood count, and liver chemistries. Additionally, busulfan drug levels will be measured after the first infusion of the drug. The patients will be discharged from the NIH when the Absolute Neutrophil Count (ANC) is greater than 500 on three consecutive days.

The gene marking levels will be monitored every 3 months for the first year post transplant, then at 18 and 24 months, followed by every 12 months. Included in the monitoring will be a complete blood count as well as linear amplification-mediated PCR (LAM PCR) to assess the clonality of marking. Assessments of neutrophil function will also be performed to assess phenotypic correction. More frequent monitoring of LAM-PCR will be done if there is any indication or need as suggested by clinical findings, or by the CBC or previous LAM PCR results.

2.1 STUDY DESIGN FLOW CHART

2.1.1 Study Entry (Month –2 to Day –2)

This is a pilot study designed for patients who have an untreatable, persistent infection and who have no sibling-matched donor for stem cell or bone marrow donation.

2.1.2 Transduction of Autologous CD34+ HSC (Day –4 to Day 0)

- *Ex vivo* culture of patient's CD34+ hematopoietic stem cells (HSC) with amphotropic-MFGS-gp91 vector per SOP.
- On Day 0, these cells will be washed and reconstituted in Plasmalyte-A with 1% human serum albumin.

2.1.3 Patient Preparation for Infusion of Autologous CD34+ HSC (Day –4 to Day –1)

Patient will be admitted to the NIH inpatient service and will have a central venous catheter (CVC) placed if one is not already present.

2.1.4 Daily Patient Management

- Infection Prophylaxis (See Supportive Care [SC] guidelines- http://intranettst2.cc.nih.gov/bmt/clinicalcare)
- Fever Regimen (See SC Guidelines- http://intranettst2.cc.nih.gov/bmt/clinicalcare)
- Bleeding Prophylaxis - Platelets will be maintained at greater than 10,000/μl.

2.1.5 First Busulfan Drug Infusion (Day –2, two days prior to transduced cell infusion)

- The patient will be administered IV phenytoin dosed at 5 mg/kg via the central venous access line prior to each daily dose of busulfan.
- 30 minutes afterwards, the patient will receive the first of two IV busulfan infusions at a dose of 5mg/kg prepared in normal saline, given as a 2-hour infusion.
- The patient will be monitored with vital signs taken every 15 minutes for one hour, then every 4 hours while awake. (See also Section 19.0 Pharmaceutical, Biologic, and Device Info: BUSULFAN)

Laboratory studies to be performed on Day –2 are:

- Drug (busulfan) levels to be obtained pre-infusion, end of infusion, 135, 150, 180, 240, 300, and 360 minutes after the first infusion of the drug for a total of eight samples on Day -2. The levels will be assessed by chromatographic analysis at a reference lab.

- Vital signs will also be recorded with each level drawn, then every 4 hours while awake.

2.1.6 Second Busulfan Drug Infusion (Day –1, one day prior to transduced cell infusion)

- The patient will be administered IV phenytoin dosed at 5 mg/kg via the CVC line. This dose will be the second and final dose. No additional doses will be administered. No maintenance dose will be prescribed.
- 30 minutes afterwards, the patient will receive the second busulfan IV infusion at a dose of 5mg/kg prepared in normal saline and given as a 2-hour infusion.
- The patient will be monitored with vital signs taken every 15 minutes for one hour, then every 4 hours while awake.

Subject receives 5mg/kg of busulfan each day for a total of 10 mg/kg total.

2.1.7 Infusion of Autologous CD34+ HSC (Day 0)

- Minimum transfusion dose of cell product of 5×10^6 cells per kg body weight.
- Transduced cell product administered intravenously over 10 minutes at NIH Clinical Center (outlined in the CC SOP for the Infusion of Products for Cellular Therapy).

Subject will remain as an inpatient at the Clinical Center post-cell product infusion. Vital signs and safety evaluations (CBC with differential and CHEM 20) will be assessed prior to discharge, which will occur after recovery from the expected neutrophil nadir. The subject will be discharged when the absolute neutrophil count (ANC) is greater than 500 for three consecutive days (usually around Day #11 or 12).

2.1.8 Patient Follow-Up Post Transfusion (Time 0 to Month 42)

- Telephone monitoring interviews (Weekly Surveillance) with patient will be conducted each week for the first 3 months, excluding weeks in which subject visits NIH for follow-up (e.g., Months 1, 2, 3 visits).
- Subject will return to NIH Clinical Center within +/- 6 weeks of Month 1 (PE and blood specimen only), within +/- 6 weeks of Month 2 (PE and blood specimen only) and within +/- 6 weeks of Months 3, 6, 12, 18, 24, 30, 36 and 42 for history, physical, blood specimen laboratory testing and Quality of Life questionnaire assessment (except for months 1 and 2). The Quality of Life Questionnaire tool will be used informally to assess the patient's perception of their clinical well-being.
- The subject may be required to return to the NIH Clinical Center within +/- 6 weeks of Months 9, 15, 21, 27, 33 and 39 for additional laboratory evaluations. In the event that the subject cannot travel to the Clinical Center for these scheduled visits, blood sampling kits and instructions will be provided for these collections to be done by the subject's private physician. Also during Months 9, 15, 21, 27, 33 and 39, a telephone call will be placed and a Medical Encounter/Medication Review will be recorded.

2.1.9 Safety Follow-Up (Starting at 3 Months and Continuing for 15 Years)

- Monitoring for insertional mutagenesis will be conducted within +/- 6 weeks of Months 3, 6, 12, and every 6-12 months thereafter for 15 years (or until withdrawal from the study). The subject will be brought to the NIH Clinical Center for history and physical exam, basic chemistries, urinalysis, and hematologic and immunologic testing including: CBC, differential, lymphocyte surface marker phenotyping (including alpha-beta and gamma-delta receptor-bearing T cells) and immunoglobulins (Ig's).
- Provirus will be assessed by PCR; if any provirus is detected, insertion site analysis will be performed by LAM PCR or an equivalent method. If a predominant insertion site (defined as >50% of the insertions in a cell lineage) is observed, a second test will be performed within 3 months to see if the clone is persistent. Attempts will be made to isolate every predominant vector insertion location, and the insertion site(s) will be sequenced and evaluated for proximity to cancer-associated genes. The subject will be clinically monitored closely for signs of malignancy. Frank malignancy or continued rise in cell number within a lineage to >5-fold above normal values will prompt reporting as a serious adverse event (see Toxicity Tables, Appendix A). However, if no provirus is detectable in any blood lineage in each of 3 consecutive samples taken at 6-month intervals, only annual follow-up as outlined here will be continued indefinitely.
- Blood will be tested for Replication Competent Retrovirus (RCR) at months 3, 6, and 12; additional blood samples will be obtained annually and stored so that RCR testing can be performed retrospectively if an indication arises.

3.0 STUDY ENDPOINTS

This is a study pilot designed to treat 5 patients with an amphotropic pseudotyped vector. The study size is based upon availability of vector at this current time and tolerance to busulfan.

3.1 PRIMARY ENDPOINTS

The primary safety and efficacy goals of this study will be considered achieved if:

1. final results of all safety studies on pre-infused cell products and the patients post-infusion are negative.
2. no reports of toxicity greater than Grade 2 related to the infusion of busulfan conditioning
3. no transduced HSC-related toxicity greater than Grade 2 is noted.
4. resolution, either complete or partial, of the infection for which the patient is enrolled, can be documented within one year of infusion of the cells.

- Complete resolution will be determined either by radiographic imaging, and/or other diagnostic testing, showing no further evidence of the targeted infection.
- Partial resolution will be defined as either a lack of further progression of the infection or diminishment but not complete resolution of the infection as measured either radiographically and/or by other diagnostic testing.

3.2 SECONDARY ENDPOINTS

The secondary safety and efficacy goals of this study will be considered achieved if:

- appearance of any neutrophils in the peripheral blood which express normal gp91.
- evidence of successful insertion of provirus into 5% or more of the patient $CD34^+$ cells *ex vivo*.
- provirus can be detected in any mature blood lineage *in vivo*.
- persistence of genetically corrected cells for a minimum of 4 months post infusion
- comparison of transduction efficiency between different recipients of the autologous genetically modified cells.
- comparison of *in vitro* transduction efficiency and *in vivo* marking levels as measured by flow cytometry and PCR analysis of the end product and peripheral blood samples obtained from each patient.

4.0 SUBJECT ENROLLMENT

4.1 PATIENT SELECTION CRITERIA

4.1.1 Inclusion Criteria

1. Have a diagnosis of X-linked CGD (*i.e.*, a gp91-*phox* gene mutation/defect).
2. Have a minimum of 5.0 x10^6 CD34+ cells per kg body weight (collected and cryopreserved, prior to enrollment) available for transduction.
3. Weight greater or equal to 20 kg.
4. Unresponsive or incurable infection as defined by either/or:
   - Continued (stable or progressive) infection despite standard antimicrobial therapy
     - Stable and/or does not completely resolve despite a minimum of 2 months of treatment

     OR
     - Progressive as shown by increase in size or new sites of infection despite therapy for a minimum of two weeks.
   - Multidrug resistant organism as determined by tissue analysis
5. Not have a suitable sibling who is HLA-matched for stem cell or bone marrow donation.
6. Males aged 3-55 years.
7. Must use two approved methods of contraception, such as barrier method (condom) with spermicide.

4.1.2 Exclusion Criteria

1. Weigh less than 20 kg.
2. Be hemodynamically unstable, or requiring pressor support.
3. Require ventilatory assistance with high levels of oxygen.
4. Have an HLA-matched suitable sibling for stem cell or bone marrow donation.
5. Intolerance to busulfan.
6. Failure to use two approved methods of contraception, such as barrier method (such as a condom with spermicide).
7. Participation in another Gene Therapy clinical trial
8. If pre-conditioning and pre-infusion evaluations are found to match a criterion for Grade 4 toxicity as defined in the Appendix A, Toxicity Table*

* When the condition or parameter has returned to the criterion for Grade 3 or less for that condition or parameter twice over an interval of at least 4 weeks prior to the infusion, eligibility may be resumed.

5.0 TREATMENT PLAN

5.1 CLINICAL AND LABORATORY EVALUATIONS

See Appendix B: Schedule Of Study Evaluations.

5.1.1 Study Entry Evaluations

The pre-infusion evaluation will be completed within the two months prior to Day –2 (i.e., busulfan conditioning). It is expected that these evaluations will require one to three clinic visits.

a. Informed consent/assent obtained.

b. Obtain health history (baseline **Quality of Life** questionnaire), including information about number of hospitalizations, missed school days over the past year, the activity of recurrent infections, presence of autoimmune diseases such as colitis and sarcoid, family history of childhood malignancy, and high-risk behaviors or other circumstances affecting the patient as reported by the patient or parents that might increase the risk for HIV and hepatitis). This questionnaire will be used as an extension of the same tool currently used for the XSCID Gene Therapy NIH protocol # 02-I-0057, with very minor revisions made to include adults as well as children in the questionnaire. These qualitative observations will be used to assess the overall condition of the patient during the specific time interval.

c. The patient will be invited to participate in other protocols for which they may be eligible.

d. Physical Examination: including height, weight, vital signs, electrocardiogram, pulse oxygenation measurement, urinalysis, and a chest X-ray or CT. Pulmonary functions studies may be performed as part of the baseline evaluation. Regardless of the results, these will not be considered as part of the exclusion criteria.

e. Clinical laboratory blood studies: Complete blood count (CBC) with differential, platelets, clotting studies (PT and PTT), Erythrocyte Sedimentation Rate, CHEM 20 chemistry profile (includes measures of liver and renal function), TTV (transfusion-transmissible viruses screen, including anti-HIV 1, anti-HIV 2, anti-HTLV 1, anti-HTLV 2, HBsAg, anti-HBc, anti-HCV and HIVp24Ag.). Antibody testing, which occurs as part of the routine transfusion-transmissible viruses screen noted above as required by the NIH Department of Transfusion Medicine, will be performed because the presence of such antibodies must be documented. However, only FDA approved antigen based tests (HBsAg and HIVp24Ag) can be used as criteria to determine if the patient has hepatitis B or HIV1, which, if positive, exclude the patient from participation in the study (as noted in Section 4.1.2 Exclusion Criteria). Requires 20 ml of blood.

f. Functional analysis of neutrophil function by dihydrorhodamine assay (DHR). Requires 3ml of blood.

g. Flow cytometry analysis of blood to measure cell surface markers for neutrophils, T, B, and NK cells (e.g. markers such as CD3, CD4, CD8, CD11, CD16, CD19 or CD20, CD56, CD132) for quantification of these cellular subsets and for analysis of gp91 expression. Requires 5 ml of blood. (4 mL for pediatric patients)

h. Quantification of immunoglobin levels in blood (IgM, IgA, IgE, and IgG) as a measure of B cell function. If the patient has been exposed either by environmental acquisition or vaccination to a known antigen, and if sera before and after the exposure are available, paired sera will be evaluated for four-fold rises in specific antibodies. Requires 10 mL of blood (2 mL for pediatric patients).

i. Serum sample will be obtained and stored frozen for a baseline comparison of specific and/or general antibody production. Requires 10 mL of blood.

j. Storage of baseline DNA from peripheral blood cells for comparison with post-transduction samples of DNA from T, B, NK, and myeloid cell lineages to be obtained later to evaluate copy number of retrovirus vector marking. Requires 10 ml of blood.

k. Peripheral blood mononuclear cells obtained for baseline RCR testing and archiving. Requires 10 ml of blood.

l. If pre-conditioning and pre-infusion evaluations (must have been performed within two months of the busulfan conditioning) are found to match a criterion for Grade 4 toxicity as defined in the Appendix A, Toxicity Table, then the patient would become ineligible for conditioning and gene therapy transfusion. When the condition or parameter has returned to the criterion for Grade 3 or less for that condition or parameter twice over an interval of at least 4 weeks prior to the infusion, eligibility may be resumed. Furthermore, if the pre-infusion evaluations demonstrate any condition, which, in the judgment of the Principal Investigator (PI) might significantly increase the risks of gene therapy for that patient, then the PI may delay such infusion or withdraw the patient prior to treatment.

m. Blood draw volume assessment: Blood sampling for study entry performed as outlined above (including any optional testing) should require a total volume of approximately 95mL (less for pediatrics). All blood draws will be tracked to ensure that specimen collections do not exceed the 450 cc per 6 week Clinical Center policy for adults. Pediatric patients will have blood draws limited to 3mL/kg/day or 7mL/kg in a 6-week time interval.

6.0 PLAN FOR MONITORING SUBJECTS AND CRITERIA FOR EARLY WITHDRAWAL

Subjects may be withdrawn from the study for the following reasons:

1. If no infusions of cultured, transduced cell product occur for any reason and there are no plans to go forward with any future infusion of gene therapy, then the patient will be considered withdrawn from the study. If any amount of cell product is infused, the patient will be maintained on study and will receive all follow-up evaluations.

2. The patient may withdraw voluntarily from this study at any time, although they will be strongly encouraged to receive the cells after busulfan has been administered, as there is increased risk of prolonged neutropenia without cellular support post chemotherapy.

3. The IRB, the NIAID, the FDA, or other government agencies, as part of their duties to ensure that research subjects are protected, may discontinue a subject from the study at any time.

6.1 MONITORING DURING TREATMENT

6.1.1 Pharmacokinetics of Busulfan Therapy

Busulfan drug levels will be measured after the first infusion of the drug, with vital signs also recorded at: pre-infusion, 120, 135, 150, 180, 240, 300, and 360 minutes post infusion (total of 8 samples). The levels will be assessed by chromatographic analysis at a reference CLIA-certified lab. Specimens will be coded with a subject identification number. Access to these results will be accessible only to investigators to ensure confidentiality (total blood volume drawn is expected to be less than 20 mL for either adults or pediatrics).

6.1.2 Monitoring During Transduced Cell Infusion

Vital signs will be recorded at: 15 minutes before, 15, 30, 60, and 120 minutes post infusion of cell product. Vital signs and safety testing (CHEM 20 and CBC with differential) to be assessed prior to discharge, and the subject will be discharged when the ANC is greater than 500 on three consecutive days (expected to be some time after Day #11 or 12).

6.2 MONITORING AND EVALUATIONS POST INFUSION TO MONTH 42

a) Complete history (with Quality of Life questionnaire assessment) and physical examination (Months 1 and 2 PE only) 3, 6, 12, 18, 24, 30, 36, and 42.

b) Interval telephone health history weekly (i.e., "Weekly Surveillance") for the first 3 months (excluding weeks in which subject is an inpatient awaiting ANC>500 or when visits NIH for follow-up at Months 1, 2, 3)

c) Medical Encounter/Medication Review at Months 9, 15, 21, 27, 33, and 39, to be recorded by telephone. If the patient is required to travel to the Clinical Center, this form supplements the History & Physical (H&P) form.

d) Peripheral Blood Mononuclear Cells (PBMC) for RCR testing by PCR for detection of envelope sequence, or by marker rescue assay, or by other accepted bioassay (Months 3, 6, 12). PBMCs to be cryopreserved annually after Month 12 if all previous RCR tests are negative.

e) Whole blood CBC with differential and Erythrocyte Sedimentation Rate Month 1, 2, 3, 6, 9, 12, and every 6 months thereafter.

f) gp91 functional analysis by DHR assay at baseline, every 3 months for the first year, and then annually.

g) CHEM 20 (Months 1, 2, 3, 6 [PRN], 9 [PRN], 12, 24, 36, and 42).

h) Urinalysis (UA) at baseline and every 6 months until month 42 and then annually thereafter.

i) PCR for provirus copy number every 6 months; if provirus detected, check insertion site diversity by LAM-PCR or equivalent method. If any insertion site clone becomes predominant, additional measures will include sequencing of insertion site(s) with identification of any activation of cancer or proliferation associated genes, along with close clinical monitoring.

j) Quantification of immunoglobin levels in blood (IgM, IgA, IgE, and IgG) as a measure of B cell function (every 6 months until Month 24 and then annually thereafter).

6.3 LONG-TERM EVALUATIONS BEYOND MONTH 42

The patient will return to NIH every 6 -12 months for a minimum of 15 years to life so that safety monitoring for insertional mutagenesis can be conducted. In addition to history and physical exam, we will obtain CBC with differential, Sedimentation Rate, CHEM 20 profile, lymphocyte surface markers, PCR for provirus copy number, insertion site diversity if provirus detected, and follow-up as above (Section 6.2) if any insertion site becomes predominant.

PBMC collected and stored annually to be tested for RCR only if clinically indicated.

6.4 TREATMENT ADMINISTRATION

If 5 x 10^6 transduced autologous hematopoietic stem cells (HSC) per kg of body weight are available and the subject is clinically stable, the subject will receive busulfan 5mg/kg infused over 2 hours, for 2 consecutive days. Following the busulfan conditioning, which will be administered on the previous two days, the cell product will be given by intravenous push over 5-10 minutes in the NIH Clinical Center (outlined in the CC SOP for the Infusion of Products for Cellular Therapy). Infusion orders will be written by the NIH-credentialed PI or by any of the Associate Investigators (AI). Transduced cell product administered intravenously over 10 minutes at NIH Clinical Center (outlined in the CC SOP for the Infusion of Products for Cellular Therapy).

All efforts will be made to infuse the product once the busulfan has been infused (the prior day). although the dose of busulfan given is such that autologous hematopoietic recovery should occur even without cellular infusion. Busulfan drug levels will be measured after the first infusion of busulfan. Vital signs will be taken within 15 minutes prior to the transfusion of the stem cells and then at approximately 15, 30, 60, 90, and 120 minutes post-transfusion. The patient will be allowed to return home when the absolute neutrophil count is greater than 500 on three consecutive days, and vital signs and safety testing results (CHEM 20, CBC with differential) are stable as compared with baseline. Prior to discharge, the subject will be shown and provided with a blood collection kit. The subject will be verbally instructed on a standardized method for specimen collection and shipment to the PI. The patient will go to his primary medical provider to obtain any blood samples, which will then be sent to NIH. A printed list of instructions will also be provided for the patient and his medical provider.

The patient will be instructed to follow the standard post-allogeneic transplant recommendations for receiving vaccinations, after being assessed for immune status 6 months post gene therapy.

A list of contact people and their phone numbers will also be provided for the medical provider in the event that AE's/SAE's, questions, or other needs arise.

7.0 HUMAN SUBJECT PROTECTION

The trial will be conducted in compliance with this protocol, International Conference on Harmonization (ICH) Guideline for Good Clinical Practices (GCP) and any applicable regulatory requirement(s).

7.1 RATIONALE FOR SUBJECT SELECTION AND RECRUITMENT STRATEGIES

The subjects selected for participation in this protocol are limited to males age 3 to 55 as, by definition, this is an X-linked disorder and females are ineligible for participation. There are no recruitment restrictions based on minority or ethnic diversity and male children over the age of 3 are not excluded from participating.

Recruitment will be made from patients who may already be participating/have participated in existing NIH protocols and are being referred by their NIH physician for inclusion in this protocol. Primary care physicians outside NIH may also refer patients for inclusion in this protocol.

Any advertising recruitment strategies that may include use of the Internet, mention in professional journals, or in the form of notices will be submitted to the NIAID IRB for approval prior to use.

7.2 CONSENT PROCESS

Either the Principal Investigator or any of the Associate Investigators may make initial patient contact regarding participation in this protocol. If the patient expresses an interest in learning more about the protocol, then additional verbal and written information about the protocol (including copies of the protocol as well as the consent and assent documents) will be provided by a member of the staff working with the Principal Investigator and Co-Investigator. Only the Principal Investigator, Dr. Elizabeth Kang, or the Co-investigator, Dr. Harry Malech, will provide the final extensive verbal discussion of the design and goals of the protocol and the risks and benefits associated prior to obtaining the signed informed consent.

The patient must be alert, oriented, and capable of asking questions and providing consent or age appropriate assent at the time of all discussions about the protocol and at the time of enrollment. The patient will be provided written copies of the protocol and the informed consent document. The patient will then be requested to consult with family, friends, home physician and/or other advisors about the protocol for as long as needed to read the documents and discuss the protocol with others. At least 24 hours after providing the written protocol and consent/assent documents to the patient, the PI (Dr. Kang) or the Co-investigator (Dr. Malech), again will explain, in detail, the goals of the protocol, procedures, risks, and benefits. This will include a point-by-point

discussion of the items in the consent document. This discussion will take place in the presence of an individual who is not a family member or an investigator associated with the protocol, and who will sign as "witness" to the consent document. Since gene therapy protocols are inherently complex, it may be difficult to be certain that the patient fully understands the procedures, the risks and the benefits of the protocol.

After these procedures, the patient will be asked to sign and date the consent document. The witness and the PI or designated Co-investigator will also sign and date the consent document. The signed original consent/assent documents will become a part of the permanent medical record at NIH. Copies will be provided to the patient and also retained in the research data records. If during the time of the discussions relating to enrollment or at any time during participation in the protocol any new information becomes available relating to new risks, adverse events, toxicities, or results from experience with other participating patients that relate to risk or benefit, this information will be provided orally or in writing to all enrolled or prospective patient participants in a timely fashion.

If the patient is a minor, when possible, both parents who sign for the minor must be legally recognized parents or guardians to indicate understanding and consent for their child to participate in the study. Where deemed appropriate by the Investigator and the child's parent or guardian, the child will also be included in all discussions about the study and the minor's assent will be obtained. The parents or guardians will sign on the designated line on the consent form attesting to the fact that the child has given assent.

7.3 RISK/BENEFIT ASSESSMENT

Based on the collected experience of the last 10 years of gene therapy clinical protocols, including our own NIH protocol #95-I-0134, which used murine retrovirus vectors for *ex vivo* transduction of autologous HSC or lymphocytes, we believe this method of gene transfer to be safe and well tolerated. Therefore, the known risks to CGD patients receiving a single transfusion of transduced autologous HSC are likely to be very low. Patients who have had recurrent infections have a high risk of death, and near certain mortality as a result of their ongoing infection. However, since 2002, leukemia has developed in three patients who received retroviral gene therapy for XSCID. The risks of insertional mutagenesis are not known at this time but are considered to be lower in the context of CGD. The actual risk will only be learned as more human trials are conducted.

7.4 PLAN FOR MAINTAINING THE PRIVACY AND CONFIDENTIALITY OF SUBJECT RECORDS

The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by a coded number only in order to maintain subject confidentiality. All records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject or subject's guardian, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, the pharmaceutical supporter(s), or the supporter's designee.

8.0 PATIENT MEDICAL MANAGEMENT AND THERAPEUTIC CONSIDERATIONS

Primary care of the subject's medical condition will continue to be under the auspices of the referring physician, when the patient is deemed able to be transferred to their care. A letter describing the study will be sent to the subject's primary care physician. Also in this letter will be details outlining what conditions must be reported to the PI/AI along with their contact information. The subject's primary care provider(s) will be encouraged to call the PI/Co-PI should any change in condition be noted as compared to the subject's baseline status. Should the occasion arise, subjects may also be treated at the NIH Clinical Center.

The patient will be asked to return to the NIH Clinical Center at Months 1, 2, 3, 6, 12 post-infusion and then at least every 6 months for 3 years, then a minimum of every 12 months for 15 years minimum. A physical exam along with basic lab evaluations (CHEM 20, CBC with a differential, Sedimentation Rate, PCR for provirus insertion, monitoring for predominance of insertional sites, and follow-up of any suspected clonal proliferation (as described in Sections 6.2 and 6.3) will be conducted. RCR testing will only be conducted after Month 12, if clinically indicated. Should the subject die for any reason, a family member will be asked to have an autopsy completed. Tissue samples will be requested to assess for RCR and the presence of transgene in biopsied specimens. Such specimens will include, if possible, peripheral blood, bone marrow, a lymph node, liver, spleen, and gonad. If available, tissues from other organs will be sampled if the cause of death is presumed to involve such other organ systems.

9.0 ADVERSE EVENT REPORTING PLAN

9.1 DEFINITION OF AN ADVERSE EVENT

An adverse event (AE) is any undesirable experience/unwanted effect that occurs in a patient or to a study participant during the course of a clinical trial (or a reasonable time after trial termination) whether or not that experience is considered related to the drug/ investigational product. An adverse event can therefore be any unfavorable and unintended physical or psychological sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal/investigational product, whether or not considered related to the medicinal/investigational product. This includes exacerbation of pre-existing conditions and intercurrent illnesses.

Stable chronic conditions which are present prior to clinical trial entry and do not worsen are not considered adverse events and will be accounted for in the subject's medical history.

9.2 GRADING ADVERSE EVENTS

AE's will be graded for severity as defined in **Table A,** Toxicity Chart for Adverse Event Monitoring. All other laboratory and clinical AE's that occur in a study participant will be

assessed (graded) for severity and then classified into one of four clearly defined categories as follows:

- **Mild (Grade 1):** Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the patient/study participant's normal functioning level.

- **Moderate (Grade 2):** Symptom results in some limitation in activity/ some impairment of functioning; did not result in loss of work or cancellation of social activities. No or minimal intervention required. It is uncomfortable or an embarrassment.

- **Severe (Grade 3)**: Symptom results in significant limitation in activity; required bed rest and/or resulted in loss of work or cancellation of social activities. Medical intervention often required. The AE produces significant impairment of functioning or incapacitation.

- **Life-threatening (Grade 4):** Extreme limitation in activity, significant assistance required; immediate medical intervention or therapy required to prevent death.

9.3 ASSESSING THE RELATIONSHIP OF AN ADVERSE EVENT TO THE STUDY TREATMENT

Any AE that occurs in a patient/study participant will be assessed for relationship to the investigational study treatment. The best estimate at the time of reporting of the causal relationship between the experimental intervention and an adverse event and the degree of certainty about causality will be graded as follows:

Definitely Related: The adverse event and administration of study agent are related in time, and a direct association can be demonstrated (e.g., disappears or decreases with reduction in dose or cessation of drug/investigational product and recurs with re-exposure).

Probably Related: The adverse event and administration of study agent are reasonably related in time and/or follows a known pattern of response, and the adverse event is more likely explained by study agent than other causes.

Possibly Related: The adverse event and administration of study agent are reasonably related in time and/or follows a known pattern of response, and the adverse event can be explained equally well by causes other than study agent (e.g., could readily have been produced by the subject's clinical state or could have been due to environmental or other interventions).

Unlikely Related: A potential relationship between study agent and the adverse event could exist (i.e., the possibility cannot be excluded), but the adverse event is most likely explained by causes other than the study agent (e.g., could readily have been produced by the subject's clinical state or could have been due to environmental or other interventions)

Not Related: Adverse event is clearly due to extraneous causes (e.g., underlying disease, environment) or exposure to the investigational product has not occurred. Such events MUST have an alternative, definitive etiology documented in the patient's medical record.

9.4 MONITORING ADVERSE EVENTS

The safety of infusing cultured and transduced CD34+ HSC will be determined by clinical and verbal monitoring of the patient who demonstrates:

- the absence of adverse events
- testing of the cultured transduced CD34+ cells
- absence of infectious agents including RCR as outlined in the Schedule of Evaluations (Appendix B).

While at home, the patient's local medical provider will be instructed verbally and with written guidelines to contact the NIH investigators immediately for any adverse event. A toll-free number will be available to provide 24-hour access to the PI or his designee. The details of all adverse events, including the dates, nature of the event, and the action taken, will be entered onto the standard NIAID IRB and Office of Biotechnology Activities (OBA) Adverse Event (AE) Reporting forms. Copies of the AE report and other required regulatory information will be provided to the 1) NIAID Clinical Director, 2) NIAID DSMB, 3) NIAID IRB, 4) NIH Institutional Biosafety Committee (IBC), 5) OBA, and the 6) FDA as per the Gene Therapy Guidelines. Reports will be submitted to each regulatory board annually with the exception of the DSMB, which will require reports at least semi-annually. The PI and site staff will maintain copies of the reports in a permanent record. An independent protocol contract monitor will review all adverse events for accurate and timely reporting at the time of the study visit. The PI and site staff will periodically (at least yearly) review all adverse events to assess trends and to ensure both the safety and full informed consent of the participant in the study.

9.5 EXPECTED ADVERSE EVENTS

Patients with CGD are expected to have recurrent bacterial, fungal, or viral infections. Furthermore, patients with CGD are expected to need recurrent treatment with antimicrobials. However, self-limited reactions to these medications will not be considered adverse events related to this protocol. Examples of such reactions include fevers, hives, pruritus, and malaise. These patients will be treated for their underlying infections during their first year of participation on the protocol. Afterwards, The PI and the research team will maintain a consultative role with the patient's referring physician. After the first year on protocol, patients will be provided only with study-related medications by the NIH research team. Commonly expected side effects due to venipuncture procedures which will not be considered adverse events and will not be reported as such include: pain, bruising and some bleeding at the site of venipuncture, lightheadedness, and fainting. Any infection that directly results from a procedure required as a part of this study (e.g., an infection at a site of venipuncture required for study blood draw or for administration of the transduced cell product) will be reported as an adverse event.